ATRAZINE

RISK CHARACTERIZATION DOCUMENT

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List of Abbreviations

AADD	Annual Average Daily Dosage
ADD	Absorbed Daily Dosage
ADI	Acceptable Daily Intake
BMD	Benchmark Dose
CDFA	California Department of Food and Agriculture
DACT	Diaminochlorotriazine
DEA	Desethylatrazine
DIPA	Desisopropylatrazine
DPR	Department of Pesticide Regulation
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GnRH	Gonadotrophin Releasing Hormone
HDT	Highest Dose Tested
IARC	International Agency for Research on Cancer
ICPEMC	International Commission for Protection against Environmental Mutagens and Carcinogens
LADD	Lifetime Average Daily Dosage
LDT	Lowest Dose Tested
LDH	Lactate Dehydrogenase
LOEL	Lowest Effect Level
MCL	Maximum Contaminant Level
MDL	Minimal Detection Limit
M/L/A	Mixer/Loader/Applicator
MOE	Margin of Exposure
MTD	Maximum Tolerated Dose
NACA	National Agricultural Chemicals Association
NOEL	No Effect Level
PCO	Pest Control Operator
PEL	Permissible Exposure Level
PMZ	Pesticide Management Zone
PPE	Personal Protective Equipment
ppm	part per million
ppb	part per billion
Q_1	Cancer Potency Factor, Maximum Likelihood Estimate (MLE)
Q_1*	Cancer Potency Factor, 95% Upper Confidence Limit of the Maximum Likelihood Estimate (MLE)
RAC	Raw Agricultural Commodity
RfD	Reference Dose
RIA	Radioimmunoassay
RT-PCR	Reverse transcriptase polymerase chain reaction
TAS	Technical Assessment Systems, Inc.
TVC	Total Vegetation Control
USDA	United States Department of Agriculture
USEPA	U.S. Environmental Protection Agency
WHO	World Health Organization

I. SUMMARY

Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is a pre or post-emergence herbicide which has been registered in the U.S.A. since 1959. Novartis has 3 registered products in California [Aatrex 4L (100-497-AA, Aatrex Nine-O (100-585-AA) and BICEP II Magnum, (100-817-AA) and Drexel has one [Drexel Atrazine 4L (19713-AA)]. Non-crop uses were canceled voluntarily in 1992.

The environmental fate of atrazine and degradation products has been extensively studied. The degradates are of interest as their persistence and toxicity are similar to that of the parent compound. Three major metabolites of atrazine are also major metabolites of other widely used triazine herbicides. Because the degradation products are more polar than parent atrazine, they have reduced soil binding characteristics and concomitantly, a greater tendency to leach into groundwater. Along with atrazine itself, they are commonly found in California groundwater samples. In addition, at low pH, triazines mixed with nitrogen fertilizers may also lead to the formation of nitrosotriazine(s).

The current Risk Characterization Document addresses potential human exposures from the California use of atrazine as an active ingredient in herbicide formulations for weed control in corn and sorghum. The potential dietary risk from the theoretical consumption of foods containing the highest legal residues of atrazine is also assessed.

The Risk Assessment Process

The risk assessment process consists of four aspects: hazard identification, dose response assessment, exposure evaluation, and risk characterization.

Hazard identification entails an evaluation of the toxicological properties of each pesticide. The dose-response assessment then considers the chemicals toxicological properties and estimates the amount which could potentially cause an adverse effect. The amount which will not result in an observable or measured effect is called the No-Observed-Effect-Level, NOEL. A basic principle of toxicology is that at a high enough dose, virtually all substances will cause some type of toxic manifestation. Although chemicals are often referred to as "dangerous" or "safe", as though these concepts were absolutes, in reality, these terms describe chemicals that require low or high doses, respectively, to cause toxic effects. Toxicological activity is determined in a battery of experimental studies which define the kinds of toxic effects that can be caused, and the exposure levels (doses) at which the toxic effect is first seen. State and federal testing requirements mandate that chemicals be tested at doses high enough to produce toxic effects, even if that testing requires dose levels many times higher than those to which people might be exposed.

In addition to the intrinsic toxicological activity of the pesticide, the other parameters critical to determining risk are the level, frequency and duration of exposure. The exposure assessment includes an estimation of the potential exposure through occupational and dietary routes on an

acute (one-time) and chronic (long-term) basis. For potential occupational exposure, the levels of exposure are determined by measurement of atrazine in air and skin absorption by people working with the pesticide. For potential dietary exposure, the levels of exposure are determined by the amount of pesticide residue on specific commodities and processed food and the consumption rate.

The risk characterization then relates the toxic effects observed in the laboratory studies, which are generally conducted with relatively high dosages of pesticides, to potential human exposures to low dosages of pesticides in the diet or work place. The potential for possible non-tumor adverse health effects in human populations is expressed as the margin of safety or exposure (MOE), which is the ratio of the dosage which produces no effect in laboratory studies to the estimated human dosage. For tumor effects, the probability of risk is calculated as the product of the cancer potency of the pesticide and the estimated human dosage.

In 1990, California listed atrazine on the groundwater protection list as a chemical which was found in groundwater [California Code of Regulations, Title 3, Division 6, Chapter 4, Subchapter 1, Article 1, Section 6800(a)]. In 1994, a Special Review was initiated by U.S. EPA. In this case, concerns were expressed about possible cancer risks, resulting not just from atrazine, but also from two other related triazine herbicides, cyanazine and simazine, either alone or in combination. The main atrazine registrants, Novartis, have completed many toxicology and environmental fate studies in order to maintain the registrations.

Toxic Effects in Animal Studies

In chronic toxicity studies conducted according to FIFRA guidelines, oncogenic effects were observed in the form of mammary gland tumors in female SD rats and interstitial cell tumors in male rats. The latter effect was observed only at the HDT and was probably secondary to increased lifespan and therefore mammary gland tumors were used for risk assessment. Tumors of the mammary gland in male rats, lymphoid tumors in rats and mice, and uterine tumors in female rats were present in non-FIFRA-guideline studies, but could not be used for regulatory purposes due to deficiencies in study design or conduct. There was no oncogenicity in either sex of the mouse or F344 rat in acceptable studies.

Based on the currently available data, DPR has concluded that the principal toxicological effects of atrazine consist of an elevated incidence of fetal skeletal variations, post-implantation loss, resorptions and decreased fetal body weight following short term exposure in the rabbit (NOEL 5 mg/kg/day); cardiotoxicity following longer term exposure, in the dog (NOEL 0.5 mg/kg/day); cancer following lifetime exposure [human Q_1= 0.064 $(mg/kg/day)^{-1}$; Q_1* = 0.12 $(mg/kg/day)^{-1}$]. The mammary tumors which occurred in female SD rats were malignant, increased in a dose-dependent manner, with high potency and have also been observed with other, related triazine pesticides. In some studies these tumors also had an earlier onset. Genotoxicity tests conducted with atrazine and metabolites indicate that, although atrazine itself is probably not genotoxic, one or more metabolites could be active. There is evidence that the rat mammary tumors arose through an endocrine mechanism, which would be expected to have a (practical) threshold, suggesting that a NOEL/MOE may be the most appropriate method for estimating risk associated with atrazine exposure. Indeed, using a weight-of-the-evidence approach, the

massive amount of data generated over the past few years make an endocrine mechanism almost inescapable, although the precise mechanism(s) remains to be determined.

Occupational Exposure

The potential occupational exposure associated with the use of technical atrazine in formulations used as herbicides was assessed for workers during the mixing, loading, and application activities. The exposure data (Sanborn and Mehler, 1999) were derived from a recent study in corn, adjusted for typical atrazine use rates in California, and using a recently conducted human dermal absorption study. ADD values were 1.8 to 6.1 μg/kg/day (95%UB values were 8.6 to >91 μg/kg/day using passive dosimmetry and >13 to 28 μg/kg/day, urinary monitoring) ; AADD were 0.07 to 0.3 (commercial applicator) or 0.02 to 0.05 μg/kg/day (farmer); LADD were 0.04 to 0.1 (commercial applicator) or 0.008 to 0.03 μg/kg/day (farmer), respectively. The margin of exposure or MOE values (calculated as Animal NOEL/ Estimated Human Exposure) for short-term (acute) exposure were 820 to 2,800; based on 95%UB exposure, MOE values were 55 to 580, for both farmers and commercial applicators. A margin of safety of $\geq$100 is generally considered to be protective of human health when the NOEL is based on toxicology data from animal studies. The MOE of 55 for short-term exposure, at 95%UB, is below 100. The potential occupational exposure of workers from long-term use was also assessed. The MOE was 1,700 (commercial applicators) or 10,000 (farmer), based on mean AADD estimates, which are above the value generally considered to be protective of human health. The excess oncogenic risk associated with lifetime exposure was 2.6 to 12 in a million (commercial applicators) and 0.5 to 3.6 in a million for farmers, based on mean LADD values. These values are greater than the generally accepted negligible risk of one in a million.

Dietary Exposure

The registrants' crop residue database suggests that residues will not be present at harvest. Calculations conducted by DPR of dietary acute and chronic (annual) exposure generally used default residue levels or tolerances. The exposure has been calculated for all crops, combined, for which there are U.S. EPA tolerances *i.e.* cane sugar, corn, guava, grain sorghum and wheat (germ oil) along with secondary residues in beef, chicken, eggs, milk and water, for which there is a MCL. Registrant data (crop residues) and DPR data (water) are the only consistent sources of residues of parent and the (3) main chlorotriazine metabolites, DIPA, DEA and DACT. Thus, in the event of a non-detection, the MDL for each (usually 0.005 ppm) has been added to give a combined atrazine plus degradates residue (usually 0.02 ppm). The dietary exposure for various population subgroups has been calculated for all commodities combined, including drinking water. Short term exposure ranged from 0.234 to 0.857 μg/kg/day, for seniors and infants (non-nursing), respectively, determined at the 95$^{th.}$ percentile of exposure. Long-term or chronic exposure ranged from 0.046 to 0.286 μg/kg/day, for nursing infants and children (1 - 6 yrs), respectively, calculated as the mean annual dietary exposure and assuming that 100% of each crop was treated with atrazine. When adjusted for percentage of each crop treated, the equivalent range of long-term exposure was 0.038 to 0.203 μg/kg/day, for nursing infants and non-nursing infants, respectively. The margins of exposure ranged from 5,830 (non-nursing infants) to 21,300 (seniors) for acute and 1,750 (children, 1-6 yrs.) to 10,900 (nursing infants) for chronic exposure. Taking the percentage of each crop treated into account, the

equivalent MOEs for chronic exposure were 2,460 and 13,100, for non-nursing infants and nursing infants, respectively. The excess oncogenic risk for lifetime dietary exposure was 4.5 to 8.4 in a million (adjusted for percent crop-treated). These values are greater than one in a million, which is generally accepted to be negligible risk. However, for the reasons given above, these are likely to be overestimates of dietary exposure.

Combined Exposure

Acute, combined exposure (occupational, ADD + dietary) was calculated as 6.4 μg/kg/day (6.1+0.251), of which occupational comprises about 95%. At 95%UB acute, occupational exposure, combined exposure would increase by only about 0.2%, remaining at 91 μg/kg/day. Chronic (annual) combined exposure (AADD + mean chronic, dietary adjusted) was calculated as 0.09 (farmer) to 0.3 μg/kg/day (commercial applicator). Lifetime combined exposure (LADD + mean chronic, dietary adjusted) 0.07 (farmer) to 0.14 μg/kg/day (commercial applicator). The combined MOE values were: for acute exposure, 675; for chronic exposure 5,000 (farmer) and 1,500 (commercial applicator). The excess cancer risk after combined exposure was calculated to be: for the farmer, 4.8 (MLE) to 17 (UB) in a million and for the commercial applicator, 6.8 (MLE) to 24 (UB) in a million. Because atrazine registrations for Home & Garden and TVC have been canceled, there are no other likely sources of exposure in California.

Tolerance Assessment

Based on the 95th percentile of the theoretical consumption of water and foods with established legal residues for atrazine, the acute margins of exposure at the highest legal residue levels (tolerances) were all greater than 100. As such, existing tolerances were considered adequate for the protection of human health. Based upon pesticide residue monitoring programs, the long-term consumption of foods containing residues at tolerance levels was considered improbable, if not impossible. Therefore, an assessment of the margins of exposure from theoretical chronic exposure to foods with tolerance levels of atrazine was not undertaken.

II. INTRODUCTION

A. CHEMICAL IDENTIFICATION

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) was first reported as a pre- and postemergence herbicide in 1957 by J.R. Geigy S.A. (subsequently known as Ciba-Geigy and now as Novartis and due to be renamed Syngenta in late 2000). It affects a variety of biochemical processes in plants including photosynthesis, plant growth regulation, nitrogen metabolism and nucleic acid metabolism (USDA, 1984). As with other triazine herbicides, atrazine interference with photosynthesis involves inhibition of the Hill reaction, in which oxygen is evolved from water in chloroplasts. This inhibition of photosynthesis is probably largely responsible for the phytotoxicity of atrazine (Corbett *et al.*, 1984). The biochemical events behind growth regulation are not completely known, but small amounts of atrazine appear to stimulate plant growth, while larger amounts inhibit it. Atrazine was reported to cause an increased uptake of mineral nutrients by plants.

B. REGULATORY HISTORY

After a 1986 study demonstrating an increased incidence of mammary tumors in the female SD rat, USEPA recommended that the registrants conduct studies to establish the mechanism of tumor formation by triazines. Atrazine label-approved uses were amended by the US EPA in July, 1992 as a result of voluntary proposals by the registrants (US EPA, 1992a). The changes only affected future manufacturing or formulation, and did not require relabeling of stocks already in the channels of trade. All noncrop uses were deleted, and the maximum application rate was set at 1.6-2.5 pounds of active ingredient per acre per year. Specific setback application distances were established for the use of atrazine near bodies of water. The U.S. EPA set the Reference Dose (RfD) for atrazine at 0.005 mg/kg/day based on a No-Observed-Effect-Level (NOEL) of 0.5 mg/kg/day for reduced pup weight in a two-generation rat reproduction study (US EPA, 1991).

As part of the pesticide reregistration process, in September 1990, the US EPA required additional data in the following areas: beginning materials and production process, formation of impurities, preliminary analysis, color, physical state, odor, melting point, density, octanol/water partition coefficient, pH, stability, nature of pure active ingredient in plants, nature of pure active ingredient and metabolites containing an intact triazine ring in livestock, residue analytical methods, storage stability, magnitude of residues in plants (macadamia nuts, corn, millet, sorghum, wheat, rye forage, sorghum forage, wheat forage, and sugarcane), magnitude of residues in meat/milk/meat by-products of cattle/sheep/goats/hogs or horses, magnitude of residues in poultry and eggs, magnitude of residues in rotational crops, photodegradation in water and on soil, aerobic soil metabolism, leaching and adsorption/desorption, soil dissipation, forest dissipation, spray drift droplet size spectrum, spray drift field evaluation, applicator dermal and inhalation exposure, rat acute inhalation toxicity, 21-day dermal toxicity, chromosomal aberration and other mechanisms of mutagenicity, general metabolism, avian reproduction, freshwater invertebrate acute toxicity, fish life-cycle, and simulated or actual aquatic field testing (US EPA, 1990b). These reports have now been completed. A 2-year rat oncogenicity study of

the atrazine degradate, hydroxyatrazine, has also been conducted (US EPA, 1991b; Chow & Emeigh Hart, 1995). Summaries of reviews of toxicology reports are to be found in Appendix V.

The current US EPA labels for atrazine products have a signal word of "CAUTION". Atrazine was classified by US EPA as a Restricted Use Pesticide in 1990. Users are required to wear long-sleeved shirts and long pants, chemical resistant gloves and waterproof boots. Also, those involved in mixing/loading operations must use chemical resistant rubber or neoprene gloves and a face shield or goggles. Written or oral warnings must be given to workers that atrazine is being used and fields may not be entered without protective clothing until sprays have dried.

The presence of atrazine in well and surface waters used for drinking is also a concern. The US EPA and the State of California have both set a maximum contaminant level (MCL) of 3 ppb for atrazine in drinking water (US EPA, 1991c; and CDHS, 1988). The chlorinated degradates DEA, DIPA and DACT are also subject to detection, in water and crops; tolerances for range and orchard grass, plus hay from the latter, include residues of these degradates (CFR40, 1999). The primary registrant, Novartis, petitioned the US EPA to set MCLs for DEA and DIPA because they are considered toxicologically similar to atrazine (US EPA, 1994). Novartis also petitioned to establish an MCL for the hydroxy metabolite of atrazine, which appears to affect a different target organ and thus may be toxicologically dissimilar. The US EPA issued a health advisory concerning atrazine in drinking water (US EPA, 1989a), indicating that atrazine may be removed from water by techniques such as filtration through activated carbon.

In California, wells which contain atrazine or other pesticides become the center of a pesticide management zone (PMZ). The PMZs are one-square-mile sections of land where a pesticide is in ground water as a result of normal agricultural practices, and which, therefore, is determined to be sensitive to pesticide contamination. Use of the detected pesticide may be prohibited or restricted in the PMZs, depending on the extent of contamination. On July 31, 1992 there were 78 PMZs for atrazine, where atrazine use is prohibited, and 6 more proposed (DPR, 1991b).

In Europe, the environmental hazard due to atrazine pollution of drinking water has led to a complete ban of the chemical in Germany and Sweden, suspension in Italy, and proposed cancellation in Austria (Ugazio *et al.*, 1991; and Seiler, 1993). In Germany, chemicals used in plant production are automatically banned if ground water levels exceed 0.1 ppb (Plott, 1991).

C. TECHNICAL/PRODUCT FORMULATIONS

Atrazine is available as a technical material (97-98.5%) for formulating end-use products, and under various trade names as wettable powders, water dispersible granules, or liquid suspensions ranging in concentrations from 8 to 90%. Currently there are 3 California registrants, for 10 different formulations, with atrazine as an active ingredient. Atrazine may be formulated with numerous other chemicals, including alachlor, propachlor, metolachlor, sodium chlorate and sodium metaborate or prometon for a variety of uses (USDA, 1984; WSSA, 1989).

D. USAGE

Atrazine is the most widely used conventional pesticide in the United States and in the world. In 1990, annual usage was over 80 million pounds active ingredient (a.i.) of which approximately

11 million pounds were for non-crop uses (US EPA, 1990). In California, the cropping profile results in limited applications of atrazine (Table 1). It has been used in the USA for weed control in corn, sorghum, milo, wheat, sudan grass, macadamia orchards, forest trees, pineapples, sugar cane and certain other crops. Outside of the United States, atrazine is registered on coffee, bananas, citrus and African oil palms (FCH, 1991; ACH, 1987). Depending on the crop or intended use, atrazine sprays may be applied preplant, preemergence or postemergence.

In California, the Pesticide Use Reports indicate usage of 38,000 to 60,000 lbs of atrazine a.i. *per annum*, during 1993-1997. The pattern of use of atrazine in California has changed, in part due to restrictions (Table 1). For example, current labels for Novartis, the principal registrant and manufacturer, prohibit non-crop uses in California (Table 1).

Table 1 Major Usage of Atrazine (a.i.) in California, 1993-1995.[1/]

CROP	1993		1994		1995	
	LBS.	ACRES	LBS.	ACRES	LBS.	ACRES
Sudangrass [2/]	3,800	2,900	12,800	8,800	12,300	7,700
Corn [2/]	24,700	15,000	27,600	18,700	7,000	5,200
Bermudagrass [2/]	211	184	180	125	6,300	3,900
Forest Trees [3/]	1,100	1,900	1,800	1,800	4,000	1,900
Rights-of-Way [3/]	8,400	8,000	1,900	N/A	2,700	N/A
Corn (human)	4,200	3,000	1,900	2,000	2,500	2,400
TOTAL LBS.	47,000		49,000		38,000	

Table 1 cont. Major Usage of Atrazine (a.i.) in California, 1996-1997.[1/]

CROP	1996		1997		
	LBS.	ACRES	LBS.	ACRES	
Sudangrass [2/]	14,400	7,400	10,500	5,900	
Corn [2/]	11,600	7,800	11,900	7,600	
Bermudagrass [2/]	10,400	7,400	4,100	2,900	
Forest Trees [3/]	11,100	3,200	15,900	5,200	
Rights-of-Way [3/]	5,400	N/A	280	N/A	
Corn (human)	4,300	4,100	4,700	4,700	
TOTAL LBS.	60,000		49,000		

1/ DPR, 1994; DPR, 1995; DPR, 1996; DPR, 1999
2/ forage/fodder 3/ These uses are no longer on the approved labels for atrazine

E. ILLNESS REPORTS

Between the years 1982-1987 there were four illnesses reported to be related to atrazine exposure in California. Two of them were effects on the eyes, and the other two were systemic. All of these incidents involved some aspect of either mixing/loading or application of atrazine (Sanborn and Mehler, 1999). Illnesses outside California include severe dermatitis of the arms and hands in a farm worker several hours after he sprayed atrazine and cleaned clogged spray nozzles, without protective gloves (Schlicher *et al.*, 1972). During the same day, he had sprayed cyanazine. The response was severe enough to be incapacitating, and recovery was not complete 28 days later. A patch test several months later, with a commercial atrazine formulation, was strongly positive. The worker had twice previously experienced dermatitis following use of propachlor (2-chloro-*N*-isopropyl acetanilide), suggesting an unusual sensitivity (Hayes, 1982). Another farm worker developed a milder rash of the hands and face a few hours after using an atrazine formulation (Stevens *et al.*, 1991). Rights-of-way applications caused dermatitis and increased numbers of eosinophils in some workers' blood (Ikonen *et al.*, 1988).

In an epidemiological study of pesticide users in a rural area (Long *et al.*, 1969), farm workers were evaluated for any relationship between the types and degree of pesticide use and various clinical chemistry parameters. Significant correlations ($p<0.01$) were obtained between the amount of atrazine applied and bilirubin 1-minute and 30-minute values. However, the number of subjects involved was only 7 and 8 respectively and the amount of bilirubin change may not have been biologically significant. Moreover, because the workers had some exposure to other pesticides, it is not possible to state that atrazine was definitely the cause of the signs. The authors stated that continued investigation would be necessary to substantiate the findings.

A number of case-controlled human studies were considered, in order to obtain some information on the carcinogenicity of triazine pesticides in general, and atrazine in particular (Vainio, *et al.*, 1991). An area of northern Italy showed slightly elevated risks for ovarian tumors among women using triazine herbicides (Donna *et al.*, 1989). In association with the use of unspecified triazines or specifically atrazine, several epidemiology studies in the mid-western U.S. showed small excess risks for cancers at a number of sites (Hoar *et al.*, 1985, 1986; Zahm *et al.*, 1988, 1992; Cantor *et al.*, 1985). However, the increases were not always statistically significant. Complex mixtures or insufficient reporting of actual exposures made it difficult to evaluate the potential human carcinogenicity of triazine herbicides (Vainio, *et al.*, 1991).

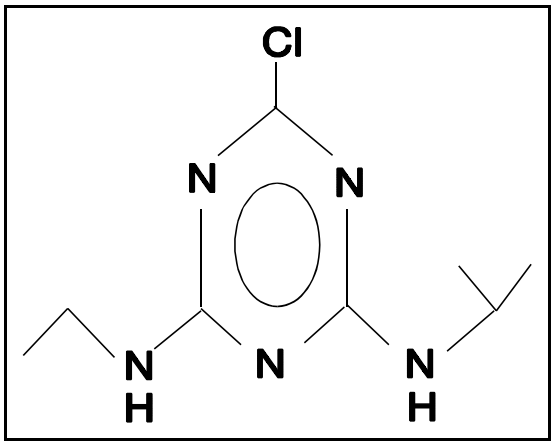
An analysis of mortality among 4,917 potentially triazine-exposed men employed at Ciba-Geigy Corporation's McIntosh and St. Gabriel chemical plants was submitted to DPR (Delzel, 1992). The observed numbers of deaths among study subjects during the period 1960 through 1986 was compared with the number expected on the basis of United States and regional (Alabama and Louisiana) mortality rates. The average follow-up period was 14 years. The study group did not show an increased incidence of death from all causes, or from cancer, as compared to national or regional expected death rates. The group did have 3 observed deaths from non-Hodgkin's lymphoma as compared to 0.78 expected, however, two of the men had spent less than one year in definite triazine-related work. The investigator concluded that this observation argued against a causal link of this disease with occupational exposure. In addition, increased chromosomal aberrations were reported in lymphocyte cultures from agricultural workers with extensive occupational exposure to various pesticides, including atrazine (Yoder *et al.*, 1973).

Similar conclusions were reached in a follow-up study of workers at the same plants, 1975-1993 (Ciba-Geigy, 1997a,b). Again, a slight increase in deaths from NHL was observed which was considered by the report's authors to be a chance event since NHL deaths did not correlate with degree or length of exposure. However, it should be noted that in the Hoar *et al.*, 1986 study (above), a significant association was noted between triazine exposure and NHL but was discounted because the *a priori* hypothesis focussed on 2,4-D as the causative agent.

In a review of 11 epidemiological studies of triazine herbicides and cancer (Sathiakumar & Delzell, 1997), ten case-control and one follow-up study were analyzed, including four of the above studies. The authors noted that the case-control studies were subject to bias (inaccurate recall) and that all studies were subject to confounding factors such as: exposure to other agricultural chemicals, poor dose-response patterns and induction time inadequacies. Limited numbers of cases added further to the difficulties of drawing conclusions from the studies, with the possible exception of non-Hodgkins lymphoma. However, it was concluded that none of the studies, in themselves, was persuasive of the presence or absence of a causal association between triazine herbicides and cancer.

F. PHYSICAL/CHEMICAL PROPERTIES (WSSA, 1989)

Chemical Name: 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine
Common Name: atrazine
Trade Names: Aatrex, Aatram, Atrasol, Crisazana, Gesaprim, Primatol, Zeazin.
CAS Registry Number: 1912-24-9
Structural Formula:



Empirical Formula: $C_8H_{14}N_5Cl$
Molecular Weight: 215.7
Physical State: white crystalline
Melting Point: 173-177°C
Ionization constant: (pKa)1.68
Density: 1.187 g/cm^3
Stability: Stable in neutral, slightly acidic/basic solutions; hydrolyzed by alkali/ mineral acids at higher temps.
Vapor Pressure: 40 μPa (3 x 10^{-7} mm Hg) at 20° C
Solubility: water ~33 ppm, methanol 18,000 ppm, chloroform 52,000 ppm, dimethyl sulfoxide 183,000 ppm.
Henry's Law Constant:: 2.6 x 10^{-9} atm m^3 mol^{-1}
Octanol/Water Coeff: 418 ± 14 at 20°C; logP = 2.6

G. ENVIRONMENTAL FATE

Summary: The environmental fate of atrazine and degradation products has been extensively studied. The degradation pathways include *N*-dealkylation and hydroxylation and/or glutathione conjugation of the triazine ring. The degradation products are of interest as their persistence and toxicity are similar to that of the parent compound. Three major metabolites of atrazine, desethylatrazine (DEA), desisopropylatrazine (DIPA), and diaminochlorotriazine (DACT), are also major metabolites of other widely used triazine herbicides: DEA is a degradate of propazine, DIPA of simazine and cyanazine and DACT, of all three. Hydroxy analogs of these triazines have also been detected. Because all of these degradation products are more polar than parent atrazine, they have reduced soil binding characteristics along with a greater tendency to leach into groundwater. At times, levels of these breakdown products in environmental samples may equal or exceed levels of atrazine itself. DEA and/or DIPA have been detected in ground water at levels of 4 times those of atrazine. Contamination of groundwater with atrazine and nitrogen fertilizers may also lead to the formation of nitrosoatrazine at low pH.

Hydrolysis

No significant hydrolysis of atrazine was observed after 30 days at pH 5 to 9 at 25ºC. Ciba-Geigy stated that hydrolysis, at environmentally relevant pH and temperature, did not appear to be an important degradation mechanism for atrazine (Spare, 1986). However, Bray *et al.*, 1997, have demonstrated the cleavage of the triazine ring of the sulfonylurea herbicide prosulfuron, incubated in sterile aqueous solution. This hydrolytic opening occurred at pH 5, but not at pH 7 or 9. The triazine-cleaved product was unequivocally identified by comparison with the chemically-synthesized authentic reference standard. Previously, an open-chain triazine had been postulated as a hydrolysis product of propazine, a close analog of atrazine, but without structural confirmation (Montgomery & Freed, 1964). It is therefore possible that triazines such as atrazine may undergo some ring cleavage, especially under acidic conditions.

Photolysis

In a soil photolysis study using California loam soil (Das, 1989), atrazine was applied to 1 mm thick soil layers on glass plates at a rate of 9.7 to 10.6 ppm. The soil plates were exposed to artificial sunlight for 12 hour/day for 30 days. Control samples were incubated in the dark. The soil plates were sampled after 0, 1, 3, 7, 14, 21, and 30 days of exposure. The estimated $t_{½}$ for atrazine and two degradates (desethylatrazine and desisopropylatrazine) under irradiated and non-irradiated conditions were 38 and 267 days, respectively. The net $t_{½}$ attributable to photodegradation was 45 days. At 30 days, desethylatrazine constituted 13.3% and desisopropylatrazine was 11.9%. The contribution of hydroxyatrazine was not determined.

A published account of aqueous photolysis (Burkhard *et al.*, 1976) has been submitted to DPR. The study was conducted using a 10 ppm aqueous atrazine solution, with or without 1% acetone, at pH 6.8. The radiation energy of artificial light was 1700-2300 $J.m.^{-2}s.^{-1}$ The results suggest that the reaction followed first order kinetics and the $t_{½}$ of atrazine was calculated to be approximately 5 hours. The $t_{½}$ of atrazine in the absence of acetone, but under the same conditions was 25 hours. This indicated that acetone had a strong photosensitizing effect. The

products of photolysis were desethylatrazine, desisopropylatrazine, diaminochlorotriazine, and the corresponding hydroxy compounds.

In a study of aqueous photolysis of atrazine in natural sunlight (Spare, 1988), no photolysis was observed after exposure to sunlight for up to 30 days at neutral pH. The calculated $t_{½}$ was approximately 335 days with a rate constant of 1.72×10^{-4} $h.^{-1}$ No single photolysis product was observed at greater than 10% of dose at any point during the study. The investigator stated that solution photolysis was not an environmentally relevant transformation process for atrazine.

Soil Metabolism: Aerobic and Anaerobic

There is a large body of literature indicating that soil microorganisms are capable of metabolizing triazines in general, and atrazine in particular (USDA, 1984). At least 4 species of bacteria and 21 fungi have been shown to be effective in metabolizing atrazine. The three major routes for microbial degradation of atrazine are dealkylation (removal of side chains and evolution of carbon dioxide), hydrolysis at the chlorine to form the metabolite hydroxyatrazine, and cleavage of the triazine ring. The highest rates of atrazine inactivation are generally found under those conditions that are favorable for microbial growth, such as warm temperatures, high moisture, and high soil organic matter content. In a laboratory study with atrazine incubated with soils taken from different depths, it was demonstrated that atrazine degraded 2 to 3 times more rapidly in topsoils than in subsoils, that each 10°C temperature increment (from 15°C-35°C) resulted in a 2- to 3-fold increase in degradation rate, and increased soil moisture content (from 0.4 to 0.8 field capacity) resulted in $C0_2$ evolution from atrazine-treated soil to increase from 0 to 6 times (USDA, 1984).

In another soil metabolism study (Rustum, 1988), after 300 days of incubation in aerobic soil, atrazine was degraded to about 27.1% of the amount applied. The major products were soil bound radioactivity (62.8%) and organosoluble metabolites, identified as desethylatrazine (4.6%), desisopropylatrazine (1.2%), hydroxyatrazine (0.2%), diamino-chlorotriazine (0.1%) and an unidentified metabolite (0.3%). Water-soluble radioactivity represented 6.7%. The calculated half-life of atrazine under aerobic conditions was approximately 146 days. After 94 days of incubation in sterile aerobic soils, atrazine represented 77.4% of the amount applied. The major products were soil-bound radioactivity (16.9%) and desethylatrazine and desisopropylatrazine, both below 1%.

After 32 days of aerobic followed by 62 days of anaerobic incubation, atrazine comprised 51.2% of the amount applied. The major residues were soil-bound (40.9%) and included desethylatrazine (1.6%), desisopropylatrazine (0.7%), and hydroxyatrazine (0.4%). The aqueous fraction contained 6.8% of the ^{14}C. The calculated $t_{½}$ of atrazine under anaerobic conditions was 159 days.

Mobility: Field Dissipation

In a field dissipation study (White, 1987a) corn plots were treated with atrazine (AAtrex Nine-O, 91% purity) at 3.96 lbs a.i./acre. The soil was sampled to a depth of 48 inches starting prior to application, and continued at scheduled times until 360 days after the application. Based on

the data submitted, DPR calculated the $t_{½}$ for atrazine from the total atrazine residue in the 0-6" soil layer plus the 6-12" layer using first-order kinetics and linear regression analysis. The $t_{½}$ was found to be 70.6 days with a rate constant of 9.82×10^{-3}/day. However, the registrant indicated problems had been encountered in the analysis for hydroxyatrazine due to an apparent binding to soil, which led to poor extractability. The results of improved analytical methods were subsequently submitted (Decker, 1988a), indicating that the $t_{½}$ for 0-6" was 141 days when hydroxyatrazine residues were added to the total. On Day 358, hydroxyatrazine residues of 0.0157 ppm were present at 24-36", the lowest depth measured.

In another field dissipation study (White, 1987b) bareground plots were treated with 18 pounds atrazine a.i./acre (AAtrex Nine-O, 91% purity). The soil was sampled to a depth of 48 inches starting prior to application, and continued at scheduled times until 360 days after the application. The $t_{½}$ for the degradation products of atrazine was calculated using first order kinetics. The degradation $t_{½}$ for atrazine in the 0-6" soil depth was estimated to be 119 days with a coefficient of 0.453 (r^2). Residues were not detected below 12". However, the registrant again indicated problems had been encountered in the analysis for hydroxyatrazine due to an apparent binding to soil, which led to poor extractability. The results of improved analytical methods were subsequently submitted (Decker, 1988b), indicating that when hydroxyatrazine residues were added to the total, no dissipation was apparent. On Day 358, hydroxyatrazine residues of 0.21 ppm were present at 24-36", the lowest depth measured.

In a published summary of atrazine persistence in subsurface soil (PTCN, 1991), it was reported that researchers found little or no adsorption or degradation of atrazine at a depth of 33 feet. The study also found that atrazine concentrations decreased less rapidly in sterile soils, biodegradation was affected by sediment type (perhaps due to lower organic matter content), and atrazine did not mineralize.

Mobility: Atmospheric Dissipation

Despite the low vapor pressure of atrazine (3×10^{-7} mm Hg at 20° C), atrazine has been detected in the atmosphere. In a report of analysis of mid-winter fog in the Central Valley of California for the presence of pesticides, atrazine was detected at levels of 0.27 to 0.70 μg/l in the aqueous component (Glotfelty *et al.*, 1987). In a summary of atmospheric deposition of herbicides in the Midwest, it was reported that atrazine was observed in almost every rainfall during the growing season, with a peak rain level of 10 ppb. Atrazine was also detected in snow and snowmelt at a level of 0.1 to 0.2 ppb, which was thought to be indicative of either long-range transport or a long residence time (Capel, 1990; PTCN, 1991). A previous study, conducted in 1964, had also detected atrazine in rain at levels of about 0.1 ppb (Cohen *et al.*, 1966).

In another study of atmospheric transport of atrazine, (Elling *et al.*, 1987), thin-layer chromatography (TLC) and enzyme immunoassays detected atrazine during the planting season. Levels as high as 5 μg/TLC plate were recorded. The authors indicated that atrazine was useful as a model for air transported pollutants, and that, despite a low vapor pressure, transport of atrazine is possible through the evaporation of small droplets.

In an assessment of atrazine levels in indoor air (Wallace, 1991), US EPA calculated exposure as 0.05 ng/m^3, based on the arithmetic mean of population-weighted and seasonally weighted

average personal exposures measured for 285 people during the years 1986-1988. House dust was mentioned as a possible source of the contamination.

In published studies of the volatility of atrazine, it was reported that atrazine may be lost from soils and plants. Up to 35% of atrazine applied to soil was lost in 48 hours at 35°C (Kearney *et al.*, 1966). Volatility is influenced by soil type, moisture, temperature, and adjuvants (Nalewaja, *et al.*, 1976). Atrazine volatility may be greater from plant surfaces. Sixty three percent of atrazine applied to Canada thistle was lost as vapor in 48 hours at 30°C. Significant volatility loss of atrazine from soil may, therefore, be less probable than from plant material (Burt, 1974).

Mobility: Soil Adsorption/Desorption

A batch equilibrium study was conducted to determine the adsorption and desorption coefficients (K_d values) of atrazine and four degradation products (Yu, 1986a-e). Four soil types - sand, California sandy loam, silty loam, and clay loam were used in the experiment. The adsorption coefficients for atrazine were found to be between 0.427 and 2.03. The K_{oc} (K_d value divided by soil organic carbon content) for maximum and minimum K_d were calculated as 135 and 90.9. The K_d values for N-dealkylated atrazines were found to be slightly lower (0.116 - 1.44) than for atrazine. Hydroxyatrazine showed a little higher adsorption to soil (K_d = 1.643 - 8.165) than atrazine and N-dealkylated products. The K_d value for diaminochlorotriazine was determined to be 0.108 - 0.800. The results indicate that atrazine and N-dealkylated metabolites were not strongly adsorbed onto soil, and they were easily desorbed. Hydroxyatrazine was, however, well adsorbed onto soil.

In another study of the adsorption/desorption of atrazine in California loam soil (Spare, 1989), the adsorption K_d, K_{oc}, and n were 0.73, 155, and 1.11 respectively. The corresponding desorption values were 4.76, 1012, and 1.05. The adsorption K_d constants for clay, sandy loam and sandy soils were 2.45, 0.79 and 0.20 respectively, with equivalent desorption K_d values of 8.48, 7.28 and 1.48.

In conclusion, the soil mobility of atrazine and its degradates can be summarized as follows: atrazine – medium to very high; chloro- metabolites – high to very high; hydroxy-atrazine – immobile to medium.

Mobility: Ground Water Contamination

Extensive ground water contamination with atrazine and metabolites has been reported across the United States and Europe. Surveys included samples from urban and rural wells, and from wells serving Grade A dairy farms (Belleck *et al.*, 1991; US EPA, 1990a,1991b; and Ciba-Geigy, 1992a-c). In instances where analysis for metabolites has not been done, it has been estimated that the metabolites may be present in concentrations up to 4 times that of the parent compound (US EPA, 1991a; Belleck *et al.*, 1991; Ciba-Geigy, 1992c).

In California, atrazine has been detected in ground water at levels up to 8.5 ppb (DPR, 1991b), and in wells supplying large public water systems at up to 2.4 ppb (CDHS, 1986). Levels of metabolites in California well water were also present at up to 3-4 times that of atrazine itself. Desethylatrazine was detected at up to 0.52 ppb, and desisopropyl-atrazine levels reached 1.8 ppb (DPR, 1993). Granular activated carbon has been identified as the best available technology to remove atrazine residues from water that is to be used for drinking (US EPA, 1991c).

Studies indicate that atrazine is stable in ground water (US EPA, 1990-91). When bottled ground water containing 600 to 1000 ppm atrazine was stored at 6 to 22°C for as long as 6 months, no decrease in atrazine concentration was observed (USDA, 1984). In a study of the use of ozone as a drinking water disinfectant, it was reported that ozonation did not remove atrazine from the water. Instead, atrazine was chemically modified to typical breakdown products, DEA and DIPA primarily (Adams *et al.*, 1992). Chlorination has been shown to have little effect on atrazine levels in water (Miltner *et al.*, 1989).

Mobility: Surface Water Contamination

In a survey of pesticides in the Sacramento River and agricultural drains (CRWQCB, 1987), atrazine was not detected, over a range of Minimum Detection Limits (MDLs) from 0.04 to 89 ppb. In a pesticide stormwater monitoring study of agricultural drains in the Sacramento Valley (CRWQCB, 1992), atrazine was not detected using a MDL of 0.1 ppb

In a review of pesticides detected in California surface waters (CRWQCB, 1989), atrazine was detected in surface waters of the Sacramento and San Joaquin River Valleys and the Delta region at levels of 0.1 to 50 ppb in various studies conducted between 1979 and 1989. In the review, some of the data was reported from the US EPA Storet database. Although the Storet database has a history of containing inaccuracies, a review of the atrazine listings did not uncover errors.

Surface water contamination with atrazine has also been reported across the United States. Generally, atrazine is detected in surface water in any areas where atrazine is applied. Surveys of water in the Mississippi river basin by the United States Geological Survey (USGS, 1991; Pereira *et al.*, 1990), have indicated that atrazine was detected in every springtime sample tested, and levels exceeding the MCL of 3 ppb were present 27% of the time. It was estimated that the annual transport of atrazine and two chlorometabolites into the Gulf of Mexico was about 500 metric tons, or almost 2% of the amount of atrazine applied each year in the Midwest.

Bioaccumulation: Aquatic

Triazine herbicides have been detected in fish in the Sacramento-San Joaquin Delta of California at levels of up to 52 ng/g of liver. Fish that were moribund had higher levels than healthy fish (Cashman *et al.*, 1992). It has been reported that atrazine does not extensively bioaccumulate in fish, but reflects an equilibrium with the concentration in the surrounding water, at a bioconcentration factor of about 2-3 (USDA, 1984,1988).

Mobility: Soil Leaching

An irrigation study was conducted by the Environmental Hazards Assessment Program of DPR in 1987 and 1988 to compare the effect of three amounts of deep percolating water (denoted by low, medium, and high) applied by four methods (drip, sprinkler, floor, and furrow) of irrigation on the leaching of atrazine (Troiano *et al.*, 1990). The amount of water added was based on a water budgeting method that used measures of evapotranspiration (ETo), which is an estimate of the amount of water required to replenish that lost from soil evaporation and plant transpiration. ETo values were obtained from the California Irrigation Management Information System (CIMIS) weather station at Fresno, California. Focusing on the sprinkler, flood, and furrow irrigation results, increases in the amount of water applied caused an increase in the downward movement of both water and atrazine. Using the location of the center of atrazine mass recovered above 3 meters (9.8 feet) as a measure of downward movement, the center of the mass was about 0.6 meters (2 feet) deeper with every 0.5% increase in the ETo used to determine the amount of water to apply. Although the slope of the relationship was similar for the three irrigation methods, the magnitude of leaching differed with irrigation method. The center of atrazine mass at each percolation treatment was about 0.4 meters (1.3 feet) deeper in basin than in sprinkler irrigation and about 0.6 meters deeper in furrow than in basin irrigation. Because the measurement of soil infiltration rates and soil texture were similar between locations, the differences were probably due to the method of water application.

A physical explanation for differences in soil water movement between sprinkler and basin irrigation was provided through simulations with the LEACHM solute movement model (Wagenet *et al.*, 1989). LEACHM models water flow and solutes in soils with respect to specific site conditions of soil texture and climatic factors. Evaporation of water during the 40 day period was greatest in the sprinkler applications as the low volume and frequent water application kept the surface soil wetter for a greater portion of the time than in basin and furrow treatments. This resulted in less water available for deep percolation. A linear relationship was measured when the depth to the center of the atrazine mass was plotted against percolated water calculated from LEACHM simulations for all sprinkler and basin treatments. The deeper the center of the mass, the greater the downward movement due to treatment. The significance is that differences in centers of mass between methods of application could be ascribed solely to differences in the amount of percolated water produced by each treatment. Depth to center of the mass after 40 days increased by approximately 0.4 m for each 0.1 m increment of percolated water. The amount of percolated water was also highly correlated ($r=0.86$) with the ETo index indicating that the values were good surrogates for one another. The result provided support for the supposition that the management of atrazine leaching could be attained through indexing of irrigation water applications. Atrazine moved the deepest under

furrow irrigation, probably because water was applied to only one-half of the soil surface compared to basin irrigation.

Plant Metabolism

The major pathway for uptake of atrazine by plants is through the roots, with both susceptible and resistant plants readily absorbing the compound. Technical atrazine applied to plant foliage does not readily penetrate, but various adjuvants will increase the rate of foliar penetration. Studies with radiolabeled atrazine have shown that once the herbicide is absorbed into the plant roots, it is rapidly translocated by the xylem into the above-ground portions of the plant (USDA, 1984). It is then distributed throughout the plant with some tendency for accumulation in the leaf margins. The rates of uptake and translocation appear to be increased by factors such as higher temperature and lower relative humidity, which tend to increase the rate of transpiration and fluid movement.

The ability of plant species to resist the herbicidal activity of atrazine is directly related to the ability to degrade atrazine, hence those species or varieties which readily degrade atrazine to nonphytotoxic or less phytotoxic compounds are less susceptible to its herbicidal activity. In plants, at least six types of reactions by which atrazine can be degraded have been identified, including hydrolysis, conjugation of atrazine with other molecules in the plant, removal of side chains from the atrazine molecule with liberation of carbon dioxide, modification of the side chain of the atrazine molecule by processes such as oxidation, replacement of the amino groups by hydroxy substituents, or cleavage of the triazine ring structure. The major pathway for metabolism of atrazine is via hydroxyatrazines, with lesser amounts converted to chlorotriazine metabolites (USDA, 1984; US EPA, 1989b).

Nitrosoatrazine Formation

Several reports have indicated the potential for nitrosoatrazine formation following ingestion of drinking water contaminated with atrazine and nitrites (Meisner *et al.*, 1993, Weisenburger *et al.*, 1987, 1988, 1990). Although nitrites are normally found in certain vegetables and cured meats, the main source of human exposure is from reduction of nitrates. Nitrate contamination of well water in rural areas may be accompanied by the presence of atrazine, leading to simultaneous ingestion with the potential formation of nitrosoatrazine in the stomach. In the laboratory, nitrosoatrazine is readily formed from atrazine and nitrite at acid pH (Weisenburger *et al.*, 1987), and N-nitroso compounds have also been shown to be synthesized *in vitro* by combination of morpholine (a secondary amine) with nitrite and gastric juices, or *in vivo*, when amines are combined with nitrites in the human stomach (Hall *et al.*, 1987). In a recent report, the formation of nitrosoatrazine was shown to occur *in vitro* following the incubation of atrazine with sodium nitrite and human gastric juice (Cova *et al.*, 1996). However, it was reported that the nitroso derivative was relatively unstable, reverting to atrazine after *ca.* 3 h incubation at pH 1.5 to 2.0. The potential formation of nitrosoatrazine is of concern as many nitrosamines are strong animal carcinogens and mutagens (Meisner *et al.*, 1993). Both atrazine and nitrates have been detected in California ground water (DPR, 1993; CDFA, 1989). However, specific investigation of the extent of any potential co-contamination of human drinking water in California has not been reported. Residue methods for nitrosoatrazine in water and crops are not available.

III. TOXICOLOGY PROFILE

A. PHARMACOKINETICS

Summary: Oral absorption of atrazine was considered complete in the rat. In both rats and humans, it was readily dealkylated following oral administration. After conjugation with glutathione, DEA, DIPA and DACT were eliminated primarily in the urine. Dechlorinated metabolites were identified in the rat. An intravenous dosing of the rhesus monkey revealed negligible accumulation of atrazine or its degradates, with total recovery in excreta over seven days. Dermal absorption of atrazine in humans, using the 4L formulation, was 5.6% based on excretion of total ^{14}C.

Oral - Human

Following a single oral atrazine dose of 0.1 mg/kg, the mono-dealklylated metabolites 2-amino-4-chloro-6-isopropylamino-triazine (DEA) and 2-amino-4-chloro-6-ethylamino-triazine (DIPA) were identified in urine of 6 volunteers for up to 48 hours (Davidson, 1988). The di-dealkylated metabolite 2,4 diamino-6-chloro-*s*-triazine (diaminochlorotriazine, DACT) was measurable for up to 96 hours . These three metabolites accounted for 5.4, 1.4, and 7.7% of the atrazine dose respectively, a total of 14.5%. A stepwise oxidative P450 dealkylation of atrazine to DEA and then to DACT was proposed. Atrazine or the dealkylated chloro metabolites may then be conjugated with glutathione leading to cysteine conjugates or mercapturic acids in urine. Blood analysis from one person detected measurable levels of DEA and DACT, but not DIPA, up to 8-24 hours post-dose (Davidson, 1988).

Renal excretion of DACT followed a single-compartment first-order model (Davidson, 1988). The kinetics paralleled its disappearance from blood. The half-life for renal elimination was 10.7 hours compared to the disappearance from blood of 17.8 hours. The metabolites DEA and DIPA were found to generally follow a two-compartment first-order model. Renal excretion half-lives for the former were 2.3±0.5 and 8.4±0.7 hours, while those for the latter were 2.4±1.0 and 36.2±6.2 hours, for the fast and slow compartments in each case, respectively.

Dermal - Human

Both *in vitro* and *in vivo* studies have been conducted. Ademola *et al.*, 1993, found that 4.6 $\mu g/cm^2$ of ^{14}C atrazine, applied to skin of 0.5 mm thickness from 4 individuals in a diffusion cell over a 20 hr. period, was absorbed 16.3% (receptor fluid plus skin residual). In a recent *in vivo* study, ^{14}C-atrazine in a 4L formulation was applied to the ventral forearms of adult males at doses of 6.7 $\mu g/cm^2$ (n=4) and 79 $\mu g/cm^2$ (n=6). The collection of ^{14}C in urine was 5.03% at the low dose and 1.11% at the high dose. Excretion in the feces was much lower. The majority of the ^{14}C remained in the skin, 95.4% and 91.0%, at low and high doses, respectively. Mass balance was 101.2% and 92.3% (Hui *et al.*, 1995). The total excretion in urine and feces at 6.7 $\mu g/cm^2$ was 5.6%. As part of this study, the clearance of ^{14}C-atrazine following IV administration was measured in an experiment with rhesus monkeys. After a single injection of atrazine, 84.8±5.6% of the total ^{14}C was recovered in urine and 11.7±1.9% in feces over the next seven days. The extremely high recovery of ~99% suggests a low tendency for atrazine to accumulate

in tissues. Therefore, the excretion value of 5.6% from the human dermal study is considered appropriate for estimating dermal absorption of atrazine in humans.

Oral - Rat

Following oral gavage, in two separate studies, rats excreted 47% and 76% of the atrazine dose in the urine and, respectively, 49% and 15% in feces, within 72 hours (Miles *et al.*, 1987; Thede *et al.*, 1987). At 72 hours, 4.7 to 7.2% of the dose remained in the tissues with the highest concentration in the erythrocytes. The reason for the wide variation was not apparent, but other studies indicated that urinary excretion is in the 70 to 75% range (Orr *et al.*, 1987; Orr, 1987; Bakke, 1972). Elimination patterns following dermal application are even more variable, with urine:fecal ratios ranging from 3:1 to 97:1 (Murphy *et al.*, 1987; Williams *et al.*, 1983; Shah *et al.*, 1987). Such large variation makes it difficult to draw conclusions about oral absorption in the rat, but it appears to be 90% - 100%, *i.e.* essentially complete (US EPA, 1990).

Sprague-Dawley rats were given an oral dose of ring-labeled atrazine at 1, 3, 7, 10, 50 or 100 mg/kg/day, followed by sacrifice at either 3 h or 72 h after the tenth dose. The mean excretion in the urine was 70%, for the 3 h rats, and 76%, for the 72 h animals. The mean fecal excretion was 13% or 15%, respectively. The proportion of each dose excreted was independent of dose level (Thede & Kahrs, 1987). Hydroxylated, *N*-dealkylated, oxidized, and conjugated urinary metabolites were identified. Subchronic dosing apparently does not alter first-order kinetics patterns for atrazine excretion. Experimental evidence supports a metabolic pathway of oxidative removal of alkyl side chains with 2,4,- diamino-6-chloro-*s*-triazine being the major metabolite. The C-Cl bond is stable to enzymatic hydrolysis, but subject to conjugation via glutathione-S-transferase. Sulfur-containing metabolites are acted upon to give 2-sulfhydryl-*s*-triazines which in turn are subject to methylation followed by oxidation to the corresponding S-oxides. A study in which Fischer rats were sacrificed for analysis of brain, liver, and kidney after receiving 6 or 12 mg/kg/day for a week, indicated similar pathways (Gojmerac *et al.*, 1989).

In female Sprague-Dawley rats which had received 0, 1, 3, 7, 10, 50, or 100 mg/kg/day of triazinyl-14C-atrazine for 10 days, an apparent plateau was reached after 8-9 days, with plasma concentrations proportional to dose (Miles *et al.*, 1987). Following cessation of dosing, concentrations declined exponentially with an estimated $t_{½}$ of 38.6 hours. The estimated apparent volume of distribution (Vd) was 4.15 L/kg body weight, and the mean steady-state concentration for a 10 mg/kg body weight dose was 5.61 mg-equivalent atrazine/ L plasma. Atrazine concentrations in erythrocytes rose with repeated dosing, but failed to achieve a steady-state within the treatment period. Concentrations in RBCs were proportional to dosage. Following cessation of treatment, the estimated $t_{½}$ was 8.14 days. The estimated Vd, $t_{½}$, and predicted mean steady-state concentrations for a 10 mg/kg/day dose, were 0.70 L/kg, 5.1 days, and 104.6 mg-equiv./L cells, respectively.

Interstrain/Interspecies Comparisons

Hepatocyte cultures from Fischer and Sprague-Dawley rats, humans, and Alpine goats were incubated with both radiolabeled (98.7% purity) and unlabeled (97% purity) atrazine (Simoneaux, 1988). Thin-layer and cation exchange chromatography indicated that Fischer rat, human and goat hepatocytes are similar in producing two major metabolites, mono-dealkylated

2-amino-4-chloro-6-isopropylamino-triazine and 2-amino-4-chloro-6-ethylamino-triazine. Less than 1% of diaminochlorotriazine was evident in histograms of the hepatocyte incubations. Thus, atrazine did not significantly affect cell function in any of these four organisms. The results do not reflect the *in vivo* results, in which 2-amino-4-chloro-6-ethylamino-triazine was a minor metabolite in urine, and was not measurable in the blood of humans, while the di-dealkylated metabolite accounted for more of the radioactivity than the combined monodealkylated metabolites. Sprague-Dawley rat autoradiograms indicated further metabolism of the two metabolites to a group of polar metabolites, with a major peak (>33%) for the didealkylated 2,4 diamino-6-chloro-*s*-triazine. Approximate 24-hour bioconversion rates for the Fischer rat, Sprague-Dawley rat, goat, or human cells were 14.5, 30.0, 31.9, and 11.0 μg metabolized/μg DNA, respectively. These results would suggest that bioconversion of atrazine by human hepatocytes is slightly slower than other species, but, as previously stated, the *in vitro* results do not necessarily reflect the *in vivo* results.

In an additional investigation (Adams *et al.*, 1990), the metabolism of three triazine herbicides (atrazine, simazine, and terbutryn) was studied in rats (Sprague-Dawley and Fischer), mouse, goat, sheep, pig, rabbit and chicken by using *in vitro* hepatic 10,000 g supernatant or microsomal systems. Principle phase I metabolites were 4- or 6-monodealkylated-*s*-triazines; several observations, including studies with purified enzymes, demonstrated that phase I reactions were cytochrome P-450 mediated. There were species-specific variations in the rates of metabolism and in the ratios of primary metabolites, but no sex-related differences were noted. Phase II products were glutathione conjugates of the parent and of the two monodealkylated metabolites. Microsomal preparations from Sprague-Dawley and Fischer rats provided comparable rates and patterns of phase I and phase II metabolism of atrazine when data were normalized to the amount of product per nanomole of P-450 or per milligram of protein. However, on a per gram of liver or per gram of body weight basis, Fischer rats metabolized atrazine 2-5 times more quickly. Experiments with Fenton's reagent, which generates hydroxyl radicals, gave dealkylated 2-chloro-*s*-triazine, supporting a possible role of active oxygen radicals in the cytochrome P-450 mediated reactions.

B. ACUTE TOXICITY

Summary: The available LD_{50} and LC_{50} values for technical and formulated atrazine and atrazine metabolites are summarized in Table 2. Clinical signs following inhalation included nasal discharge or wet muzzle. Clinical signs after oral exposure included decreased activity, constricted pupils, epistaxis, piloerection, polyuria, salivation, muscle weakness, prostration, facial swelling, weight loss, ataxia, tremors, ptosis, sensitivity to touch, and dyspnea. Following dermal exposure, local erythema and edema were recorded. Necropsy findings included discolored lungs, mucoid material in the intestines, hemorrhages in the gastrointestinal tract, pale liver, occult blood in the urinary bladder and renal cysts.

A suitable oral NOEL to characterize the acute risks from atrazine exposure was not identified from submitted lethality studies or from published literature. Instead, the US EPA and DPR have used a NOEL of 5 mg/kg/day, for maternal and developmental toxicity in a rabbit teratology study (see Section IIIG: Developmental Toxicity) to assess potential human acute oral and dermal exposure (Arthur, 1984; US EPA, 1990b).

Table 2. The Acute Toxicity of Technical and Formulated Atrazine and Metabolites.

Route/Species	Sex	Dose/Effect	References[a]
		Atrazine Technical Grade (97-98%)	
Inhalation LC_{50}[b]			
Rat	NA[c]	>710 mg/m^3 (>85 mg/kg)	1
Oral LD_{50}			
Rat	M/F	672-4,200 mg/kg	1-4
Mouse	M/F	>1,332-3,992 mg/kg	5-6
Hamster	NA	1,000 mg/kg	7
Dermal LD_{50}			
Rat	M/F	>2,500->3,100 mg/kg	1,3
Rabbit	M/F	>4,500 mg/kg	8
Ocular Irritation			
Rabbit	M/F	Non-Irritant	1,9
Dermal Irritation			
Rabbit	M/F	Non/Mild Irritant	1,10
		Atrazine Liquid Formulations (41-53.2%)	
Inhalation LC_{50}			
Rat	M/F	>2,380->8,400 mg/m^3 (>184->672 mg/kg)	2,11,12
Oral LD_{50}			
Rat	NA	1,075 - 3,080 mg/kg	2,12
	M/F	>5,000 mg/kg	13
Dermal LD_{50}			
Rabbit	M/F	>2,000->10,200	2,12,14
Ocular Irritation			
Rabbit	NA	Non/Mild Irritant	2,12,15
Dermal Irritation			
Rabbit	NA	Non/Mild Irritant	2,12,16
Skin Sensitization			
Guinea Pig	NA	Positive	12

a/ References: 1 Pesticide Manual, 1991; 2 FCH, 1991; 3 Gaines *et al.*, 1986; 4 CINFR, 1973; 5 Kuhn, 1988; 6 Sachsse & Bathe, 1975; 7 Cabral *et al.*, 1979; 8 van Beek,1973; 9 Rosenfield, 1983; 10 Rosenfield, 1984; 11 Rosenfield, 1981a; 12 NACA,1992; 13 Rosenfield, 1981b; 14 Rosenfield, 1981d; 15 Rosenfield, 1981e; 16 Rosenfield, 1981c.
b/ The calculation used for inhalation studies was: (exposure, mg/m^3) x (rat respiration rate, 0.96 m^3/kg/day) x (exposure duration/24 hrs) x (50% respiratory retention).
c/ Not Available.

Table 2. The Acute Toxicity of Technical and Formulated Atrazine and Metabolites. (continued)

Route/Species	Sex	Dose/Effect	References[a]
		Atrazine Granular Formulations (8-90%)	
Inhalation LC_{50}[b]			
Rat	M/F	>1,300-5,200 mg/m^3 (4 hr) (>104-416 mg/kg)	2,12,17-20
Oral LD_{50}			
Rat	M/F	1,600->5,000 mg/kg	12, 21-23
Dermal LD_{50}			
Rabbit	M/F	>2,000->20,000 mg/kg	2,12,21,24-5
Ocular Irritation			
Rabbit	M/F	Minimal/Moderate Irritant	2,12,21,26-7
Dermal Irritation			
	M/F	Minimal/Moderate Irritant	2,12,21,28
		Desisopropylatrazine	
Oral LD_{50}			
Rat	NA[c]	798 mg/kg	29
		Desethylatrazine	
Oral LD_{50}			
Rat	NA	750 mg/kg	29
		Nitrosoatrazine	
Oral LD_{50}			
Rat	NA	3,525 mg/kg	30
Mouse	NA	1,320 mg/kg	30

a/ References: 17 Jessup, 1978; 18 IBT, 1971a; 19 RTECS, 1984; 20 Maedgen, 1984a; 21 IBT, 1971c; 22 Maedgen, 1984b; 23 Kepler, 1972; 24 Maedgen, 1984c; 25 Griffiths, 1978; 26 Maedgen, 1984d; 27 Griffiths, 1977; 28 Maedgen, 1984e; 29 Ciba-Geigy, 1992a-c; 30 Weisenburger, 1987.

C. SUBCHRONIC TOXICITY

Summary: Reports have been submitted on the subchronic toxicity of atrazine (14d - 90d) in the rat *via* diet or gavage (n=3) and in the rabbit by dermal exposure (n=1). Similarly, atrazine metabolites have been studied in the rat (n=6) and the dog (n=3), by dietary administration. In all studies, reductions in body weight have been reported, usually with concomitant falls in food intake. Two of the most readily observed effects in the rat were increased extramedullary hematopoiesis (EMH) in the spleen (and sometimes also, liver) and renal toxicity, often manifest as calcification. Hematological abnormalities were often reported in the rat. Alterations in the estrus cycle was noted with the metabolite, DACT, in the SD rat, with a lower NOEL than for the reduction in body weight. In the dog, reductions in body weight have also been reported, for the metabolites DIPA, DEA and hydroxy-atrazine (n=3). For DIPA and DEA, cardiac toxicity was also demonstrated, in the form of reduced absolute heart weight and reductions in amplitude of the P-wave of the EKG, in both sexes. Also observed in some individuals were paroxysmal atrial fibrillation, in a female and hyperplasia and inflammation of the right atrium, in a male. NOEL values determined from the studies are summarized in Table 3. Additional subchronic toxicity studies, designed to investigate endocrine effects of atrazine, are described in Section J.

Atrazine

Gavage-Rat

In a published account of a 14-day renal toxicity study (Santa Maria *et al.*, 1986), it was reported that ten male Wistar rats/dose received 0, 100, 200, 400, or 600 mg/kg/day of analytical grade atrazine (purity unstated) in an arabic gum vehicle via oral tube. The animals were housed in metabolism cages for collection of urine and sacrificed on day 14. The authors reported a significant dose-related increase in the urinary excretion of sodium, potassium and chloride ions in all treated groups. Proteinuria was also increased in all treated groups, and creatinine clearance was depressed at 200 mg/kg/day and above. Dose-related increases in total lactate dehydrogenase (LDH) and renal α-hydroxybutyrate dehydrogenase were believed to indicate renal toxicity. No other urinary parameters were evaluated. Based on proteinuria and increased urinary ion excretion, the LOEL was 100 mg/kg/day.

Gavage-Rat

In a published account of a 14-day hepatic toxicity study (Santa Maria *et al.*, 1987), it was reported that ten male Wistar rats/dose received 0, 100, 200, 400 mg/kg/day of analytical grade atrazine (purity unstated) in an arabic gum vehicle via gavage. Another group of ten male Wistar rats received 600 mg/kg/day for 7 days. The animals were sacrificed at the termination of dosing. A dose-related decrease in body weight and blood glucose was present in all groups. Dose-related increases in total serum lipids, alkaline phosphatase and alanine aminotransferase were also noted. No significant ultrastructural changes were observed in hepatocytes at 100 mg/kg/day. However, a dose-related proliferation and degeneration of smooth endoplasmic reticulum, lipid accumulation, mitochondrial malformation and alteration of bile canaliculi were present in the other atrazine-treated groups. No other hepatic parameters were evaluated. Based on decreased food consumption and body weight, the LOEL was 100 mg/kg/day.

Dietary-Rat

In a summary of a 90-day feeding study of technical atrazine (98% atrazine and related compounds), it was reported that ~20 Sprague-Dawley rats/sex/dose received 0, 200, 1000, or 5000 ppm in the diet (~0, 20, 100 or 500 mg/kg/day) (Tisdel, 1977). At 5000 ppm, clinical signs of emaciation, poor muscle tone and ruffled hair coat appeared as soon as day 4. By week six, 13 males and 9 females of the 5000 ppm group had died. Reduced body weight and food consumption were present throughout the study. At 1000 ppm, reduced food consumption and body weight were present for most of the treatment period, while at 200 ppm, food consumption was reduced only during the first week. Clinical pathology examinations conducted at weeks 4 and 13 revealed increased numbers of neutrophils, elevated alkaline phosphatase levels, and differences in urinary pH and protein content at 5000 ppm. Histological examinations were conducted on all animals in the 5000 ppm group, and one half of the animals in the control and 1000 ppm groups. Eye and nerve tissues were not examined in those groups, and no tissues from the 200 ppm group were examined. Histologic alterations identified in terminally sacrificed animals included testicular atrophy, squamous metaplasia and/or hyperplasia of renal epithelium, squamous metaplasia of urinary bladder epithelium, calcium deposits in the kidneys, extramedullary hematopoiesis in the spleen and erythroid hyperplasia of the bone marrow. The changes were confined to 5000 ppm animals except for spleen hematopoiesis and kidney calcification, which were also present in several 1000 ppm males. The incidence in the 200 ppm group was unknown, due to the lack of histological examination. A NOEL based on reduced body weight and food consumption was 200 ppm. However, the inadequate histological examinations conducted in the study precluded determination of a definitive NOEL for spleen hematopoiesis and kidney calcification.

Dermal Rabbit

In a brief summary of a 5-day dermal toxicity study in rabbits (IBT,[1] 1971b), it was reported that groups of 2 rabbits/sex/dose (breed unstated) were treated with 0, 250, 500, or 1000 mg/kg/day of AAtrex 90W (90% atrazine and related compounds). The repeated application of AAtrex 90W to the skin of rabbits was reported to be mildly irritating. No deaths or untoward behavioral reactions were observed. A decrease in body weight was noted in one or more groups. Barely perceptible erythema was noted after one application, and pale red to red, well defined erythema with mild edema was noted after 4 applications. The irritation was slightly worse on abraded areas (superficial eschar). Information on a no-effect level was not presented.

Atrazine Metabolites

Dietary- Rat

There have been three subchronic dietary studies of desisopropylatrazine in rats (Ciba-Geigy, 1992a). In a summary of the first study, it was reported that rats were fed 0, 500, or 1000 ppm (~0, 25, or 50 mg/kg/day) for 90 days. A significant reduction in male body weight occurred at 1000 ppm. No clinical signs, clinical chemistry or histopathologic changes associated with treatment were noted.

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[1] IBT is Industrial Biotest

In a summary of the second study, it was reported that rats were fed 0, 50, 500, and 1500 ppm (~0, 2.5, 25, or 75 mg/kg/day) of desisopropylatrazine for 28 days. Males and females at 500 and 1500 ppm had dose-related decreases in body weight; food consumption was also decreased at 1500 ppm. Various clinical chemistry and hematology parameters were affected; absolute and/or relative thymus, heart, liver, kidney, adrenal and spleen weights were altered in one or both sexes at 500 and 1500 ppm. Sporadic effects in organ weight were noted at 50 ppm. Histologically, crystallizations in the renal pelvis were present at 1500 ppm. Based on alterations in organ weight, the study LOEL was apparently 50 ppm (2.5 mg/kg/day).

In the third study, SPF rats (10/sex/dose) were fed 0, 10, 50 or 500 ppm (~0, 0.6, 3.2 or 34.9 mg/kg/day, M, 0, 0.64, 3.34 or 37.5 mg/kg/day, F) of desisopropylatrazine for 3 months (Schneider, 1992). In both sexes, there were reductions in body weight of 10 to 13% which were not significant for males but were ($p<0.01$) for females at both 5 and 13 weeks. In males, significant effects were reported only at 500 ppm. These included an increased incidence in rats with hypertrophy of TSH-producing cells in the pituitary ($p<0.05$, Fisher's exact test) and thyroid cells ($p<0.05$); an increased incidence in rats with fatty adrenal cortex ($p<0.01$). In females none of these effects were recorded but instead, there was an increased incidence of liver extramedullary hematopoiesis (EMH) at 500 ppm (n.s.); there was also an increase of moderate splenic EMH at 50 ppm ($p<0.05$) and 500 ppm ($p<0.01$). The LOEL and NOEL are therefore considered to be 500 ppm and 50 ppm for males and 50 ppm and 10 ppm for females, for the effects described. The study LOEL and NOEL are thus 3.34 and 0.64 mg/kg/day, respectively.

Dietary-Rat

In a summary of a 90-day oral toxicity test of desethylatrazine, it was reported that rats were fed 0, 500, or 1000 ppm (~0, 25, or 50 mg/kg/day). Significant reductions in body weight and food consumption were present at both dose levels and were attributed by the investigators to poor palatability. No treatment-related clinical signs, clinical chemistry, or histological abnormalities were reported. However, decreased red blood cell numbers and neutrophil counts were present in both sexes at 1000 ppm. White blood cell numbers were reported to be both increased and decreased. Considering only the hematology results, the NOEL was stated by the investigators to be 500 ppm (25 mg/kg/day). However, based on reduced body weight and food consumption, 25 mg/kg/day was the LOEL (Ciba-Geigy, 1992b).

Dietary-Rat

A 90-day feeding study of diaminochlorotriazine was conducted using SD-rats (15/sex/dose) receiving 0, 10, 100, 250, or 500 ppm, equivalent to 0, 0.7, 6.7, 16.7 or 34.1 mg/kg/day, males and 0.7, 7.6, 19.7 or 40.2 mg/kg/day, females (Pettersen *et al.*, 1991). There was a decrease in body weight, but not food consumption, in both sexes at 250 and 500 ppm, which was significantly lower ($p<0.01$), by 13%, only for males at 500 ppm. There was no clinical pathology, organ weight, necropsy, or histological evidence of toxicity. However, treatment-related effects on the estrus cycle were reported at 100 ppm and above. The changes included alterations in the cycle length and an increase in the incidence of animals with irregular cycles that were variable, indeterminate and/or less than 4 or greater than 5 days in length. The incidence was significant at the three top doses at 42 to 56 days and at the top two doses at 70 to 85 days. In addition, there was an increased incidence of animals with persistent estrus and/or persistent diestrus, which was generally significant at the top two doses. Serum levels of estradiol, progesterone, and prolactin were unaffected by treatment. Based on estrus

effects, the LOEL was 100 ppm (7.6 mg/kg/day) and the NOEL was 10 ppm (0.7 mg/kg/day), whereas the LOEL and NOEL for reduced body weight were higher, 250 and 100 ppm, respectively.

Dietary-Rat

In a thirteen-week feeding study, hydroxyatrazine was fed to 15 SD rats/sex/group at 0, 10, 100, 300, or 600 ppm (~0, 0.5, 5, 15, or 30 mg/kg/day). Numerous effects were seen at 600 ppm including decreased body weight and food/water consumption, decreased erythroid parameters and increased platelet counts, increased serum urea nitrogen, creatinine and electrolyte concentrations, decreased urine specific gravity, increased kidney weights and discolored or pitted kidneys. Histologically, the kidney changes included minimal to marked renal tubular dilation and basophilia with subacute/chronic interstitial inflammation at 300 ppm, cellular casts and crystals in the papillary tubules were also present at 600 ppm. Based on the kidney changes, the NOEL was 100 ppm, 5 mg/kg/day (Ciba-Geigy, 1992c).

Dietary-Dog

In a 14-week study, desisopropylatrazine was fed to 4 beagles/sex/dose at 0, 15, 100, 500 and 1000 ppm, equivalent to 0, 0.6, 3.8, 18.9 and 33.4 mg/kg/day for males and 0, 0.6, 3.8, 18.0 and 33.4 mg/kg/day for females (Thompson *et al.*, 1992). There were no clinical signs or deaths during the study. There was a dose-related reduction in body weight, reaching significance at 1000 ppm ($p<0.05$). This was correlated with lower food ingestion. Reductions in mean, absolute organ weights were dose-related and were most marked for the heart in males, $p<0.05$ and $p<0.01$, at the two highest doses respectively, and females; the testes ($p<0.05$ and $p<0.01$); the prostate ($p<0.05$ and $p<0.05$) along with substantial reductions in absolute organ weights which were not significant, for ovary (33%), uterus (66%) and thymus (55%, females). The physiological status of the heart was expressed as EKG measurements. At the two top doses, in both sexes, there was a dramatic fall in amplitude of the P wave, suggesting disfunction of the atrial muscle. This reduction, of 45% to 67%, was recorded at each time point, for each dog, of each sex. There was little effect on the R wave or on the time course of the EKG components. The LOEL and NOEL for cardiac effects, reduced body weights and reduced absolute organ weights were 500 ppm and 100 ppm, respectively, equivalent to 18 and 3.8 mg/kg/day. The report's authors failed to mention these cardiac effects they but were noted in the DPR review. Because of this oversight, the report was considered unacceptable to DPR,

Dietary-Dog

In a 13-week study, desethylatrazine was fed to 4 beagles/sex/dose at 0, 15, 100 and 1000 ppm, equivalent to 0, 0.6, 3.7 and 28.8 mg/kg/day for males and 0, 0.5, 3.9 and 32.2 mg/kg/day for females (Rudzki *et al.*, 1992). There were no clinical signs or deaths during the study. There was a fall in body weight, reaching significance for females (but not for males), at 100 ppm (16%, $p<0.05$) and 1000 ppm (30%, $p<0.01$). This was correlated with lower food intake in both sexes at 1000 ppm. Reductions in mean, absolute organ weights were restricted to 1000 ppm and were most marked for the heart (19%, males, $p<0.05$ and 20%, females, n.s.); the testes (29%⇓, n.s.); the epididymis (29%⇓) and prostate (22%⇓) along with substantial reductions for females, in ovary (46%⇓), uterus (92%⇓, $p<0.05$), pituitary (37%⇓, n.s.) and thymus (66%⇓, $p<0.05$). Hematology data indicated a fall in RBC count of 16%, males (n.s.) and 25%, females ($p<0.01$); hemoglobin of 20%, males ($p<0.05$) and 23%, females ($p<0.01$); hematocrit of 22%,

males ($p<0.05$) and 24%, females ($p<0.01$) along with cholesterol of 25%, males ($p<0.05$) and 24%, females (n.s.). Some of these effects could have been secondary to the fall in food intake/body weight. Renal toxicity was noted at the HDT in the form of a higher incidence of hyperplasia and/or basophilia of the tubular epithelium, in both sexes. The physiological status of the heart was expressed as EKG measurements. At the HDT, in both sexes, there was a dramatic fall in mean amplitude (50%) of the P wave, suggesting disfunction of the atrial muscle. This reduction in amplitude was recorded at each time point, for each sex. There was a corresponding increase in mean duration of the P wave, of 33%, in both sexes. Paroxysmal atrial fibrillation was noted in the EKG for one female, at two time points, at 1000 ppm. There was little effect on the R wave but there was a marked increase in mean heart rate (tachycardia) in males (31%) and females (20%). There was also a slight increase in males (15%) at 100 ppm. Histopathological examination revealed angiomatous hyperplasia and multifocal inflammation of the right atrium in one male at 1000 ppm. The LOEL and NOEL for cardiac effects, reduced body weights and reduced absolute organ weights were 1000 ppm and 100 ppm, respectively, equivalent to 28.8 and 3.7 mg/kg/day. The effects on the EKG, with the exception of atrial fibrillation, were not mentioned in the report but were noted in the DPR review. Because of this oversight by the authors of the report, it was considered unacceptable to DPR.

Dietary-Dog

In another 13-week study, hydroxyatrazine was fed to 4 beagle dogs/sex/group at 0, 15, 150, 1500, or 6000 ppm (~0, 0.375, 3.75, 37.5, or 150 mg/kg/day). Signs of emaciation were present in one female at 6000 ppm. Changes present at both 1500 and 6000 ppm, in both sexes, included slight decreases in food consumption and body weight, increased urine volume and decreased urine specific gravity and pitted or rough kidneys with microscopic evidence of minimal to severe tubular and interstitial chronic nephropathy along with intra tubular crystals. Based upon these changes, the NOEL was 150 ppm (3.75 mg/kg/day) (Chad *et al.*, 1990).

Table 3. The Subchronic Toxicity of Atrazine and Metabolites.

Species/ Sex	Exposure	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
		Atrazine			
Rat (M)	14-day gavage	Proteinuria & ⇑ urinary ion excretion (Na, K, Cl)	-	100[a]	Santa Maria, 1986
Rat (M)	14-day gavage	⇓ Food consumption & b.wt.	-	100[a]	Santa Maria, 1987
Rat (M/F)	90-day diet	⇓ Food consumption & b.wt. ⇑ EMH in spleen - male Inadequate histological exam	20	100	Tisdel, 1977
		Desisopropylatrazine			
Rat (M/F)	13-week diet	⇓ b. wt.	25	50	Ciba-Geigy, 1992a
	4-week diet	⇓ b.wt.; altered organ weights	-	2.5[a]	Ciba-Geigy, 1992a
Rat (M/F)	3-months, diet	⇓ b. wt. - male ⇑ EMH in spleen - female	3.2 0.64	34.9 3.34	Schneider *et al.*, 1992
Dog (M/F)	14-week diet	cardiotoxicity	3.8	18	Thompson *et al.*, 1992
		Desethylatrazine			
Rat (M/F)	13-week diet	⇓ Food consumption & b.wt.	-	25[a]	Ciba-Geigy, 1992b
Dog (M/F)	13-week diet	cardiotoxicity, b.wt.⇓	3.7	28.8	Rudzki *et al.*, 1992
		Diaminochlorotriazine			
Rat (M/F)	13-week diet	Alterations in estrus cycle	0.7	7.6	Pettersen *et al.*, 1991
		Hydroxyatrazine			
Rat (M/F)	13-week diet	Kidney degeneration & inflammation	5	15	Ciba-Geigy, 1992c
Dog (M/F)	13-week diet	Kidney degeneration & inflammation	3.75	37.5	Chad *et al.*, 1990

[a] Lowest dose tested

D. CHRONIC TOXICITY/ONCOGENICITY

Summary: Toxic effects were identified in chronic toxicity/oncogenicity studies of atrazine or metabolites in dogs, rats, and mice. Non-neoplastic changes included decreased food consumption and body weight, hematological abnormalities and degenerative, hyperplastic, or inflammatory changes in mammary gland, heart, kidney, skeletal muscle, prostate, or eye. Altered reproductive hormone levels were also measured. Neoplastic changes resulting from atrazine administration were restricted to an increased incidence of mammary tumors in the female SD rat; no increases in tumors were consistently found in the male SD rat or in Fischer rats or mice, of either sex. In an acceptable FIFRA Guideline oncogenicity study of atrazine, a dose-related increased incidence and decreased latency of benign and/or malignant mammary tumors were reported in female Sprague-Dawley rats; an increased incidence of benign testicular interstitial cell tumors in males was also noted, at the highest dose tested (HDT) only. A supplemental study using Sprague-Dawley rats also showed an increased incidence of mammary tumors in females. In some studies there was also a reduced latency of tumor onset. In only one report using this strain of rat was there no increase in mammary tumors in females and this was a brief summary of a non-FIFRA guideline study. In another non-FIFRA guideline study using the Fischer rat, there was no increase in mammary tumors, although there was an increased incidence of uterine tumors and leukemia/lymphoma at the HDT. In a sixth, supplementary non-FIFRA guideline study, using the Fischer rat, there was no evidence of an increased level of tumors of any type. In an acceptable FIFRA guideline study using the Fischer rat, there was no increased incidence of any type of tumor, in either sex. In another non-FIFRA guideline study, using an unspecified strain of albino rat, there was no increased incidence of any tumors. An increased incidence of mammary carcinomas was also observed in a 2-yr. study using female SD rats which were analyzed for estrus cyclicity (Morseth, 1998, described in Section III.J.). No oncogenic effects were present in mice in an acceptable study or in two other reports submitted in summary form. A 2-year study of the atrazine metabolite, hydroxyatrazine, did not present evidence of oncogenicity. NOELs determined from chronic studies are summarized in Table 18. The value of 0.5 mg/kg/day was used as the critical chronic NOEL for risk characterization, based on cardiotoxicity in a dog 1-year study and supported by a BMD for EMH in the spleen of the female SD rat, in a 2-year study.

Atrazine: Dietary-Rat

Atrazine technical (95.8% purity) was administered in the feed to 70 Sprague-Dawley rats/sex/dose at 0, 10, 70, 500, or 1000 ppm (~0, 0.33, 2.28, 17.4 or 36.2 mg/kg/day, males or 0, 0.41, 2.86, 21.8, or 45.3 mg/kg/day, females) for 24 months (Mayhew, 1986). The 0 and 1000 ppm groups also contained an additional 20 rats/sex of which 10 were sacrificed after 12 months, and 10 were sacrificed at 13 months after receiving a control diet for a month. Clinical pathology determinations were evaluated at 3, 6, 12, 13, 18, and 24 months. Dosed females had reduced survival; for example, after two years at 500 and 1000 ppm, 63% ($p<0.01$) and 57% ($p<0.01$) had died *vs.* 38% of control rats. This was apparently related to mammary tumor formation as the average survival in non-tumor-bearing animals was longer in 1000 ppm females than in controls. Survival in males was significantly increased; for example, after two years at 1000 ppm, only 26% of rats had died *vs.* 44% of controls ($p<0.01$). Weight gain and food consumption were reduced in the 500 and 1000 ppm groups of both sexes. In males, final body weight was 9% below control at 500 ppm and 19% below ($p<0.01$) at 1000 ppm. For females, the corresponding reductions in body weight were 18% ($p<0.01$) and 26% ($p<0.01$). In part because of these falls in body weight, the relative weight of several organs, including brain,

kidney and liver, was significantly increased by atrazine, in both sexes. In females at 1000 ppm, the 6, 12, and 18 month data showed significant reductions in hematocrit (6.5%, 9.2% and 15%, $p<0.01$), RBC counts (5.1%, 11% and 19%, $p<0.01$) and hemoglobin levels (6.4%, 7.8% and 14%, $p<0.01$). Serum glucose levels were decreased at 1000 ppm at 3, 6, and 12 months of study; for example, at 12 months, the glucose was reduced by 18% ($p<0.01$) for both sexes. The serum triglyceride concentration tended to be decreased at 1000 ppm throughout the study; for example, at 12 months, it was reduced by 50% ($p<0.01$) for males and 59% ($p<0.01$) for females. There was also an increase in platelet count at 6, 12 and 18 months in females; at 1000 ppm, this was 7.5% ($p<0.05$) above control at 6 months, 18% ($p<0.01$) at 12 months and 16% ($p<0.05$) at 18 months. In males, the corresponding increases in platelet count were 3.6%, 6.6% and 3.8% respectively, none of which were significantly different from the control group.

Histologically, non-neoplastic lesions which increased significantly at 1000 ppm in males included mammary gland hyperplasia (12% of control rats to 32% of rats at 1000 ppm), kidney calculi (23% to 46%), prostate hyperplasia (18% to 44%), skeletal muscle degeneration (9% to 42%) and retinal degeneration (3.1% to 10%). In females, skeletal muscle degeneration (7.5% to 20%), hyperplasia and inflammation of the kidney (25% to 48%), urinary bladder (6.0% to 16%) and retinal degeneration (18% to 34%) were present at 1000 ppm only. Bone marrow hyperplasia in the femur and sternum and extramedullary hematopoiesis (EMH) of the spleen were increased in 500 and 1000 ppm females and smaller dose-related increases in EMH were also present in 10 and 70 ppm females (Table 4A). Increased incidences were not observed in males (Table 4B). The report attributed the hyperplasia and EMH to ulcerated and/or necrotic mammary tumors, but there was little correlation between mammary tumors and either hyperplasia or EMH (chi^2 = 0.1 to 1.7). However, although rats with fibroadenoma(s) show little correlation with those showing EMH in either the interim or final sacrifice animals, there is a correlation for adenocarcinomas at the interim sacrifice. All females with EMH at 12 (n=2) and 13 (n=1) months had mammary gland adenocarcinomas and none of these had fibroadenomas. However, at each timepoint, four rats had adenocarcinomas without EMH. Thus, all females with EMH had adenocarcinomas but not all the rats with adenocarcinomas had EMH. It is thus possible that the EMH in the spleen of females is related to the increased blood supply required by the mammary carcinomas. Furthermore, the biological significance of the correlation is probably greater for the interim rats (than at 2 years) because secondary effects related to age and inadequate diet would be less marked. At the study conclusion, at the HDT (1000 ppm), 19 females had fibroadenoma and EMH, 23 had fibroadenoma without EMH and 9 had EMH without fibroadenoma, out of a total of 65 rats examined. Also, 19 had adenocarcinoma with EMH, 16 had adenocarcinoma without EMH and 8 had EMH without adenocarcinoma. Out of 65 rats considered, 53 had fibroadenoma and/or adenocarcinoma and 28 had EMH; 25 out of these 28 had fibroadenoma and/or adenocarcinoma and 3 had neither. Thus, there appears to be little correlation between EMH and mammary tumors at two years.

The non-neoplastic NOEL was initially established at 70 ppm (2.86 mg/kg/day) based on increased EMH, in the original review of the study, but since EMH incidence was apparently dose-related throughout the dose range (r = 0.985), the benchmark dose approach (Kimmel, 1990; Crump *et al.*, 1991; Beck *et al.*, 1993) was used to establish a value for chronic risk characterization. Using the probit model, the ED_{10} (the dosage estimated to affect 10% of the rats above control) for EMH was 2.57 mg/kg/day, with a 95% lower bound of 0.471 mg/kg/day. The latter value was used to assess potential chronic human exposure hazards in a previous interim risk assessment of atrazine (DPR, 1990).

Neoplastic lesions which increased in treated groups included testicular interstitial cell tumors (adenomas), which were present in 1.5% of controls and 10.5% of males at 1000 ppm ($p<0.05$, Fisher's exact test). Because this is an age-related tumor (Capen, 1996), it may be a direct result of the significantly longer survival of males at 1000 ppm, but not at lower doses. In females, the incidence of all mammary tumors, and the incidence of mammary adenocarcinoma, were increased significantly at 70 ppm and above (Table 5). The neoplasms occurred at a lower dose level than the decreased body weight and food consumption, indicating that carcinogenic activity was present at a dose level well below a MTD. Mammary tumors were also increased at the interim sacrifice and in animals which died after exposure to atrazine for 12 months, either without or with a 1-month recovery period on undosed diet (Tables 6 & 7). A variety of tumors, both benign and malignant, were present in other organs. Although most tumors were found at the HDT, there was no clear dose-response relationship for any tumor aside from mammary gland ones in females. The quantitative risk assessment for oncogenicity used the multi-stage (Global 86) model, with the latter data (Section IV A). Although the interim sacrifice animals showed an earlier onset time (Tables 6,7), the rats in the 2-year study did not. The essential conclusions of the study pathologist were confirmed in a supplemental report (Hardisty, 1987).

Table 4A: Chronic Effects of Atrazine in the Female SD Rat: Activity in Hematopoietic Organs at 2 Years.[a]

EFFECT	DOSE, ppm[b]				
	0	10	70	500	1000
Bone marrow femur myeloid hyperplasia	25/68+++ (37%)	23/65 (35%)	24/69 (35%)	38/65** (58%)	52/64*** (81%)
Bone marrow sternum myeloid hyperplasia	21/68+++ (31%)	21/65 (32%)	20/69 (29%)	33/65* (51%)	46/65*** (71%)
Spleen extramedullary hematopoiesis	12/67+++ (18%)	14/65 (22%)	18/69 (26%)	22/65* (34%)	28/65** (43%)

Table 4B: Chronic Effects of Atrazine in the Male SD Rat: Activity in Hematopoietic Organs at 2 Years.[a]

EFFECT	DOSE, ppm[b]				
	0	10	70	500	1000
Bone marrow femur myeloid hyperplasia	23/65 (35%)	30/63 (48%)	21/67 (31%)	27/67 (40%)	27/67 (40%)
Bone marrow sternum myeloid hyperplasia	23/65 (35%)	28/65 (43%)	21/66 (32%)	27/67 (40)	26/66 (39%)
Spleen extramedullary hematopoiesis	6/65 (9.2%)	10/65 (15%)	14/67 (21%)	9/67 (13%)	9/67 (13%)

a/ Mayhew, 1986
b/ equivalent to 0, 0.41, 2.86, 21.8 or 45.3 mg/kg/day (F) and 0, 0.33, 2.28, 17.4 or 36.2 (M), respectively, based on time-weighted averages.

* significantly different from control at p<0.05 (Fisher's exact test)
** significantly different from control at p<0.01 (Fisher's exact test)
*** significantly different from control at p<0.001 (Fisher's exact test)
+++ significant trend at p<0.001 (Peto's trend test)

Table 5. Incidence of Mammary Tumors in Female Sprague-Dawley Rats on Dietary Atrazine for Two Years.[a/]

Tumor type	Dose, ppm 0	10	70	500	1000
No. of rats at risk[b]	66	64	68	65	64
Malignant[c]					
Adenocarcinoma[d]	15+++ (23%)	15 (23%)	26* (38%)	27* (42%)	35*** (55%)
Carcinosarcoma	0	0	0	0	2 (3%)
Benign					
Adenoma[e]	1 (1.5%)	0	1 (1.5%)	1 (1.5%)	2 (3.1%)
Fibroadenoma[f,g]	29++ (44%)	29 (45%)	35 (51%)	38 (58%)	42** (66%)
Malignant + Benign Tumors[h]	35+++ (53%)	39 (63%)	47* (69%)	47* (72%)	56*** (89%)

a/ data taken from Mayhew, 1986; for historical control tumor incidence, see Appendix I.
b/ incidences are expressed as the number of animals bearing a particular tumor type, as defined at necropsy and excluding interim sacrifice animals.
c/ in addition, miscellaneous tumors, which were identified in mammary tissue without necessarily originating there, were as follows: at 1000 ppm, one rat had hemangiosarcoma and another had histiocytic sarcoma along with fibroadenoma and (3) adenocarcinomas; at 10 ppm, one rat had lymphoma.
d/ rats with multiple tumors were: 13% (control), 40%, 46%, 48% and 57% with increasing dose; the others had single tumors.
e/ includes one rat with fibroadenoma (0 ppm), one with adenocarcinoma (70 ppm), one at 500 ppm and another at 1000 ppm with fibroadenoma and (two) adenocarcinoma(s).
f/ rats also having adenocarcinoma(s) were: 14% (control), 8%, 21%, 28% and 38%, with increasing dose.
g/ rats with multiple fibroadenomas were: 24% (control), 34%, 31%, 50% and 43% with increasing dose; the others had single tumors.
h/ includes adenocarcinoma, carcinosarcoma, fibroadenoma and adenoma.

***** $p < 0.05$; ****** $p<0.01$; ******* $p<0.001$ (Fisher's Exact Test).
++ $p<0.01$; **+++** $p<0.001$ (Peto's Trend Test).

Table 6. Incidence of Mammary Tumors in Female Sprague-Dawley Rats on Dietary Atrazine for up to 12 Months (Mayhew, 1986).

	Dose, ppm				
Tumor type	**0**	**10**	**70**	**500**	**1000**
No. of rats at risk	12	5	1	5	15
Mammary Adenocarcinoma	0/12[b] (0%)	1/5[c] (20%)	1/1[c] (100%)	0/5[c] (0%)	3/15[b] (20%)
All Mammary Tumors[a]	0/12[b] (0%)	1/5[c] (20%)	1/1[c] (100%)	1/5[c] (20%)	4/15[b] (27%)

a/ Includes adenoma, fibroadenoma, or adenocarcinoma.
b/ Includes 10 rats from interim sacrifice, the remainder died intercurrently.
c/ Rats died intercurrently

Table 7. Incidence of Mammary Tumors in Female Sprague-Dawley Rats on Dietary Atrazine for 12 Months followed by 1-Month Recovery (Mayhew, 1986).

	Dose, ppm				
Tumor type	**0**	**10**	**70**	**500**	**1000**
No. of rats at risk	10	-	-	-	10
Mammary Adenocarcinoma	0/10[b++] (0%)	NE	NE	NE	5/10*[b] (50%)
All Mammary Tumors[a]	0/10[b++] (0%)	NE	NE	NE	5/10*[b] (50%)

a/ Includes adenoma, fibroadenoma, or adenocarcinoma. NE = Not histologically examined.
b/ Rats at interim sacrifice.
***** $p < 0.05$ by Fisher's exact test.
++ $p < 0.01$ by Peto's trend test.

Dietary - Rat

In a subsequent, non-FIFRA guideline study in which reproductive hormone levels were measured during the cancer process, technical atrazine (97% purity) was administered in the feed to 70 female Sprague-Dawley rats/dose at 0, 70, or 400 ppm (~0, 4.1, or 26.0 mg/kg/day) for up to 24 months (Thakur, 1991a, McConnell, 1995). Ten rats per group were sacrificed at 1, 3, 9, 12, 15, 18 months, and survivors at 24 months. Only pituitary, mammary glands, uterus, and ovary were examined histologically. In treated animals, plasma estradiol levels were increased by month 3 and by month 9 the time spent in estrus was increased. At 9 months, progesterone blood level was reduced by 36% in SD rats; estradiol was elevated by 37% and prolactin by 157% at 400 ppm (Table 8). Lesions induced by prolactin, such as increased galactoceles, were also significantly elevated at 400 ppm, at 9 months. Ovarian effects, such as increased incidence of antral and atretic follicles, were increased significantly at both 70 and 400 ppm. There was a non-significant increase in lesions of the pituitary gland, such as focal hypertrophy and adenoma, along with a significant increase in mean, absolute pituitary weight of 13% at 70 ppm and 54% ($p<0.05$) at 400 ppm. Mean group body weights were decreased by only 0.8% and 3.7% at 70 and 400 ppm, respectively, at 9 months. Slight though significant ($p<0.05$, Dunnett's test) decreases in food consumption were noted in both treated groups (3%-8% during the first month). During the full 24 months of the study, there was an earlier onset of both fibroadenomas ($p<0.05$, Gehan-Breslow test) (Table 9) and adenocarcinomas ($p<0.01$, Gehan-Breslow test) (Table10).

The study confirmed the effect of tumor induction seen in the Mayhew, 1986 study. Data were generally consistent with the possibility that atrazine caused elevated estradiol levels in early adult life, which may have resulted in elevated incidence or earlier onset of these tumors. DPR considered the report supplementary due to the unusual non-FIFRA study design.

Dietary-Rat

In a parallel study in the F-344 rat, the role of reproductive hormones in atrazine carcinogenesis was examined. Atrazine technical (97% purity) was administered in the feed to 70 female Fischer-344 rats/dose at 0, 10, 70, 200, or 400 ppm (~0, 0.7, 4.4, 12.9, or 25.9 mg/kg/day) for up to 24 months (Thakur, 1991b, McConnell, 1995). Ten rats per group were sacrificed at 1, 3, 9, 12, 15, 18 months and survivors at 24 months. Pituitary, mammary glands, uterus and ovary were examined histologically. Plasma estradiol, prolactin, or progesterone levels were not affected by treatment. The number of days spent in estrus was also unchanged (Eldridge *et al.*, 1992). Mean body weight and body weight gain values for the 200 and 400 ppm groups were generally lower than control throughout the study, with a 6% and 11% weight gain depression present at one year (Table 11 shows 9 months). No other effects were observed during the study, giving 200 and 400 ppm as the NOEL and LOEL, respectively. The incidence of mammary tumors was comparable across groups, and no evidence of an effect on time-to-tumor was noted. DPR considered the report supplementary, due to the unusual non-FIFRA study design.

Table 8. Data from 9-month interim sacrifice animals (SD Rat, F) in a 2-yr. atrazine study.[a/]

Endpoint	0	70	400 ppm
Body weight (g) (n=10/dose)	399.8±49.2	396.4±47.8 (-0.8%)	384.9±36.1 (-3.7%)
Mammary gland tumors carcinoma fibroadenoma any tumor	 0/10 0/10 0/10	 0/10 0/10 0/10	 3/10 2/10 5/10*
Hormone levels (n=10/dose) estradiol (pg/ml) progesterone (ng/ml) prolactin (ng/ml)	 22.8±20.6 11.6±11.0 17.8±12.4	 20.7±26.1 8.2±6.6 24.3±10.4	 31.2±28.1 (+37%) 7.4±4.1 (-36%) 45.8±20.0** (+157%)
Mammary gland lesions **Prolactin effects** galactocele (mod+marked) acinar/lobular development secretory activity dilated duct w/secretion	 0/10 0/10 1/10 1/10	 0/10 2/10 2/10 2/10	 6/10** 7/10** 9/10*** 8/10**
Ovarian lesions antral follicles, marked atretic follicles (mod+marked) interstitial clear cells (mod.+marked)	 0/10 1/10 0/10	 4/10* 4/10 0/10	 8/10*** 9/10*** 4/10*
Uterine lesions irregular cycle (extended P/E)[b/]	 6/10	 7/10	 10/10*
Pituitary gland lesions focal hyper-plasia/trophy[c/] adenoma combined	 1/10 0/10 1/10	 2/10 0/10 2/10	 2/10 2/10 4/10
Pituitary gland wt., (g) (n=10/dose)	0.024±0.006	0.030±0.006 (+13%)	0.037±0.008* (+54%)

a/ data from Thakur, 1991a and McConnell, 1995
b/ proestrous and estrous
c/ minimal or mild
*** significantly different from control at p<0.05 (Fisher's exact test or *t* test)**
**** significantly different from control at p<0.01 (Fisher's exact test or *t* test)**
***** significantly different from control at p<0.001 (Fisher's exact test)**

Table 9. Incidence of Mammary Fibroadenoma in Female Sprague-Dawley Rats on Dietary Atrazine for Up To Two Years.[a/]

	Dose (ppm) [b/]		
Sacrifice Time	**0**	**70**	**400 [c/]**
1 Month	0/9 (0%)	0/9 (0%)	0/9 (0%)
3 Months	0/10 (0%)	0/10 (0%)	0/10 (0%)
9 months	0/10 (0%)	0/10 (0%)	2/10 (20%)
12 Months	1/10 (10%)	0/9 (0%)	0/10 (0%)
Unscheduled Deaths [d/] (months 1-12)	0/0 (0%)	0/0 (0%)	2/4 (50%)
Total 1-12 Months	1/39 (2.6%)	0/38 (0%)	4/43 (9%)
15 months	2/10 (20%)	5/10 (50%)	1/10 (10%)
18 months	2/10 (20%)	4/10 (40%)	4/10 (40%)
24 months	1/5 (20%)	2/3 (67%)	2/2 (100%)
Unscheduled Deaths (months 13-24)	2/5 (40%)	1/6 (17%)	2/4 (50%)
Total 13-24 Months	7/30 (23%)	12/29 (41%)	9/26 (31%)
Total 0-24 Months	8/69 (12%)	12/67 (18%)	13/69 (19%)

a/ from Thakur, 1991a
b/ equivalent to mean measured dosages of 0, 4.1 and 26.0 mg/kg/day
c/ Onset time analysis was significantly positive ($p<0.05$) by the Gehan-Breslow test.
d/ The unscheduled deaths for months 1- 12 includes animals dying or sacrificed moribund during weeks 1-54. This range was selected to encompass the same range of weeks as for the interval sacrifices selected for months 1-12.

Table 10. Incidence of Mammary Carcinoma in Female Sprague-Dawley Rats on Dietary Atrazine for Up To Two Years.[a/]

	Dose (ppm)[b/]		
Sacrifice Time	**0**	**70**	**400[c/]**
1 Month	0/9 (0%)	0/9 (0%)	0/9 (0%)
3 Months	0/10 (0%)	0/10 (0%)	0/10 (0%)
9 Months	0/10 (0%)	0/10 (0%)	3/10 (30%)
12 Months	0/10 (0%)	1/9 (11%)	1/10 (10%)
Unscheduled Deaths[d/] (Months 1-12)	0/0 (0%)	0/0 (0%)	2/4 (50%)
Total 1-12 Months	0/39 (0%)	1/38 (2.6%)	6/43 (14%)*
15 Months	2/10 (20%)	0/10 (0%)	0/10 (0%)
18 Months	3/10 (30%)	2/10 (20%)	4/10 (40%)
24 Months	2/5 (40%)	0/3 (0%)	0/2 (0%)
Unscheduled Deaths (Months 13-24)	2/5 (40%)	1/6 (17%)	1/4 (25%)
Total 13-24 Months	9/30 (30%)	3/29 (10%)	5/26 (19%)
Total 0-24 Months	9/69 (13%)	4/67 (6%)	11/69 (16%)

a/ Thakur, 1991a.
b/ equivalent to mean measured dosages of 0, 4.1 and 26.0 mg/kg/day
c/ Onset-time analysis was significantly positive (p<0.01 by the Gehan-Breslow test).
d/ The unscheduled deaths for Months 1-12 include animals dying or sacrificed moribund during weeks 1-54. The range was selected to encompass the same range of weeks as for the interval sacrifices selected for Months 1-12.
*** p = 0.017 by Fisher's exact test.**

Table 11. Data from 9-month interim sacrifice animals (F-344 Rat, F) in a 2-yr. atrazine study.[a/]

Endpoint	0	10	70	200	400 ppm
Body weight (g) (n=10/dose)	202.7±10.5	196.5±9.2 (-3.1%)	201.5±7.6 (-0.6%)	191.4±11.5* (-5.6%)	191.5±8.7* (-5.5%)
Mammary gland tumors					
carcinoma	0/10	0/10	0/10	0/10	0/10
fibroadenoma	0/10	0/10	0/10	0/10	0/10
any tumor	0/10	0/10	0/10	0/10	0/10
Hormone levels (n=10/dose)					
estradiol (pg/ml)	15.4±4.9	N.D.	N.D.	N.D.	17.4±16.4 (+13%)
Mammary gland lesions					
Prolactin effects					
galactocele (mod+marked)	0/10	0/10	0/8	0/8	0/10
acinar/lobular development	0/10	0/10	0/8	0/8	0/10
secretory activity	0/10	0/10	0/8	0/8	0/10
dilated duct w/secretion	0/10	0/10	0/8	0/8	0/10
Ovarian lesions					
antral follicles, marked	0/10	0/10	0/10	0/10	0/10
atretic follicles (mod+marked)	0/10	0/10	0/10	1/10	2/10
interstitial clear cells (mod.+marked)	0/10	0/10	0/10	0/10	0/10
Uterine lesions					
irregular cycle (extended P/E)	0/10	0/10	2/10	2/10	1/10
Pituitary gland lesions					
focal hyper-plasia/trophy[b/]	2/10	0/10	1/9	1/10	0/10
adenoma	0/10	0/10	0/9	1/10	0/10
combined	2/10	0/10	1/9	2/10	0/10
Pituitary gland wt., (g) (n=10/dose)	0.015±0.002	0.014±0.002	0.015±0.003	0.014±0.004	0.016±0.002

a/ data from Thakur, 1991b and McConnell, 1995
b/ minimal or mild
*** significantly different from control at p<0.05 (*t* test).**

Dietary - Rat

In a published account of a carcinogenicity study conducted in Hungary (Pinter *et al.*, 1990), it was reported that 50-56 F344/LATI rats/sex/dose received 0, 500, or 1000 ppm atrazine in the diet. After eight weeks of treatment, the concentrations were lowered from 500 and 1000 ppm to 375 and 750 ppm (~18.8 and 37.5 mg/kg/day), because of decreased body weight gain and increased water consumption. The decreases in body weight gain at that time were 12.1 and 8.2% for males and females respectively. As the study was designed to be a life-long experiment, it ended at 126 weeks for males and 123 weeks for females. Food consumption was decreased 7% in 750 ppm males and 17 and 13% in 750 ppm and 375 ppm females measured through 104 weeks. Decreased body weight gain was reported in treated animals of both sexes, but the exact amount was not presented. Survival of both atrazine-treated male (but not female) groups was significantly increased compared to controls. Based on limited histological examination, no non-neoplastic lesions were increased in treated groups, but 750

ppm males did have statistically significant increases in mammary adenoma/ adenocarcinoma, and leukemia/ lymphoma. Analysis of the tumor incidence was hindered because up to 30% of the animals apparently did not have a complete histological examination, or were not examined at all. An increased latency was also stated to be present for tumors of the mammary region. The increased survival and tumor latency in 750 ppm males did not permit meaningful comparison with the control group without the use of individual animal data, which were not provided. In females, statistically significant increases in uterine adenocarcinoma and leukemia/lymphoma were present at 750 ppm, with comparable survivals and tumor latency. The total number of animals with malignant tumors of any type was also significantly increased at 750 ppm for both sexes. No historical data were said to exist for F344/LATI rats. DPR did not consider the study acceptable nor of regulatory value due to variances from FIFRA guidelines, the lack of individual and historical data and the inadequate histological examination.

In another non-FIFRA guideline study, groups of 60 female SD rats were exposed to atrazine, technical (97% pure) in the diet at 0, 70 and 400 ppm, for two years. Oncogenicity was noted along with histopathology of uterus, pituitary, ovary and mammary glands (Thakur, 1992a). Reduced body weight gain and increased mortality (p=0.07) during the study were observed at 400 ppm. There was an increased incidence of rats with carcinomas among animals dying in the first year at 400 ppm (p=0.013). However, there was not a significant increase at the end of the study, perhaps because of the high concurrent control incidence (29%). Mammary fibroadenoma incidence or onset was unaffected by atrazine (Table 12). The LOEL for the effects described was 400 ppm and the NOEL 70 ppm. The study was considered supplementary by DPR.

Table 12. Chronic Toxicity of Dietary Atrazine in the Female SD Rat.[1/]

Endpoint	0 ppm	70 ppm	400 ppm
Mammary tumor incidence, 1yr.			
Carcinoma	0/58	1/56 (1.8%)	6/57*(11%)
Fibroadenoma	2/58 (3.5%)	1/56 (1.8%)	4/57 (7.0%)
Mammary tumor incidence, 2yr.			
Carcinoma	17/58 (29%)	13/56 (23%)	22/57(39%)
Fibroadenoma	39/58 (67%)	30/56 (54%)	41/57 (72%)
Fibroadenoma + Carcinoma	46/58 (79%)	34/56 (61%)	49/57 (86%)
Unscheduled mortality	29/60 (48%)	35/60 (58%)	38/60 (63%)
Body weight changes, g			
0 - 4 wks.	78.8 ± 15.6	77.0 ± 14.7	68.8 ± 13.1* (13%)
0 - 13 wks.	149 ± 25.2	146 ± 23.8	131 ± 20.8* (12%)
0 - 24 wks.	185 ± 30.7	185 ± 30.9	163 ± 29.0* (12%)
0 - 52 wks.	232 ± 52.7	247 ± 54.6	212 ± 46.9 (8.6%)
0 - 76 wks.	273 ± 63.1	283 ± 55.4	237 ± 54.9*(13%)
0 - 104 wks.	283 ± 64.9	327 ± 67.2*	271 ± 78.5 (4.2%)

1/ from Thakur, 1992a; * significantly different from control @ p<0.05.

In an analogous study, groups of 60 male and female Fischer-344 rats were exposed to atrazine, technical (97% pure) in the diet at 0, 10, 70, 200 and 400 ppm, for two years (Thakur, 1992b). A complete histopathological examination (18 tissues) showed no compound-related increases in any tumors during the study (Table 13B). Reduced body weight and body weight gain for both sexes were observed at 200 and 400 ppm (Table 13A). It was concluded that the LOEL for the loss of body weight is 200 ppm and the NOEL 70 ppm. The study was acceptable to DPR under FIFRA guidelines.

Table 13A. Chronic Toxicity in the F-344 Rat on Dietary Atrazine for up to 2 yrs. [1/]

Time		Mean body weight change, % from control				
		0 ppm	10 ppm	70 ppm	200 ppm	400 ppm
0 - 4 wks.	M	0	+0.6	-3.5	-10.4*	-15.5*
	F	0	-6.9*	-6.1*	-15.2*	-17.5*
0 - 13 wks.	M	0	-1.2	-6.1*	-5.5*	-15.9*
	F	0	-2.4	-3.9	-12.2*	-13.9*
0 - 24 wks.	M	0	-1.8	-4.9*	-6.3*	-15.1*
	F	0	-4.4*	-3.5	-9.5*	-12.4*
0 - 52 wks.	M	0	-0.6	-2.2	-6.4*	-14.8*
	F	0	-1.5	-3.0	-10.1*	-13.7*
0 - 76 wks.	M	0	+1.4	-2.2	-8.1*	-16.4*
	F	0	+1.7	-2.0	-7.6*	-10.8*
0 - 104 wks.	M	0	+2.9	-0.6	-6.6	-11.4*
	F	0	-2.2	-0.4	-4.9	-11.6*

1/ Data from Thakur, 1992b
* significantly different from control @ $p<0.05$.

Table 13B. Mammary Tumors in the Female F-344 Rat on Dietary Atrazine for up to 2 yrs. [1/]

Tumor type	0 ppm	10 ppm	70 ppm	200 ppm	400 ppm
Mammary carcinoma	2/60 (3.3%)	0/60	2/60 (3.3%)	3/60 (5.0%)	2/59 (3.4%)
Mammary fibroadenoma	2/60 (3.3%)	5/60 (8.3%)	5/60 (8.3%)	7/60 (12%)	6/59 (10%)
Combined	4/60 (6.7%)	5/60 (8.3%)	7/60 (12%)	10/60 (17%)	8/59 (14%)

1/ Data from Thakur, 1992b

Dietary - Rat

In a brief summary of a rat oncogenicity study conducted at Industrial Biotest Laboratories, it was reported that Sprague-Dawley rats were fed 0, 10, 100, or 1000 ppm atrazine for two years. An increased incidence of benign and malignant mammary tumors was present in females at 1000 ppm. Because of missing or incomplete laboratory data, the study was determined by the US EPA to be of insufficient quality for regulatory use (Ciba-Geigy, 1986a,b; Ciba-Geigy 1988a,b; US EPA, 1989c).

Dietary - Rat

Atrazine 50W (48.25% purity) was administered in the feed to 30 albino rats/sex/dose at 0, 1, 10, or 100 ppm (~0, 0.05, 0.5 or 5.0 mg/kg/day) for 24 months (Keller, 1961). Five animals from each group were sacrificed at 26 and 52 weeks for interim evaluation. After 65 weeks of feeding the 10 ppm group was changed to 1000 ppm (~50 mg/kg/day). The investigators reported that no atrazine-related adverse effects were detected. The study was not acceptable to DPR based on FIFRA guidelines due to the limited numbers of animals tested, inadequate histology, non-technical test material, changed dose levels and poor viability due to infections.

Dietary - Rat

In a brief summary of an ancillary rat oncogenicity study, it was reported that 20-30 female Sprague-Dawley rats exposed to 0, 10, 50, or 500 ppm atrazine, both *in utero* and continuously during their lifetimes, had no apparent increase in mammary tumors (Ciba-Geigy, 1988a,b). This study was not acceptable to DPR due to limited number of animals, non-FIFRA protocol, and limited histological examination.

Dietary - Rat

In a 1-year ancillary study, female SD rats (55/group) were administered dietary atrazine at 0, 15, 30, 50, 70 and 400 ppm, with interim sacrifices at 3, 6 and 9 months, and 25/group being maintained until the terminal sacrifice at 12 months (Pettersen and Turnier, 1995). Tissue samples were taken and hormone assays conducted at each sacrifice to determine the stage of estrus. The results of the hormone assays, according to the authors, would be reported in a subsequent publication. Reduced body weight at 400 ppm ($\leq$9%) from weeks 5 to 18 ($p<0.01$) and at week 34 ($p<0.05$) indicate that a MTD had been reached. The NOEL and LOEL for body weight loss were 70 and 400 ppm, respectively, equivalent to ~3.5 and 20 mg/kg/day. The only significant adverse effect was an increase in the number of rats with mammary tumors. At the HDT, the percentage of rats with adenocarcinoma combined with adenoma (28%) was higher than control (4%), $p<0.05$ (Fisher's exact test). Similarly, at the HDT, the percentage of rats with all combined mammary tumors (44%) was above control (12%), $p<0.05$ (Fisher's exact test).

Dietary - Mouse

Atrazine technical (97.6% purity) was administered in the feed to 60 Crl:CD-1(ICR)BR mice/sex/dose at 0, 10, 300, 1500, or 3000 ppm (~0, 1.2, 38.4, 194.0, or 385.7 mg/kg/day) for 91 weeks (Hazelette *et al.*, 1987). Treatment-related reductions in mean body weight and percent body weight gain were statistically significant for most of the measured intervals at

1500 and 3000 ppm and intermittently so at 300 ppm. Decreased food and water consumption, decreased red blood cell numbers and decreased hematocrit and hemoglobin were noted at 1500 ppm for males and 3000 ppm for both sexes, similar to the results obtained in the rat by Mayhew, 1986. Absolute brain weight was decreased at 3000 ppm for both sexes and absolute spleen weight was also decreased in 3000 ppm females. Atrial thrombosis of the heart was increased in 1500 ppm females and at 3000 ppm for both sexes, producing increased mortality in 3000 ppm females. No oncogenic effects were observed. Based on decreased body weight and body weight gain, the NOEL was 10 ppm (~1.2 mg/kg/day). The study was acceptable to DPR, based on FIFRA testing guidelines.

Oral - Mouse

In a published summary of a carcinogenicity study in mice (Innes *et al.*, 1969), it was reported that atrazine administration did not cause a significant increase in tumor incidence. Groups of 18 male and female (C57BL/6 x C3H/Anf)F1 and (C57BL/6 x AKR)F1 mice received 21.5 mg/kg/day via gavage from days 7-28 of age, followed by 82 ppm (~12 mg/kg/day) in the diet until 18 months of age. The dose was selected as the highest amount that would cause zero mortality after 19 daily doses. No oncogenic effects were reported, although only limited necropsy and histological examinations were performed. The study was not acceptable to DPR due to numerous variances from FIFRA guidelines, and lack of individual data.

Dietary - Mouse

In a brief summary of a mouse oncogenicity study conducted at Industrial Biotest Laboratories, it was reported that CD-1 mice were fed 0, 10, 300, or 1000 ppm atrazine for 22 months. Survival and body weight were reported to be decreased at 1000 ppm. Because of missing or incomplete laboratory data, the study was determined by the US EPA to be of insufficient quality for regulatory use (Ciba-Geigy, 1986a,b; Ciba-Geigy 1988a,b; US EPA, 1989c).

Intraperitoneal - Mouse

In a brief published account of a 13-month study (Donna *et al.*, 1986), it was reported that 30 male Swiss albino mice were given a series of 13 intraperitoneal injections of technical atrazine, for a total dose of 0.26 mg/kg. Two groups of 50 animals served as saline and untreated controls. By the end of a 375 day observation period, 6 of the atrazine group had died of lymphoma (4 of plasma cell type and 2 histiocytic type). One untreated control had a histiocytic type lymphoma, and no saline controls were affected. The authors stated that the increased incidence of all lymphomas was statistically significant ($p<0.001$), by the chi^2 test. The study was not acceptable to DPR due to major variances from FIFRA guidelines.

Subcutaneous/Intraperitoneal - Mouse

In another published account of an oncogenicity study in mice (Donna *et al.*, 1981), a combination of 25% atrazine and 37.5% simazine was administered by subcutaneous or intraperitoneal injection to groups of 25 female Swiss albino mice. A series of 13 injections delivered a total dose of 0.0065 mg atrazine/simazine (~0.26 mg/kg). Two groups of 50 animals served as saline controls. The animals were kept under observation for 7 months from the beginning of treatment. One animal from each group was sacrificed at 15-day intervals starting one month after the end of treatment. The authors reported a slight though statistically

significant increase in the incidence of lymphoma. However, excessive mortality due to concurrent disease in the control groups severely limited the usefulness of the data. The study was not acceptable to DPR due to major variances from FIFRA guidelines.

Dietary-Dog

Atrazine technical (97% purity), was administered in the feed to 4-6 Beagle dogs/sex/dose at 0, 15, 150, or 1000 ppm (~0, 0.48, 4.97, or 33.73 mg/kg/day) for 52 weeks (O'Connor *et al.*, 1987). There were no compound-related effects in dogs fed 15 ppm. Effects at 1000 ppm included cachexia, decreased food consumption and body weight gain, decreased erythroid parameters, total protein, albumin, and calcium levels, and increased liver and ovary weights. Electrocardiographic measurements were made every 3 months and abnormalities in the EKG included reduction in the PII wave amplitude (Tables 14, 15), which is considered to reflect depolarization of the atria, in both sexes. There was little effect on the R wave, which is primarily associated with ventricular depolarization. Although there appeared to be an increased amplitude at 150 and 1000 ppm in males, but not females, this is probably due to the appreciably higher R-wave amplitude in these two groups than in controls at the start of the experiment, in males but not in females (Table 14). Severe and moderate atrial dilation were found at necropsy in all dogs except for one male at 1000 ppm (Table 16). A reduction in absolute and relative (to brain weight) heart weight ($p<0.01$ in female, absolute) was recorded at 1000 ppm (Table 17). One dog of each sex died prematurely during the study (Day 250, M; Day 113, F) and necropsy indicated cardiotoxicity for the female. It is also possible that degenerative lesions of the right and left atrial myocardium contributed to mortality of the male (Table 17).

At the mid-dose, 150 ppm, some of the same effects were observed as at 1000 ppm. Cachexia was accompanied by a reduction in the PII wave of the EKG (Table 14, 15), particularly in one male (#12). The same dog was found at necropsy to have moderate atrial dilation, along with a pale lesion in a ventricle (Table 16). A single male death also occurred at 150 ppm (Table 15) at 75 days. Although this mortality was claimed to be due to "spontaneous disorder of beagle" it was characterized by arterial fibrinoid necrosis and arteritis, for which a role for atrazine cannot be excluded. Effects on absolute heart weight (Table 17) included an increase at 150 ppm, for males (+8.6%) and females (+11%, $p<0.05$). The decrease in absolute heart weight at 1000 ppm could have resulted from tissue degeneration as a secondary consequence of effects such as body weight loss, because heart weight relative to body weight was the same as control at 1000 ppm, for both sexes (Table 17). Relative to brain weight, there was also an increase at 150 ppm, of +4.9%, M and +12%, F ($p<0.05$). Based on cardiotoxicity at 150 ppm, the NOEL was established at 15 ppm (0.48 mg/kg/day). The study was acceptable to DPR, based on FIFRA testing guidelines. This NOEL, rounded to 0.5 mg/kg/day was used as the critical value for chronic, non-oncogenicity risk characterization (see Section IV, A - Hazard Identification).

Dietary-Dog

In a brief summary of a chronic toxicity study, it was reported that Atrazine 80W (80% atrazine and related compounds) was fed to 3 Beagle dogs/sex/dose at 0, 15, 150, or 1500 ppm (~0, 0.45, 4.5, or 45 mg/kg/day) for 105 weeks (Woodard Research Corporation, 1964). Criteria for toxicity were reported to be: survival, body weight gain, food intake, appearance and behavior, hemograms, clinical chemistry determinations, qualitative urinalysis, and histopathology. The report's authors concluded that no compound-related signs of toxicity were present at 15 ppm.

Decreased food consumption was present at 150 ppm. At 1500 ppm, signs of toxicity included decreased food intake, decreased body weight gain, increased adrenal weights, and reduced hematocrit and hemoglobin. Clinical signs noted during the study included muscular tremors or stiffness of the rear limbs at 1500 ppm, and increased lacrimation at all doses. A convulsive spasm was also noted in one dog at 150 ppm. No treatment-related histological changes were recorded at any dose. Based on lacrimation, the LOEL was established by DPR at 15 ppm. The study was unacceptable to DPR due to lack of complete individual and summary data.

US EPA calculated slightly different dose levels for the (above) study (0, 0.35, 3.54, or 35.38 mg/kg/day), and indicated a study NOEL below 0.35 mg/kg/day based on lacrimation. A NOEL for systemic toxicity of 0.35 mg/kg/day was based on increased heart and liver weights in females at 3.54 mg/kg/day (US EPA, 1991a, 1992c). Effects observed at 35.38 mg/kg/day included reduced food intake, decreased body weight, and reduced hemoglobin and hematocrit.

Table 14. Effects of Atrazine on the EKG[a/] of the Dog during a 52-week Dietary Study[b/]

Time, wks.	DOSE ppm 0	15	150	1000
PII-wave(mV) M 0 52 wks.	(n=6) 0.250±0.022 (0.2 - 0.3) 0.283±0.031 (0.2 - 0.4)	(n=4) 0.225±0.025 (0.2 - 0.3) 0.300±0.0 (6.0%⇑) (0.3)	(n=3)[&] 0.200±0.0[#] (0.2) 0.267±0.033 (5.6%⇓) (0.2 - 0.3)	(n=5)[+] 0.233±0.021 (0.2 - 0.3) 0.060±0.024** (79%⇓) (0.0 - 0.1)
PII-wave(mV) F 0 52 wks.	(n=6) 0.250±0.022 (0.2 - 0.3) 0.283±0.031 (0.2 - 0.4)	(n=4) 0.250±0.029 (0.2 - 0.3) 0.325±0.025 (6.0%⇑) (0.3 - 0.4)	(n=4) 0.267±0.025 (0.2 - 0.3) 0.275±0.025 (2.8%⇓) (0.2 - 0.3)	(n=5)[=] 0.217±0.017 (0.2 - 0.3) 0.080±0.020** (72%⇓) (0.0 - 0.1)
R-wave(mV) M 0 52 wks.	(n=6) 0.983±0.111 (0.7 - 1.4) 1.350±0.099 (1.1 - 1.7)	(n=4) 0.975±0.239 (0.3 - 1.4) 1.600±0.168 (19%⇑) (1.2 - 2.0)	(n=3)[&] 1.275±0.165[##] (0.9 - 1.5) 1.967±0.067* (46%⇑) (1.9 - 2.1)	(n=5)[+] 1.350±0.213 (0.7 - 2.0) 1.920±0.124** (42%⇑) (1.6 - 2.3)
R-wave(mV) F 0 52 wks.	(n=6) 1.217±0.151 (0.7 - 1.5) 1.683±0.192 (1.0 - 2.1)	(n=4) 1.400±0.108 (1.1 - 1.6) 2.000±0.187 (19%⇑) (1.6 - 2.5)	(n=4) 1.725±0.193 (1.3 - 2.1) 2.125±0.144 (26%⇑) (1.9 - 2.5)	(n=5)[=] 1.100±0.124 (0.6 - 1.4) 1.700±0.184 (1.0%⇑) (1.2 - 2.2)

a/ mean amplitude (±s.d.), percent change from control and range of values, at each time point.
b/ data from O'Connor *et al.*, 1987
***** significantly different from control, p<0.05 (Dunnett's test)
****** significantly different from control, p<0.01 (Dunnett's test)
# the dog (#12) which had moderate atrial dilation and a pale lesion in a ventricle at necropsy had a PII-wave amplitude of 0.200 mV at each time point.
the dog with pathological changes (#12) had a R-wave amplitude of 1.1 mV, 2.2 mV, 2.0 mV, 1.7 mV and 1.9mV at the 5 time points from -9 to 361 days.
& male #14 died after 75 days; **+** male #16 died after 250 days; **=** female #39 died after 113 days

Table 15. Summary of Percent Changes in EKG Amplitude in the Dog after Dietary Atrazine, 52 wks

EKG parameter	DOSE ppm 0	15	150	1000
PII-wave(%) M -9 to +361 days	13%⇑ (n=6)	33%⇑ (n=4)	34%⇑ (0%)# (n=3)@	74%⇓** (n=5)$
PII-wave(%) F -9 to +361 days	13%⇑ (n=6)	30%⇑ (n=4)	3%⇑ (n=4)	63%⇓** (n=5)+
R-wave(%) M -9 to +361 days	37%⇑ (n=6)	64%⇑ (n=4)	54%⇑ (75%⇑)## (n=3)@	42%⇑** (n=5)$
R-wave(%) F -9 to +361 days	38%⇑ (n=6)	43%⇑ (n=4)	23%⇑ (n=4)	55%⇑ (n=5)+

** significantly different from control, p<0.01 (Dunnett's test, using raw data).
# the dog (#12) which had moderate atrial dilation and a pale lesion in a ventricle at necropsy had a PII-wave amplitude which did not increase during the study.
dog (#12) with pathological changes had R-wave amplitude which increased by 75%.
@ mortality on day 75 (#14) due to "spontaneous disorder of beagle," unrelated to atrazine administration, according to study authors, characterized by arterial fibrinoid necrosis and arteritis.
$ mortality on day 250 (#16) was correlated with myocardial abnormalities at necropsy, which could have resulted from atrazine administration, and dermatitis.
+ mortality on day 113 (#39) was associated with EKG abnormalities, and was atrazine-related.

Table 16. Incidence of Atrial Dilation# in the Dog after Dietary Atrazine, 52 wks.

Endpoint	DOSE ppm 0	15	150	1000
Dilation, M	0/6	0/4	1/3## (33%)	4/5 * (80%)
Dilation, F	0/6	0/4	0/4	5/5** (100%)
Combined, M+F	0/12	0/8	1/7	9/10*** (90%)

moderate and severe atrial dilation are included.
dog #12 had moderate dilation of the right atrium and minimal dilation of the left atrium, (along with a pale epicardial lesion of the left ventricle).
* significantly different from control, p<0.05 (Fisher's exact test).
** significantly different from control, p<0.01 (Fisher's exact test).
*** significantly different from control, p<0.001 (Fisher's exact test).

Table 17. Mortality and Changes in Heart Weights in the Dog after Dietary Atrazine, 52wks

Endpoint	DOSE ppm 0	15	150	1000
Mortality, M	0/6	0/4	1/4 (25%)	1/6 (17%)
Mortality, F	0/6	0/4	0/4	1/6 (17%)
Mortality, M+F	0/12	0/8	1/8 (13%)	2/12 (17%)
Heart wt. (g), M (% over control) **Heart:Brain wt.** (% over control) **Heart:Body wt.** (% over control)	96.7±6.8 (n=6) 114±7.0% 0.85±0.04%	96.8±5.0 (n=4) (0) 120±4.7% (+5.5%) 0.86±0.05% (+1.2%)	105±5.1# (3) (+8.6%) 120±4.6%## (+4.9%) 0.94±0.05% (-8.2%)	80.2±3.7 (n=5) (-17%) 103±5.2% (-10%) 0.86±0.03% (+1.2%)
Heart wt. (g), F (% over control) **Heart:Brain wt.** (% over control) **Heart:Body wt.** (% over control)	79.6±2.3 (n=6) 101±3.8% 0.84±0.04%	84.2±2.5 (n=4) (+5.8%) 105±1.3% (+3.4%) 0.86±0.03% (+2.4%)	88.7±3.2*(4) (+11%) 113.7±3.5* (+12%) 0.96±0.07% (+14%)	68.2±0.7** (5) (-14%) 93.1±3.1% (-8.2%) 0.84±0.05% (0.0%)

* significantly different from control, p<0.05 (Dunnett's test, using raw data).
** significantly different from control, p<0.01 (Dunnett's test, using raw data).
\# dog #12 had a heart weight of 113 g.
\#\# dog #12 had a heart:brain weight ratio of 119%.

Atrazine Metabolites/Degradation Products

Dietary-Dog: diaminochlorotriazine

Beagle dogs (8-10 /sex/dose) were fed diaminochlorotriazine (DACT) (98.7% purity), in the diet at 0, 5, or 100 ppm (0, 0.2, or 3.62 mg/kg/day) for 52 weeks (Thompson *et al.*, 1990). Ten additional males (Group 4) received 1500 ppm (weeks 1-6; ~44.5 mg/kg/day) and 750 ppm (weeks 7, 8, 14-52; ~ 21.5 mg/kg/day) with an intervening period of 0 ppm (weeks 9-13). Ten females in Group 4 received 1500 ppm (weeks 1-6) and 750 ppm (weeks 7-52). At 13 weeks, an interim sacrifice was performed and two Group 4 females were placed on 0 ppm for weeks 14-52 (recovery). There were no compound-related effects in dogs fed 5 or 100 ppm. Treatment-related findings in both sexes at ≥750 ppm included: a) moribund sacrifice of five males and two females starting as early as day 36; b) clinical signs of inactivity, inappetence, labored breathing, cachexia, hunched posture, abnormal gait, abdominal distention, hypothermia, paleness, fecal changes (beginning at week 2), lethargy, recumbency, vocalization, emaciation, and tremors; c) fall in group mean body weight gain of 9.2%, males (p<0.05, Dunnett's test) ; d) EKG evidence of atrial fibrillation; e) reductions in erythroid parameters with reticulocytosis at 13 and 52 weeks; f) decreased albumin, calcium, and cholesterol levels, and increases in lactic dehydrogenase values; g) necropsy changes of atrial or general cardiac enlargement, discoloration or thrombosis; enlarged liver, fluid accumulation in the thoracic and abdominal cavities; h) elevated absolute and relative spleen, liver and kidney weights at 13 and 52 weeks, and a trend for increased relative heart weights in males at 52 weeks; I) histological alterations of the heart (inflammation of various types, necrosis,

hemorrhage, hemosiderosis, thrombosis and chronic myocarditis), passive congestion of the liver, hypospermatogenesis of the testes, immaturity of the prostate, thymic atrophy and hyperplasia of the bone marrow. Based on these effects, the 13- and 52-week NOELs were 100 ppm (3.62 mg/kg/day). The study was acceptable to DPR and considered supplementary.

Dietary - Rat: Hydroxyatrazine

Hydroxyatrazine technical (97.1% purity) was administered for up to 24 months in the feed to 70-80 CD (Sprague-Dawley derived) rats/sex/dose at 0, 10, 25, 200, or 400 ppm (~0, 0.39, 1.0, 7.8, or 17.4 mg/kg/day for males, and ~0, 0.48, 1.2, 9.5, or 22.3 mg/kg/day for females (Chow & Emeigh Hart, 1995). Twenty rats/sex (0 and 400 ppm groups), and 10 rats/sex (from the other groups) were scheduled for sacrifice at 12 months. Clinical pathology determinations were evaluated at 3, 6 and 12 months. Significantly decreased body weight, and food consumption were recorded at 400 ppm for both sexes. For example, mean body weight gains for survivors were reduced from 1 month onwards and at 18 months, were reduced by 35% (male) and 61% (female) ($p< 0.01$, both sexes, Dunnett's test). Mean body weight was reduced at 400 ppm by 28% (male) and 41% (female) at 18 months ($p<0.01$, Dunnett's test). Mortality was increased at 400 ppm, resulting from renal failure; at 1 yr., 25% mortality was evident at 400 ppm *vs.* ≤6% in all other groups. As a result, surviving rats at 400 ppm were sacrificed at 18 months. Altered hematologic parameters at 400 ppm included decreased numbers of erythrocytes, and increased numbers of leukocytes. Numerous clinical chemistry and urinalysis changes indicative of kidney dysfunction were also present at that dose level. At 12 months, absolute mean kidney weight was elevated by 19% (male, $p<0.01$, Dunnett's test) and 10% (female) at 400 ppm compared with controls. Relative to body weight, these increases were also significant, for both males (50% increase) and females (52% increase), both $p< 0.01$, (Dunnett's test). Statistically significant decreases in erythropoietic parameters were also present in 10, 25 and 200 ppm males, but were discounted by the investigators as a reflection of unusually high control values. Statistically significant increases in γ-glutamyl transferase, a marker for bile duct epithelial proliferation, were present in 400 ppm males and in females at ≥ 25 ppm. However, no bile duct pathology was reported and this effect must be considered to have uncertain toxicological significance because, although a significant increase was observed at 6 and 24 months, it was not increased significantly at 12 or 18 months. The increase at 24 months in females was 17% at 25 ppm and 26% at 200 ppm. Histologically, kidney damage occurred at 200 and 400 ppm in both sexes. Also in both sexes, deposition of crystalline material occurred in renal ducts and tubules ($p<0.01$, Dunnett's test, females) and also in pelvis. The weighted incidence of the appearance of crystalline material was 0% for both sexes at 0, 10 ppm and 25 ppm. However, at 200 and 400 ppm, for females, there was a weighted increased incidence of 15/69 (22%) at 200 ppm and 78/80 (98%) at 400 ppm ($p<0.01$, at both doses, Dunnett's test). For males the analogous figures were 5/70 (7%) at 200 ppm and 99% (79/80) at 400 ppm ($p<0.01$ at both doses). The crystals were accompanied by ductular dilatation, inflammation, or epithelial hyperplasia, along with interstitial fibrosis. An additional lesion characterized by interstitial swelling and eosinophilia was present in 200 ppm males. The incidence of chronic/progressive nephropathy, an aging change in rats, was also increased ($p<0.01$) at 200 and 400 ppm, in both sexes. Microscopic changes in the urinary bladder included fibrosis and mucosal hyperplasia at 200 and 400 ppm and urinary calculi. No oncogenic effects were present. Based on urinary system toxicity, DPR determined that the NOEL was 25 ppm (~0.97 mg/kg/day).

Rats/Mice - Diet/Gavage: Nitrosoatrazine

In a summary of a carcinogenicity study, Weisenburger *et al.*, 1990 reported that nitrosoatrazine or atrazine was administered to 250 female Swiss mice and 250 female Wistar rats. Groups of 50 animals were treated as follows: 1 - atrazine in feed at 1500 ppm for mice (~225 mg/kg/day), and 500 ppm for rats (~25 mg/kg/day); 2 - sodium nitrite in drinking water (3 g/l) *ad libitum*; 3 - atrazine plus sodium nitrite in drinking water as above; 4 - nitrosoatrazine by gavage twice weekly (65 mg/kg mice, 175 mg/kg rats); 5 - untreated. Due to "excessive" toxicity, doses of atrazine were decreased over time in both species and all treatments were discontinued at 67 weeks. At the end of the study, no significant increase in tumors was present in any of the treatment groups. The study was not acceptable to DPR under FIFRA guidelines due to non-standard protocol and lack of data.

Table 18. Summary of the Chronic Toxicity/Oncogenicity Studies of Atrazine and Metabolites.

Species/ Sex	Exposure Regimen	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
		Atrazine			
Rat (M/F) SD	2-yr. diet	**Non-neoplastic**: EMH, spleen (F) **Neoplastic:** Mammary Adenocarcinoma (F)	0.47[a/] N/A	N/A N/A	Mayhew, 1986[b/]
Rat (F) SD	2-yr. diet	**Non-neoplastic:** Fluid-filled uterus, mammary galactocele **Neoplastic:** Mammary Fibroadenoma and/or carcinoma	- N/A	4.1[c/] N/A	Thakur, 1991a[d/]
Rat (F) SD	2-yr. diet	**Non-neoplastic:** ⇓body wt. gain, ⇑mortality **Neoplastic:** Mammary Carcinoma⇑at 1-yr. at 20 mg/kg/d	3.5 N/A	20 N/A	Thakur, 1992a[d/]
Rat (M,F) F-344	2-yr. diet	**Non-neoplastic:** ⇓body wt. and body wt. gain **Neoplastic:** no effects	3.5	10	Thakur, 1992b[b/]
Rat (M/F)	2-yr. diet	No adverse effects reported at HDT	5	-	Keller, 1961[e/]
Rat (F) SD	*In utero*, 2-yr. diet	**Neoplastic:** No increase in mammary tumors at any dose	N/A	N/A	Ciba-Geigy, 1988a,b[e/]
Rat (F) SD	1-yr. diet	**Non-neoplastic:** ⇓ body wt. **Neoplastic:** mammary adenoma/ adenocarcinoma	3.5 N/A	20 N/A	Pettersen & Turnier, 1987
Rat (F)	2-yr. diet	**Non-neoplastic:**	-	>25[f/]	Weisenburger 1990
Mouse (F)	96-wk. diet	**Non-neoplastic:**	-	>225[f/]	Weisenburger 1990

a/ Probit analysis of the data (BMD approach) gave an ED_{10} of 2.57 mg/kg/day, with a lower bound of 0.471 mg/kg/day (DPR, 1990), used for regulatory purposes.
b/ Acceptable to DPR under FIFRA testing guidelines.
c/ Lowest dose tested.
d/ Supplementary.
e/ Unacceptable to DPR under FIFRA testing guidelines. N/A is not applicable.
f/ Dose lowered during study.

Table 18. Summary of the Chronic Toxicity/Oncogenicity Studies of Atrazine and Metabolites.
(continued)

Species/ Sex	Exposure Regimen	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
Mouse	18-month	No effect at highest	12[a]	-	Innes *et al.*, 1969[e]
Mouse	22-month diet	**Nonneoplastic:** ⇓ b.w.	15	150	Ciba-Geigy, 1986a
Mouse	13-month I/p	**Non-Neoplastic:** None	_	0.26[c,g]	Donna *et al.,* 1981
Mouse (F)	7-month s.c./i.p.[h]	**Non-neoplastic:** None **Neoplastic:** Lymphoma	_ N/A	.0065[g] N/A	Donna *et al.,* 1981[e]
		atrazine/simazine (mix. 25:37.5%)			
Dog (M/F)	2-year diet 80W	**Non-Neoplastic:** Lacrim. _ **Neoplastic:** None		0.045[c]	Woodard, 1964[e]
Dog (M/F)	1-year diet	**Non-Neoplastic:** EKG changes, ⇑ heart wt., atrial dilation. **Neoplastic:** None	0.48	4.97	O'Connor *et al.*, 1987[b]
		Diaminochlorotriazine			
Dog (M/F)	1-year diet	**Non-Neoplastic:** Cardiac & related toxicity **Neoplastic:** None	3.62	21.5	Thompson *et al.*, 1990[b]
		Hydroxyatrazine			
Rat (M/F) SD	2-year diet	**Non-Neoplastic**: ⇑ chronic nephropathy; bladder fibrosis and hyperplasia. **Neoplastic:** None.	1.0	7.8	Chow & Emeigh Hart, 1995.
		Nitrosoatrazine			
Rat (F) Wis.) Mouse (F)	2-year gavage 96-week	**Non-Neoplastic & Neoplastic** no effects in either species	65[g]	-	Weisenburger, 1990[e]

g/ Only dose tested. **h/** s.c. = subcutaneous, i.p. = intraperitoneal.

E. GENOTOXICITY

Summary: In studies submitted to DPR, no adverse effects were indicated for gene mutation, chromosomal aberration, or other mutagenic mechanisms. A US EPA review of published and unpublished data concluded that atrazine does not induce genotoxicity *in vitro* with or without rat liver microsomal activation, but can be converted by plant microsomes to a form that is mutagenic in prokaryotes and eukaryotes (*i.e.* a plant-activated promutagen). Generally, *in vivo* somatic and germinal cell cytogenic assays were negative, however, in one study atrazine produced bone marrow and lymphocyte chromosomal aberrations. The atrazine metabolites desisopropylatrazine, desethylatrazine, hydroxy-atrazine, and diaminochlorotriazine were not mutagens in the Ames test and were negative in micronucleus and DNA repair tests. In a literature report, the metabolite nitrosoatrazine produced gene mutation in an Ames assay when hamster liver microsomal activation was used, was positive in the Chinese hamster V79 assay (Weisenburger, 1988) and produced chromosome breakage in cultured human lymphocytes (Meisner, 1993). Published literature for atrazine has been described in IARC Monographs Vol.53 (IARC, 1991) and reviewed by Deerfield (1993). Positive results were obtained for mutation and numerical aberrations in fungi, and mutation, numerical aberrations and dominant lethality were observed in insects, exposed to atrazine. A mouse micronucleus assay with negative results was submitted to the US EPA for atrazine. While there was no available published micronucleus assay for comparison, positive results were reported in the literature for the mouse dominant lethal assay. The data from this assay were not presented in the report; only the lowest effective dose was provided. In a literature review of (37) non-plant genotoxicity studies, it was concluded that atrazine does not pose a mutagenic hazard (Brusick, 1994). In conclusion, from a human health perspective, it appears that although atrazine itself is probably not genotoxic, there are indications that plant metabolites and/or other environmental degradates may present genotoxic potential.

Gene Mutation

Only one acceptable report was submitted to DPR; atrazine technical (purity 98.3%) was used in a mutagenicity test with *Salmonella typhimurium* strains TA 98, TA100, TA1537, TA1538, with and without activation at levels of 0 (DMSO vehicle), 20, 78, 313, 1250 and 5000 μg/0.1 ml DMSO. Plates were incubated for 48 hours at 37°C. Positive controls with and without activation were included. No mutagenicity was observed (Deparade, 1986).

In a US EPA review of gene mutation assays, it was reported that three unpublished studies evaluated atrazine in the *S. typhimurium* mammalian/ microsome reverse mutation assay (Ames test) (US EPA, 1990a). Simmon *et al.*, 1977 exposed *S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100 to 6 doses of atrazine (5 to 5,000 μg/plate) in both the presence and absence of 10% Aroclor 1254-induced rat liver S9 mix. Arni and Muller (1978) used the same tester strains to evaluate an atrazine dose range of 10 to 810 μg/plate; the S9 mix in the study contained 30% liver homogenate. Utilizing a similar protocol, Sutou *et al.*, 1979, performed Ames tests on seven non-activated and S9-activated doses of atrazine (50 to 10,000 μg/ml). Although the studies were individually flawed by technical deficiencies, collectively the results indicated that at a concentration range of 10 to 5,000 μg/plate, either nonactivated or in the presence of rat S9, atrazine was not mutagenic in *S. typhimurium* TA1535, TA1538, TA98, and TA100. Sutou *et al.*, 1979

also found atrazine (50 to 5,000 μg/plate) to be nonmutagenic with or without S9 activation in a reverse mutation *E. coli* WP2 *hcr* assay. Results from *in vitro* bacterial assays summarized by Adler (1980) confirmed the absence of mutagenicity.

Singh *et al.*, 1982 exposed maize kernels to 0, 30, 60, or 90 ppm Aatrex (80% atrazine) and allowed them to germinate; plants were harvested and extracts prepared. The extracts caused increased gene conversion in *Saccharomyces cerevisiae* at the adenine (ade 2) and tryptophan (trp 5) loci; however the effect was not pronounced. The dose causing the greatest response (60 ppm) yielded 2.7 and 2.2-fold increases in conversion frequencies at the ade 2 and trp 5 loci, respectively. The authors demonstrated clear dose-related responses at both loci with a methanol extract of atrazine in the same protocol. The 30, 60 and 90 ppm methanol extracts increased gene conversion 2.4, 2.5 and 3.4-fold at the ade 2 locus and 1.7, 2.4 and 4.2 fold at the trp 5 locus.

Plewa *et al.*, 1976 provided similar evidence showing that extracts of maize exposed to 15, 20 and 25 ppm atrazine induced gene conversion at the ade 2 and trp 5 loci in *S. cerevisiae* D4. Using a different plant metabolic activation system (*Nicotiana alata*) and a higher eukaryote, Benigni *et al.*, 1979 demonstrated a marked increase in mutation and somatic segregation following exposure of *Aspergillus nidulans* to an unreported concentration of atrazine. In the presence of mammalian metabolism, *in vitro*, it was reported (Adler, 1980) that atrazine was not mutagenic in yeast or fungi. However, in the presence of potato microsomes, mutagenic activity was observed in *A. nidulans* and two yeast species (Adler, 1980). A commercial grade of atrazine, or atrazine combined with metolachlor or Eradicane,® assayed *in situ* with emerging maize seedlings, induced mutations at the wx locus in *Zea mays* (Plewa *et al.*, 1984). The same authors presented data indicating that *in vitro* plant-activated extracts from corn treated with 1.66×10^{-5} M metolachlor/1.37×10^{-4} atrazine were mutagenic in *S. typhimurium* TA98. The study was compromised by the unusually high negative control value and the weak effect (2.3-fold increase) at the highest reactive dose, therefore, the results were considered inconclusive.

Several *in vivo* gene mutation assays were also available for review. Simmon *et al.*, 1977 used acute (six doses ranging from 500 to 2,200 mg/kg) or subacute (five consecutive daily doses of 275 to 1,100 mg/kg) oral administration of atrazine to male mice and induced a cytotoxic but not mutagenic response. The well-controlled study assayed atrazine to a level demonstrating cytotoxicity but no mutagenicity was evident. In contrast, atrazine (100 to 600 mg/kg) administered orally to mice induced a mutagenic response in *E. coli* (increase in ampicillin-resistant mutants). Although the results of the study were initially presented as an abstract by Solt *et al.*, 1980 and subsequently published by Adler (1980), neither author indicated that the effect was dose-dependent, or had been confirmed. Also reported by Adler were negative results for a series of *S. typhimurium*/ mouse host-mediated assays with 1,000 mg/kg atrazine.

Murnik *et al.*, 1977 presented data showing a significant ($p<0.05$) increase in the rate of lethal mutations in *Drosophila melanogaster* (adult) after feeding 0.01% atrazine to the larvae. Since the authors reported that atrazine was fed throughout the larval stage, it was assumed that the offspring scored for mutations represented germ cells in the early stages of spermatogenesis. The same authors found that 0.012% atrazine injected into adult males did not increase lethal mutations in germ cells sampled at intervals timed to correspond with different stages of spermatogenesis. However the number of

chromosomes examined from later broods, arising from spermatids and spermatogonia at treatment, was too small to provide confidence in the results. Adler (1980) listed atrazine (5 to 10 mg/kg) as negative in that system. The available data were insufficient to refute the larval study results, however, and so the relevance of the finding is therefore uncertain.

In test systems involving germinal cells, Adler (1980) reported an induction by atrazine of dominant lethal mutations in mouse spermatids; no further details were provided. An increase in preimplantation loss, 4 weeks after treatment of male mice with 6 mg/kg atrazine, was reported in an abstract by Chollet *et al.*, 1982. The more critical index of dominant lethality, postimplantation loss, was not increased. At the same dose level, no adverse effects were noted in spermatogonia and spermatocyte metaphases collected from mice treated acutely or subchronically. In a mouse dominant lethal assay, a dose of 444 or 1,332 mg/kg by gavage was not clastogenic (Hool *et al.*, 1981); however the US EPA believed that a MTD was not achieved. Although not specifically a measurement of clastogenicity, the mouse-sperm morphology assay of Osterloh *et al.*, 1983 demonstrated that atrazine has no genotoxic effect on spermatogenesis. The assay provided clear evidence of toxicity (no animals survived five consecutive daily intraperitoneal injections of 1,200 or 2,400 mg/kg/day), but no testicular cytotoxic or genotoxic effects were observed in animals dosed with 600 mg/kg/day, all of which survived.

In a literature report (Venkat *et al.*, 1995), the PQ37 strain of *E. coli* was used with 47 pesticides in a SOS microplate assay, both in DMSO (10%) and in taurocholate solution. The latter simulated conditions in the small intestine. Mutagenicity was assessed as the potency of induction of the gene for β-galactosidase compared with the standard mutagen, 4-nitroquinoline oxide. Atrazine was inactive in both solutions, as also were DEA and simazine. However, cyanazine was the most potent mutagen in the DMSO solution, but it ranked only 37$^{th.}$ when assayed in taurocholate, and ametryn, the S-methyl analog of atrazine, was 9$^{th.}$ in DMSO solution.

The overall conclusions drawn from the gene mutation studies were that atrazine probably does not induce gene mutations in the presence or absence of exogenous metabolic activation derived from mammals. However, plant microsomes may be able to convert atrazine to a reactive form(s) that is mutagenic in bacteria, yeast, and fungi. The observations are consistent with the assessment that atrazine is a plant-activated promutagen.

The atrazine metabolites desisopropylatrazine, desethylatrazine, diaminochloro-triazine, and hydroxyatrazine, were non-mutagenic in the Ames test (Ciba-Geigy, 1992b,c). However, nitrosoatrazine was mutagenic in a modified Ames assay using hamster liver microsomal activation and positive in a Chinese hamster V79 assay (Weisenburger, 1988). The reports on the effects of atrazine on gene mutation are summarized in Table 19.

Structural Chromosome Aberrations

In the mouse dominant lethal test, atrazine technical (purity 98.9%) was administered as a single oral gavage dose at 0, 444 and 1332 mg/kg to 20 male Tif.MAGf(SPF) mice/group. Six hours after treatment the males were mated to untreated females. At the end of one week the females were removed from the cages and replaced by other untreated females.

This procedure was repeated for 6 consecutive weeks. The females were autopsied on day 14 of gestation. No effects on mating ratio or resorptions were observed at any treatment level (Hool, *et al.*, 1981).

In the US EPA review of chromosome aberration assays (US EPA, 1990b), it was reported that in test systems involving somatic cells, no acceptable chromosome aberration studies were found (but see Hertner, 1993, below). Adler (1980) reported that an oral dose of 2,000 mg/kg produced a significant increase in the frequency of chromatid breaks in mouse bone marrow cells. Loprino *et al.*, 1980 found similar evidence that 1 and 2 g atrazine elicited clastogenic responses in mouse bone marrow cells; no further information was furnished.

Meisner *et al.*, 1992 reported that 20 ppm atrazine in the drinking water of mice produced bone marrow chromosomal aberrations and 1 μg/ml produced chromosomal aberrations in human lymphocyte cultures. Nitrosoatrazine also produced chromosome breakage and an increased mitotic index in human lymphocyte cultures (Meisner *et al.*, 1993).

In contrast, Chollet *et al.*, 1982 reported that the intraperitoneal administration of 6 mg/kg atrazine did not increase the incidence of chromosome aberrations in mouse bone marrow metaphases sampled over the entire cell cycle; the results, presented in abstract, were not detailed. Atrazine did not increase the frequency of micronucleus induction in male or female Chinese hamsters administered oral doses equivalent to 282, 564, or 1,128 mg/kg (Hook *et al.*, 1981). The sensitivity of the assay to detect clastogenic activity at different stages in the cell cycle was, however, questionable. Brusick (1987) reported that a mouse micronucleus assay with atrazine administered at 2,000 mg/kg by oral gavage was negative; however, no further details were provided.

The US EPA stated that no definitive conclusions could be reached regarding the potential of atrazine to induce clastogenic effects in somatic or germinal cells. Although the majority of the studies were negative, they did not offset the positive results for two reasons: (1) the Office of Pesticides Programs (OPP) US EPA considered many of the *in vivo* studies to be scientifically unacceptable; and (2) the negative *in vivo* studies either assayed low doses of atrazine or used less sensitive endpoints (*i.e.* micronucleus assay). As previously mentioned, the US EPA required a repeat dominant lethal study in male mice (US EPA, 1990a). DPR found this repeat study to be acceptable (Hertner, 1993). Atrazine (G 30027; 97.1% pure) was given by gavage at 500, 1,000, 2,000 or 2,400 mg/kg to 30 mice/group and was found not to induce dominant lethal mutations. The NOEL for chromosomal aberrations in this study was thus 2,400 mg/kg.

Chromosome damage was reported as increased clastogenicity caused by triazines, using flow cytometric analysis in CHO cells (Biradar & Rayburn, 1995a,b; Taets *et al.*, 1998). The measures of clastogenicity were twofold: the CV (coefficient of variation) of the G1 peak of whole cells and the change in CV of the largest chromosome in the flow karyotype. The increase in CV is due to chromosomal breakage causing an uneven distribution of DNA in daughter cells. In the most recent publication (Taets *et al.*, 1998), the triazines atrazine, cyanazine and simazine were tested alone and in combination, at two concentrations: the MCL and the highest concentration detected in Illinois water supplies. These concentrations were 3 ppb and 18 ppb for atrazine, which served as the positive control based on the earlier work (Biradar & Rayburn, 1995a,b). Alone, simazine caused a significant effect in the whole cell assay, at both concentrations, unlike in the earlier work

where simazine failed to cause clastogenicity at the MCL, but it did not have an effect in the chromosome assay. Cyanazine did not have an effect in either assay. No synergy was observed with any of the combinations. Instead, when all three triazines were tested together, the degree of clastogenicity was significantly increased only for the whole cell assay at the MCL, to about the same level as for atrazine alone. In each of the other assays (whole cell at higher concentrations, chromosome test at both concentrations) there was no significant clastogenicity *i.e.* less effect than atrazine alone. The study also demonstrated that, for atrazine alone, increased clastogenicity was reported ($p<0.01$) in the whole-cell assay at both 3 ppb and 18 ppb; in the chromosome assay, a significant increase ($p<0.01$) was found at 3 ppb but a significant decrease ($p<0.1$) in CV of the largest chromosome was reported at 18 ppb. Thus, taken together, the demonstration of a clastogenic effect for atrazine or simazine, is unconvincing.

In a micronucleus test, negative results were obtained for the atrazine metabolites desisopropylatrazine (Ciba-Geigy, 1991a), desethylatrazine (Ciba-Geigy, 1991b), diamino-chlorotriazine and hydroxyatrazine, (Ciba-Geigy, 1992c). The reports on the effects of atrazine on structural chromosome aberrations are summarized in Table 20.

Other Genotoxic Effects

In the US EPA review of assays for other genotoxic mechanisms (US EPA, 1990), it was reported that in a test system involving DNA damage/repair in bacteria, six concentrations of atrazine (ranging from 100 to 10,000 μg/well) did not cause preferential inhibition of growth of repair-deficient *B. subtilis* M45 without activation when compared to the parental and repair-competent H17 strain (Sutou *et al.*, 1979). The concentrations evaluated in the assay were considered acceptable in the absence of a positive response; however, the lack of exogenous metabolic activation rendered the study incomplete.

Two unpublished unscheduled DNA synthesis assays (rat hepatocyte and human fibroblast) reported by Puri *et al.*, 1984a,b were considered by the US EPA to have technical flaws which prevented use of the data and required additional testing (US EPA, 1990b) An upgraded version of the rat hepatocyte report was submitted to DPR, and considered acceptable. In that assay atrazine technical (98.2% purity) was incubated with rat hepatocytes *in vitro* at 1.2, 6, 30 or 150 μg/ml medium (4 cultures/group) for 5 hours. No adverse effects were observed at any dose.

A sub-chronic study was conducted of possible cytogenetic damage in (female) mice and (male) rats resulting from the consumption of contaminated drinking water (Kligerman *et al.*, 1993). The solution, which was designed to mimic California drinking water, and which contained atrazine at 0.5 (1-X), 5 or 50 ppb, along with six other pesticides and fertilizers, resulted in a concentration-dependent increase in sister-chromatid exchanges in splenocytes of the male Fischer rat; the female B6C3F1 mouse showed the effect only at 50 ppb. However, because drinking water designed to mimic Iowa water did not cause this effect, despite containing the same concentrations of atrazine, it is unlikely that it was the cause of the genetic toxicity of the California water (Kligerman *et al.*, 1993).

In DNA repair tests, negative results were obtained for the atrazine metabolites desisopropylatrazine (Ciba-Geigy, 1991c), desethylatrazine (Ciba-Geigy, 1991d), diaminochlorotriazine and hydroxyatrazine, (Ciba-Geigy, 1992c).

Aneuploidy has been reported with atrazine, as follows: mitosis/meiosis systems in a higher plant, grain sorghum (Liang *et al.*, 1969) and a meiotic system in *Neurospora* (Griffiths *et al.*, 1985). Negative results were obtained in a meiotic system in *Drosophila* (Liang and Brinkley, 1985). There are few validated mammalian systems available to measure aneuploidy directly in mammals. The reports on the effects of atrazine on DNA damage/repair are summarized in Table 21.

Table 19. The Effects of Atrazine and Metabolites on Gene Mutation.

Test Type/System[a/]	Strain	Dose	S9[b/]	Result	Comments/(Ref.)
		Atrazine			
S.typhimurium	TA98,TA100,TA 1537,TA1538	20 to 5000μg /0.1ml DMSO	±	Neg	Acceptable - DPR (Deparade, 1986)
S.typhimurium	TA98,TA100,TA 1535,TA1537TA 1538	5-5,000 μg/plate	±	Neg	Minor technical flaws; Acceptable - US EPA, (Simon, 1977)
S.typhimurium	TA98,TA100,TA 1535,TA1537TA 1538	10-810 μg/plate	±	Neg	Minor technical flaws; Acceptable - US EPA, (Arni & Muller, 1978)
S.typhimurium	TA98,TA100,TA 1535,TA1537TA 1538	50-10,000 μg /plate	±	Neg	Minor technical flaws; Acceptable - US EPA, (Sutou, 1979)
S.typhimurium	TA98,TA100	Not reported	±	Neg	Lack of data - deficient, (Weisenburger, 1988)
S.typhimurium	TA98	Extract from corn dosed w/ atrazine +metolachlor (see text)	±	Pos	Inconclusive single dose, weak response, technically deficient - US EPA, (Plewa, 1984)
E. coli	WP2	50-50,000 μg /plate	-	Neg	Acceptable US EPA (Sutou, 1979) Minor flaws
E. coli	PQ37	Not reported	±[c/]	Neg	(Venkat *et al.*, 1995)
S. cerevisiae	D4	Water-soluble extracts of maize exposed to 30,60, or 90 ppm AAtrex	-	Pos	No dose-response; Inconclusive - US EPA, (Singh, 1982).

a/ *S. typhimurium* = *Salmonella typhimurium*; *E. coli* = *Escherichia coli*; *S. cerevisiae* = *Saccharomyces cerevisiae*
b/ Rat S9 fraction used for activation, except for Weisenburger, 1988, with hamster S9.
c/ SOS repair system: pesticides in DMSO or taurocholate solution (bile salt micelle).

Brusick (1994) has reviewed a multitude of (37) publications and reports (non-plant) on atrazine, of which five were positive, using two approaches. One was the "expert judgement" in which conflicts were resolved by thoroughly reviewing each study and critically assessing the detailed data. The second approach used a computer assisted "weight-of-the-evidence" method. The conclusions reached about the genotoxicity of atrazine were "equivocal" using the first method and "negative" using the second. The weight-of-the-evidence (computer) model, which was originally developed by ICPEMC, was considered by the author to be more relevant for assessing atrazine using such a large database.

Table 19. The Effects of Atrazine and Metabolites on Gene Mutation (continued)

Test Type/System[a/]	Strain	Dose of Atrazine	Result	Comments/(Reference)
S. cerevisiae	D4	Methanol extracts of above	Pos	Dose response; Acceptable - US EPA (Singh, 1982)
S. cerevisiae	D4	Extracts of maize dosed with15,20, or 25 ppm	Pos	Dose response; Acceptable - US EPA (Plewa, 1976)
A. nidulans	-	Unreported dose and plant metabolic activation.	Pos	Supportive result; Insufficient data- US EPA, (Benigni, 1979)
A. nidulans	-	Not reported	Pos	Adler, 1980 (review)
Z. mays	-	In situ treatment of corn w/ atrazine or atrazine+metolachlor (Eradicane)	Pos	Results confirmed; Acceptable - US EPA, (Plewa, 1984)
Mouse (male)/*S. typhimurium*	TA1535,TA1538	Six oral acute doses (500-2,200 mg/kg)	Neg	Acceptable - US EPA; assayed to a cytotoxic level; Simon, 1977)
		Subacute oral doses (275-1,100 mg/kg/d 5 days)	Neg	Acceptable - US EPA; assayed to a cytotoxic level, (Simon, 1977)
Mouse (male)/ *E. coli*	-	Oral administration (100-600 mg/kg)	Pos	Supportive result - US EPA; Abst.,(Solt,1980)
D.melanogaster	-	Larval feeding	Pos	Acceptable-US EPA; (Murnik,1977)
		0.012% by injection to adults	Neg	Unacceptable - US EPA; insufficient sample size.
Chinese hamster	V79	100 μg/ml	Pos	Weisenburger, 1988.

a/ *S. typhimurium* = *Salmonella typhimurium*; *S. cerevisiae* = *Saccharomyces cerevisiae* ; *D. melanogaster* = Drosophila *melanogaster*; *Z. mays* = *Zea mays*; *A. nidulans* = *Aspergillus nidulans*; *E. Coli* = *Escherichia coli*.

Table 19. The Effects of Atrazine and Metabolites on Gene Mutation (continued)

Test Type/System[a/]	Strain	Dose	Result	Comments/(Reference)
		Nitrosoatrazine		
S. typhimurium	TA98,TA100	50-1000 μg/plate	Pos	Hamster S9 activation; (Weisenburger, 1990).
		Desisopropylatrazine		
S. typhimurium *E. coli*	TA98, 100, 1535, 1537 WP2uvrA	3.13-50 mg/ml	Neg	Ciba-Geigy, 1990
		Desethylatrazine		
S. typhimurium *E. coli*	TA98, 100, 1535, 1537 WP2uvrA	3.13-50 mg/ml	Neg	Ciba-Geigy, 1989
		Diaminochlorotriazine		
S. typhimurium	Not reported	Not reported	Neg	Ciba-Geigy, 1992c
		Hydroxyatrazine		
S. typhimurium	Not reported	Not reported	Neg	Ciba-Geigy, 1992c

a/ *S. typhimurium* = *Salmonella typhimurium*; *E. Coli* = *Escherichia coli*.

Table 20. The Effects of Atrazine and Metabolites on Structural Chromosomal Aberrations.

Test Type/System	Strain	Dose	Result	Comments/(Reference)
		Atrazine		
GERMINAL CELLS				
Dominant Lethal Mouse	Tif.MAGf(SPF)	444, 1332 mg/kg	Neg	Acceptable - DPR; Unacceptable - US EPA; MTD not tested (Hool, '81)
Dominant Lethal Mouse	-	1,500-2,000 mg/kg	Pos	To be repeated - US EPA; abstract; (Adler, 1980)
Dominant Lethal Mouse	-	6 mg/kg	Neg	Supportive result - US EPA; abstract; (Chollet, 1982)
Dominant Lethal Mouse	Tif:Magf(SPF)	500-2,400 mg/kg	Neg	Acceptable (Ciba-Geigy, 1993)
Sperm Morphology Mouse	-	600-2,400 mg/kg, 5 days	Neg	Acceptable - US EPA; Assayed to a toxic level; (Osterloh, 1983)
Clastogenicity CHO cells	-	3 ppb and 18 ppb	Neg./ Pos.	Biradar & Rayburn, 1995a,b Taets *et al.*, 1998
SOMATIC CELLS				
Chinese Hamster Micronucleus	-	282, 564, 1128 mg/kg	Neg	Inconclusive - US EPA; Entire cell cycle not assayed; (Hook, 1981)
Mouse Bone Marrow	-	1 & 2 g/kg	Pos	Supportive result - US EPA; abstract; (Loprieno, 1980)
Mouse Bone Marrow	-	2 g/kg	Pos	Adler, 1980
Mouse Bone Marrow	$B6C3F_1$	20 ppm, drinking water	Pos	(Meisner, 1992)

Table 20. (continued) The Effects of Atrazine and Metabolites on Structural Chromosomal Aberrations.

Test Type/System	Strain	Dose	Results	Comments/(Reference)
		Atrazine (continued)		
SOMATIC CELLS (continued)				
Mouse Bone	-	6 mg/kg	Neg	Supportive - US EPA;
Mouse Bone Marrow	-	2000 mg/kg	Neg	Insufficient data;(Brusick, 1987)
		Nitrosoatrazine		
Human Lymphocyte	-	0.0001-5.0 μg/ml	Pos	Meisner, 1993
		Diaminochlorotriazine		
Micronucleus	Not reported	Not reported	Neg	Ciba-Geigy, 1992c
		Desisopropylatrazine		
Micronucleus	Tif: MAGf(SpF)	120-480 mg/kg	Neg	Ciba-Geigy, 1991a
		Desethylatrazine		
Micronucleus	Tif: MAGf(SpF)	120-480 mg/kg	Neg	Ciba-Geigy, 1991b
		Hydroxyatrazine		
Micronucleus	Not reported	Not reported	Neg	Ciba-Geigy, 1992c

Table 21. The Effects of Atrazine and Metabolites on DNA Damage/Repair.

Test Type/System	Strain	Dose	Result	Comments/ (Reference)
		Atrazine		
DNA damage/repair *Bacillus subtilis*	M45, H17	100 - 10,000 μg/well	Neg	Acceptable-US EPA; no S9 (Soutou, 79)
DNA repair rat hepatocytes	Tif:RAlf (SPF)	15.5 - 1,670 μg/ml	Neg	Acceptable (Hertner, 1992)
UDS rat hepatocytes	_	1.2 - 150 μg/ml	Neg	Acceptable - DPR Unacceptable - USEPA, Puri, 1984a
		Diaminochlorotriazine		
DNA Repair	not reported	Not reported	Neg	Ciba-Geigy, 1992c
		Hydroxyatrazine		
DNA Repair	Not reported	Not reported	Neg	Ciba-Geigy, 1992c
		Desisopropylatrazine		
DNA Repair (rat hepatocyte)	Tif:RAlf (SPF), male	7.4 - 800 μg/ml	Neg	Ciba-Geigy, 1991c
		Desethylatrazine		
DNA Repair (rat hepatocyte)	Tif:RAlf (SPF), male	9.25 - 1,000 μg/ml	Neg	Ciba-Geigy, 1991d

F. REPRODUCTIVE TOXICITY

Summary: In an acceptable FIFRA-guideline reproductive toxicity study, decreased pup weight and maternal toxicity were reported. Parental food intake, body weight and body weight gain were all reduced with a systemic LOEL of 25 mg/kg/day and a NOEL of 2.5 mg/kg/day. No signs of reproductive toxicity were observed, even at the HDT, giving a reproductive NOEL ≥25 mg/kg/day. A summary of the NOEL/LOEL information is listed in Table 23.

Dietary-Rat

In a two-generation reproductive toxicity study, atrazine technical (97.6% purity) was administered to 30 CRCD, VAF/PLUS rats/sex/group in the diet at 0, 10, 50, or 500 ppm (~0, 0.5, 2.5, or 25 mg/kg/day, based on default values) continuously for 2 parental generations of animals and their offspring throughout all phases of the study (Mainiero *et al.*, 1987). Significantly decreased body weight, body weight gain and food consumption was present in parental animals of both sexes at 500 ppm. For example, mean body weight of F_1 parental males during the premating and postmating periods was 86% of control and in F_1 females during premating, gestation and lactation, body weight averaged 89% of control. A statistically significant increase in relative testis weight was seen in both parental generations, but was believed to be secondary to the decreased body weights. Decreased postnatal F_2 pup body weight was present in all treated male groups at birth (3-6%) and continued throughout the observation period, reaching statistical significance at day 21 (7-10%). U.S. EPA noted this effect at ≥50 ppm at 21 days. It was suggested by the study authors that the weight decrease was due to pup consumption of the maternal diet. Although no data were submitted to support this suggestion, DPR considered that it probably had some validity: pup weight loss was not noted at 14 days, when exposure would have been predominantly *via* the dams' milk, but only at 21 days, when consumption of the diet and dams' milk would have resulted in an 'overdose' for pups, on a mg/kg basis. Pup weight loss was therefore not considered toxicologically relevant as an adverse reproductive effect. Based on decreased body weight, body weight gain and food consumption, the parental NOEL was 50 ppm (2.5 mg/kg/day). The reproductive NOEL was ≥500 ppm (25 mg/kg/day), based on a lack of reproductive toxicity at this dose. The study was acceptable to DPR under FIFRA testing guidelines.

In a 3-generation reproductive toxicity study (Hollingsworth *et al.*, 1966), Atrazine 80W (80% atrazine) was administered to 10 male and 20 female Charles River albino rats/dose in the diet at 0, 50, or 100 ppm (~0, 2.5, or 5.0 mg/kg/day). No parental or reproductive effects were reported. The study was not acceptable to DPR due to numerous deviations from FIFRA guidelines.

G. DEVELOPMENTAL TOXICITY

Summary: The developmental toxicity of atrazine and metabolites was studied in rats and rabbits. Delayed skeletal ossification and maternal toxicity were present in both species. The LOEL for acute rabbit maternal toxicity (blood on vulva/cage, increased abortions, body weight loss and abnormal stools) and developmental toxicity (increased resorptions, decreased viable fetuses, post implantation loss, decreased body weights and delayed ossification) was 75 mg/kg/day and the NOEL, 5 mg/kg/day. This study was used to assess risks associated with acute exposure to atrazine. In the rat, loss of body weight and delayed or incomplete ossification were also reported with a LOEL of 100 mg/kg/day and a NOEL of 25 mg/kg/day. There was a single case of cryptophthalmia at the HDT. Four metabolites of atrazine were also tested in the rat, all causing similar effects to atrazine. DEA had LOEL/NOEL values of 100 and 25 mg/kg/day, respectively; DIPA of 25 and 5 mg/kg/day; DACT of 75 and 25 mg/kg/day (maternal) and 25 and 2.5 mg/kg/day (developmental); hydroxy-atrazine of 125 and 25 mg/kg/day. In addition, in the latter case, two litters presented cases of malformations at the HDT, gastroschisis and umbilical hernia. A summary of the developmental NOELs/LOELs is given in Table 23.

Gavage-Rat

In a rat teratology study, 26 female Crl:COBS CD(SD)BR rats/dose received atrazine technical (97.6% purity) as a 0, 0.05, 0.25 or 1.00% suspension (~0, 5, 25 or 100 mg/kg/day) in 3% aqueous cornstarch containing 0.5% Tween 80 via gavage on gestation days 6-15 (Giknis, 1989). This study is based on the one of Infurna, 1984 (below). Clinical signs of post-dosing salivation were present at 100 mg/kg/day in 18/26 (69%) of dams *vs.* 0% in all other groups. Decreased food consumption (averaging 13% below control, 6-16 days, $p<0.05$), body weight (averaging 5-6% below control, days 6-16, $p<0.05$) and body weight gain (*e.g.* on days 6-8, a mean weight loss of 3.9 g/dam *vs.* a mean weight gain of 7.5 g/dam in controls, $p<0.05$) were also present at that dose level. The incidence of delayed fetal ossification was increased in all atrazine-treated groups. At 5 mg/kg/day and above, a statistically significant, 3-5 fold, dose-related increase in incomplete ossification of the interparietal bones of the skull was detected. The numbers of litters exhibiting this variation were: 10/26 (38%) control, 15/25 (60%) at 5 mg/kg/day, 14/24 (58%) at 25 mg/kg/day and 20/21 (95%) at 100 mg/kg/day, $p<0.05$ at all dosages. Because of the high level in control rats (38%), it was concluded that this effect was toxicologically significant only at 100 mg/kg/day. Also at this dose, significant increases in incomplete ossification of the hyoid, occipital and parietal bones of the skull were present, without a significant reduction in fetal body weight. Based on salivation, decreased food consumption, body weight and body weight gain, the maternal NOEL was 25 mg/kg/day. Based on incomplete and delayed ossification, the developmental NOEL was also 25 mg/kg/day. The study was acceptable to DPR under FIFRA testing guidelines.

Gavage-Rat

In another rat teratology study, 27 pregnant female [Crl. COBS CD(SD)BR] rats/dose received atrazine technical (96.7% purity) as a 0, 0.10, 0.70, or 7.00% suspension (~0, 10, 70 or 700 mg/kg/day) in 3% aqueous cornstarch containing 0.5% Tween 80 via gavage on gestation days 6-15 (Infurna, 1984). Mortality occurred in 21/27 animals at 700 mg/kg/day. Decreased food consumption, body weight, and body weight gain were present at 70 and 700 mg/kg/day, although the effects at 70 mg/kg/day occurred only briefly at the beginning of dosing. The

reduction in body weight gain was 46% on days 1 and 2 of dosing (p<0.01) and 13% over days 0 to 20 (p<0.01). Clinical signs of toxicity were also present at 700 mg/kg/day and included salivation (13/27 dams), ptosis (11/27), swollen abdomen(8/27), oral/nasal discharge (12/27) and bloody vulva (7/27) and mortality at this dose was 81% (22/27). Necropsy changes were recorded only in the 700 mg/kg/day group and included enlarged stomachs and adrenals (12/27) and discolored lungs (3/27). Fetal size and weight were reduced by ~45% (p<0.01) in the 700 mg/kg/day group. Increased incidences of fetal skeletal and visceral variants were present in all atrazine-treated groups. For example, skeletal variations which were significantly elevated at 70 mg/kg/day compared with 10 mg/kg/day or concurrent control included unossified teeth in 10/25 litters *vs.* 4/23 and 1/23 (p<0.01) at the three doses, respectively; unossified forepaw metacarpals (p<0.05) bipartite forepaw metacarpals (3/25 *vs.* 0/23 and 0/23, p<0.05) and unossified distal phalanges of the hindpaw (3/25 *vs.* 0/23 and 0/25, p<0.05); unossified hyoid (p<0.05); incompletely ossified skull (p<0.01). Based on mortality, significantly decreased body weight, body weight gain, and food consumption, the maternal NOEL was 70 mg/kg/day. Based on decreased fetal body weight and size, the developmental NOEL was also 70 mg/kg/day. It is possible that the increased skeletal variations at 70 mg/kg/day were secondary to the maternal body weight loss. This study was not acceptable to DPR, according to FIFRA guidelines, due to excessive maternal mortality and incomplete fetal examination.

Gavage-Rat

In a summary of a rat teratology study it was reported that pregnant rats received atrazine (G 30 027) at 0, 100, 500 or 1000 mg/kg day during days 6-15 of gestation (Fritz, 1971). Treatment-related effects were present in dams and fetuses at 500 and 1000 mg/kg/day. Maternal toxicity consisted of mortality at 1000 mg/kg/day, and decreased weight gain at 500 and 1000 mg/kg/day. Necropsy observations included stomach ulceration. Fetal toxicity included an increased incidence of embryonic and fetal deaths, decreased body weight, and retarded skeletal development. The maternal and developmental NOELs were both considered to be 100 mg/kg/day. The study was not acceptable to DPR due to variances from FIFRA guidelines including lack of individual data, inadequate characterization of dosing material, and excessive mortality.

Gavage-Rabbit

In a rabbit teratology study, 19 pregnant New Zealand White rabbits/dose received 0, 1, 5, or 75 mg/kg/day atrazine technical (96.8%) suspended in 3% corn starch containing 0.5% Tween 80, via gavage on gestation days 7-19 (Arthur, 1984). At 75 mg/kg/day, blood on vulva or cage (in 4/19 dams), abnormal stools, abortion and decreased locomotor activity were present (Table 22). At 5 mg/kg/day, maternal toxicity included abortion (1/19 dams) on day 21, along with decreases in food consumption, body weight gain and body weight (all significant at p<0.05). Because maternal food consumption and body weight gain were significantly reduced only after 7 to 12 daily administrations of atrazine, it is inappropriate to consider them as acute effects. Fetal toxicity was present at 75 mg/kg/day and included increased incidences of resorptions, decreased numbers of viable fetuses, post implantation loss, decreased body weights and delayed ossification (all significant at p<0.01). There was a single case of ablepharia (no eyelids) or cryptophthalmia at 75 mg/kg/day, in 1/15 litters. In order to assess the possibility that this was secondary to maternal toxicity, the individual dam was considered, as follows: body weight loss of 566 g, 7 to 14 days, which covers the 8 to 12 day period of eye development in the rabbit (Edwards, 1968), compared with the group mean loss of 522 g, along with abnormal stools, alopecia and vasodilation of the ears. None of the other maternal signs, listed above, was observed for this individual. Thus, this dam was not as severely affected as the other dams at this dose level. The maternal and developmental NOEL values were established at 5 mg/kg/day based on the effects described. US EPA originally considered a

maternal NOEL of 1 mg/kg/day to be the most appropriate for assessing the effects of potential human acute atrazine exposures by (US EPA 1990b). However, the study was re-evaluated and the maternal and developmental NOEL values were changed to 5 mg/kg/day (US EPA, IRIS, 1997). The study was acceptable to DPR based on FIFRA testing guidelines.

Table 22. Incidence of Maternal and Developmental Toxicity after atrazine dosing in the Rabbit.[a/]

Effect	Dosage, mg/kg/day [b/]			
	0	1	5	75
Maternal Toxicity				
Abortions	0/19	0/19	1/19 [d/]	2/19 [e/]
Blood on vulva/cage	0/19	1/19	0/19	4/19*
Abnormal stools	9/19	4/19	10/19	19/19**
Decreased activity	0/19	0/19	0/19	1/19
Food consumption 17d [c/]	182±10(12)	157±14 (11)	134±15*(14)	6±2**(15)
Food consumption 19d [c/]	164±9 (14)	136±10*(11)	129±12* (14)	14±7**(14)
Body wt. gain 14-19d [c/]	120±15(16) [g/]	105±17 (14) [h/]	45±23* (15) [l/]	-204±26** (16) [j/]
Mean body wt. 14d (g)	4151±19	4049±93	4083±47	3659±88*
Mean body wt. 19d (g)	4270±100	4153±104	4128±43	3454±84*
Developmental Toxicity (incidence per litter or % of litters)				
Mean resorptions	1.3	1.4	1.4	4.8**
Mean live fetuses	8.8	8.9	9.1	5.9**
Post-implant. loss (%)	12.0	11.4	13.0	42.6**
Mean fetal wt.(g) M	46.1	44.0	43.2	35.7**
Mean fetal wt.(g) F	44.0	43.3	43.1	35.8**
Unoss. phalanx f/paw	0/16	0/14	1/15 [f/]	7/15**
Unoss. phalanx h/paw	0/16	0/14	1/15 [f/]	4/15*
Unoss. patella h/paw	5/16	4/14	5/15	10/15**

a/ data from Arthur, 1984
b/ dosed on days 7 - 19 of gestation
c/ mean food eaten in g/rabbit (no. of dams)
d/ abortion occurred on day 21
e/ abortions occurred on day 20 and 25
f/ same litter had 1 fetus with forepaw and hindpaw unossified phalanges and another fetus with unossified hindpaw phalanges (n=9 in litter).
g/ 3 dams not pregnant
h/ 2 dams not pregnant; 3 dams found dead (intubation accidents)
l/ 3 dams not pregnant; 1 dam aborted
j/ 1 dam not pregnant; 2 dams aborted
* significantly different from control, $p<0.05$ (Fisher's exact test)
** significantly different from control, $p<0.01$ (Fisher's exact test)

Atrazine Metabolites

Gavage-Rat: desethylatrazine (DEA)

Desethylatrazine (97.5%) was administered to pregnant rats (24/dose) at 0, 5, 25 or 100 mg/kg/day by gavage on days 6 to 15 of gestation. Decreased maternal food consumption and mean body weight, hunched posture (one dam), increased ($p<0.01$) fetal incidence of fused sternebrae (1 and 2) and delayed skeletal ossification ($p<0.05$) were present at 100 mg/kg/day. No teratogenicity was noted. The NOEL for developmental and maternal toxicity was 25 mg/kg/day (Ciba-Geigy, 1992f).

Gavage-Rat: desisopropylatrazine (DIPA)

Desisopropylatrazine (97.4%) was administered to pregnant rats (24/dose) at 0, 5, 25 or 100 mg/kg/day by gavage on days 6 to 15 of gestation. Decreased maternal food consumption (30%, $p<0.01$) and mean maternal body weight each day during the dosing period of $\leq$8.1% ($p<0.02$), was noted at the highest dose. At 25 mg/kg/day, reduced, mean maternal body weight ($p<0.05$) was noted on days 9 and 13 only, of 3% and 4%,respectively and was associated with a 8.7% fall ($p<0.01$) in food consumption during the 6-11 day period. Developmental effects included an increased incidence ($p<0.01$) of fused sternebrae (1 and 2) at 25 and 100 mg/kg/day. This showed a clear dose response in terms of the percentage of litters affected of 0, 0, 25% and 67% in control and dosed rats, in ascending order. Other ossification delays were noted at the HDT only. NOELs for developmental and maternal toxicity were 5 mg/kg/day and 25 mg/kg/day, respectively (Ciba-Geigy, 1992g).

Gavage-Rat: diaminochlorotriazine (DACT)

Diaminochlorotriazine (98.1%) was administered by gavage in aqueous 3% cornstarch to dams (26/dose) on gestational days 6-15 at 0, 2.5, 25, 75 or 150 mg/kg/day (Ciba-Geigy, 1989a; US EPA, 1992c). Treatment-related reductions in maternal feed consumption and/or mean body weight were observed at 75 and 150 mg/kg/day. The reductions were slight and transient at 75 mg/kg/day: food consumption was reduced by 20% ($p<0.05$) only during the initial (6-8 day) period of dosing and mean body weight was reduced ($p<0.05$) on days 8,12, and 16 by 6-7%. At the HDT, food consumption was reduced during the entire dosing period, by 14-48%, and mean, maternal body weight was reduced at each weighing from day 8-20, by 9-13%. Fetal effects included increased resorptions and post-implantation loss at 150 mg/kg/day. Decreased fetal weights were observed at 75 ($p<0.05$) and 150 mg/kg/day ($p<0.05$) and an increase in skeletal variations was present at 25 mg/kg/day and above. Based on reduced body weight and food consumption, the maternal NOEL was 25 mg/kg/day. Based on incompletely ossified or unossified bones, including hyoid, interparietal and parietal, the developmental NOEL was 2.5 mg/kg/day.

Gavage-Rat: hydroxyatrazine

Hydroxyatrazine (97.1%) was administered to dams (26/dose) by gavage, in 3% aqueous cornstarch, on days 6-15 of gestation at 0, 5, 25 or 125 mg/kg/day. Slightly decreased mean maternal body weight (~1.5%) and food consumption (12%, days 8-12, $p<0.05$) were recorded at 125 mg/kg/day. Slightly reduced mean fetal weights (4%, M, $p<0.05$; 5%, F, $p<0.05$) and an increased incidence of incomplete skeletal ossification were noted at 125 mg/kg/day. Malformations were observed in two litters at the highest dose tested or HDT (gastroschisis and umbilical hernia). Based on the above findings, the maternal and developmental NOEL were both 25 mg/kg/day (Ciba-Geigy, 1989b; US EPA, 1992c).

Table 23. Reproductive and Developmental Toxicity of Atrazine and Metabolites

Species/ Sex	Exposure Regimen	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
		Atrazine			
Rat	2-gen Repro	**Parental:** ⇓ food intake & body wt. & body wt. gain	2.5	25.0	Mainiero, 1987 [a/]
		Reproductive: no effects	≥25[b/]	-	
Rat (M/F)	3-gen Repro diet	No effect at highest dose tested (see text).	≥5.0[b/]	-	Hollingsworth, 1966
Rat (F)	Teratology	**Maternal:** ⇓ food intake & body wt., salivation	25.0	100	Giknis, 1989 [a/]
		Developmental: incomplete and delayed ossification	25.0	100	
Rat (F)	Teratology	**Maternal:** Mortality, ⇓ food intake & body wt.	70	700	Infurna, 1984
		Developmental: ⇓ fetal body wt. & size	70	700	
Rat (F)	Teratology	**Maternal:** ⇓ body wt. gain	100	500	Fritz, 1971
		Developmental: Fetal death, retarded skeletal development, ⇓ pup wt.	100	500	
Rat (F)	Teratology	No effect at highest dose tested.	6.8[b/]	-	George, 1992[c/]
Rabbit (F)	Teratology	**Maternal:** ⇓ body wt./food intake, ⇑ abortions	5	75	Arthur, 1984 [a/]
		Developmental: ⇓ fetal wt. & litter size, ⇑ resorptions, post-implantation loss, (fetal) skeletal variations	5	75	

a/ Acceptable to DPR under FIFRA testing guidelines.
b/ Highest dose tested.
c/ Pesticide mixture.

Table 23. Reproductive and Developmental Toxicity of Atrazine and Metabolites cont.....

Species/ Sex	Exposure Regimen	Effects at LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
		Desethylatrazine			
Rat (F)	Teratology	**Maternal:** ⇓ food intake **Developmental:** ⇓ fetal wt., fused sternebrae & delayed ossification.	25 25	100 100	Ciba-Geigy, 1992f
		Desisopropylatrazine			
Rat (F)	Teratology	**Maternal:** ⇓ mean body wt. **Developmental:** fused sternebrae	25 5	100 25	Ciba-Geigy,1992g
		Diaminochlorotriazine			
Rat (F)	Teratology	**Maternal:** ⇓ food intake & body wt. **Developmental:** incomplete skeletal ossification.	25 2.5	75 25	Ciba-Geigy,1989a US EPA, 1992c.
		Hydroxyatrazine			
Rat (F)	Teratology	**Maternal:** ⇓ food intake & body wt. **Developmental:** incomplete skeletal ossification.	25 25	125 125	Ciba-Geigy,1989b

H. NEUROTOXICITY

In a brief published account of a neurotoxicity study of atrazine (Castano *et al.*, 1982), it was reported that Sprague-Dawley rats were treated with intraperitoneal injections of atrazine twice a week for 30 days. One group of 14 animals received injections of 40 mg of atrazine dissolved in 1 cc of dimethylsulfoxide (DMSO), a second group of 14 animals received injections of 20 mg atrazine in DMSO, and a control group of 12 animals received only DMSO. Half of the animals in each group was sacrificed at the end of the 30-day treatment period; the other half was sacrificed after a recovery period of another 30 days. Samples of sciatic nerve, spinal ganglia and spinal cord were studied by electron microscopy and liver, kidney, lung and lymph node were examined by light microscopy. No morphological abnormalities were observed by light or electron microscopy. However, a slight decrease in the axon areas of the sciatic nerve was detected by statistical evaluation of rats dosed at 40 mg which were sacrificed at the end of the treatment period. By the end of the 30-day recovery period, the axon areas of the 40 mg group were slightly larger than the other groups. The authors were unsure of the meaning of the results and indicated that additional study was needed.

I. IMMUNOTOXICITY

Summary: The immunotoxicity of atrazine has been studied in mice and rats. In mice, atrazine exposure produced a transient suppression of humoral and cellular immunity and stimulation of macrophage activity. In rats, atrazine was associated with decreased numbers of lymphocytes in the blood and various organs.

Oral-Mice

A published account of an immunotoxicity study in mice (Fournier *et al.*, 1992), reported that the technical atrazine formulation AAtrex (85.5%) was given via single oral gavage to female C57Bl/6 mice at ~0, 27, 109, 437, or 875 mg/kg. The animals were sacrificed 7-40 days after exposure. No dose-related changes were recorded for body weight, changes in spleen cell number and cell viability. Cytoflurometric studies showed no significant changes in the frequency of L3T4-positive and Lyt-2-positive T-cells and functional *in vitro* assays of mitogen activation displayed no marked effects on lymphocyte stimulation by lipopolysaccharide, phytohemagglutinin, and concanavalin A. Exposure to atrazine did not affect interleukin-2 production by spleen cells. A transient significant suppression of a primarily humoral IgM response to sheep red blood cells and a transient inhibition of a specific T-cell response to alloantigens in a mixed lymphocyte reaction, was present at all dose levels. Also reported was an atrazine-related activation of macrophage phagocytic activity expressed as an increased uptake of fluorescent microspheres by the cells, at all but the lowest dose. Based on the reductions in IgM and T-cell responses, the LOEL for immune effects was 27 mg/kg. However, these preliminary experiments need to be repeated, and the basis for the lack of a clear dose-response in the altered parameters investigated, before a direct chemical-related effect can be proven.

Oral - Rat

In another published account of atrazine immunotoxicity (Vos *et al.*, 1983a), 6 male RiV:TOX(M) Wistar rats/dose received 100, 300, or 900 mg/kg/day in the diet for 3 weeks. Body and organ weights, histological examination, and total blood leucocyte and differential counts were conducted. At termination, statistically significant decreases in food consumption, body weight, and relative thymus weight were present at 900 mg/kg/day. The relative weights of thyroid and mesenteric lymph node were slightly increased at that level. The number of blood lymphocytes was significantly depressed by 24-27% at all three dose levels. Histologically, one instance of slight lymphoid depletion of the spleen and thymus, and liver cytoplasmic alteration was recorded at 900 mg/kg/day. Based on decreased blood lymphocytes, the LOEL was 100 mg/kg/day. Based on the effects in the thymus and blood lymphocytes, and the relative lack of effects in other areas and tissues, the authors considered atrazine's effects on these immunological criteria as the most sensitive parameters of toxicity (Vos *et al.*, 1983b).

In several rat subchronic (Section C) and chronic (Section D) studies, there was a dose-related increase in EMH in the spleen of males and/or females. The spleen is the major filter of antigens in the blood and the major site at which an immunological response occurs; it also removes damaged blood cells and is a site of EMH. It is unclear whether the EMH observed following atrazine administration was part of an immunological response.

J. OTHER TOXICITY STUDIES

Pesticide/Fertilizer Mixtures

The National Toxicology Program (NTP) has performed toxicity studies with pesticide and fertilizer mixtures representative of groundwater contamination found in California and Iowa (NTP, 1993; Heindel *et al.*, 1994). The California mixture was composed of the pesticides aldicarb, atrazine, 1,2-dibromo-3-chloropropane, 1,2-dichloropropane, ethylene dibromide, simazine and the fertilizer ammonium nitrate. The mixture was administered in drinking water, with 512 ppm propylene glycol, to F344/N rats and B6C3F1 mice at a range of concentrations. These were from 0.1X to 100X, where 1X represented the median concentration of the individual chemicals found in studies of groundwater contamination from normal agricultural activities. The concentrations of atrazine were reported to be based on a median groundwater value of 0.5 ppb; and were 0.05, 0.5, 5.0 and 50 ppb.

The NTP report focused on 26-week toxicity studies describing histopathology, clinical pathology, neurobehavior/neuropathology and reproductive system effects. The genetic toxicity of the mixture was assessed by determining the frequency of micronuclei in peripheral blood of mice and evaluating micronuclei and sister chromatid exchanges in splenocytes from female mice and male rats. Additional studies reviewed in the report included teratology studies with Sprague-Dawley rats, and continuous breeding studies with CD-1 Swiss mice.

In a 26-week drinking water study of the California mixture, all rats (10/sex/group) survived to the end of the study, and there were no significant effects on body weight gains. Water consumption was not affected by the pesticide/fertilizer contaminants, and there were no clinical signs of toxicity or neurobehavioral effects as measured by a functional observational battery, motor activity evaluations, thermal sensitivity evaluations, and startle response. There were no adverse effects noted in clinical pathology (including serum cholinesterase activity), organ weight, reproductive system, or histopathologic evaluations.

In a 26-week drinking water study in mice, one male receiving the California mixture at 100X died during the study. It could not be determined if the death of the mouse was related to the consumption of the mixture. Water consumption and body weight gain were not affected and no signs of toxicity were noted in clinical observation or in neurobehavioral assessments. No adverse effects were noted in clinical pathology, reproductive system, organ weight or histopathologic evaluations.

The pesticide/fertilizer mixture, when tested over a concentration range similar to that used in the 26-week studies, was found to have no effects in the rat teratology study or the mouse continuous breeding assay examining reproductive and developmental toxicity.

The California mixture was negative for induction of micronuclei in the peripheral blood erythrocytes of female mice. Sister chromatid exchange frequencies were marginally increased in rats and mice, but the NTP did not consider the change to be biologically important. Neither species exhibited increased frequencies of micronucleated splenocytes.

In summary, studies of potential toxicity associated with the consumption of a mixture of pesticides and a fertilizer representative of groundwater contamination of agricultural areas in California failed to demonstrate any significant adverse effects in rats or mice receiving the mixture in drinking water at concentrations as high as 100 times the median concentration of the individual chemicals as determined by groundwater surveys.

Miscellaneous Studies

The *in vitro* binding of atrazine and other *s*-triazines to red blood cells has been reported in various animal species in a published study (Hamboeck *et al.*, 1981). The level of covalent binding to hemoglobin of triazine metabolites was reported as: rat > guinea pig > dog > cow > mouse > human or sheep > pig. The addition of reactive metabolites resulted in greatly enhanced binding to rat RBCs although not to human RBCs. It was further stated that the binding was due to covalent binding of the triazine ring to an exposed cysteine of rat hemoglobin which was apparently unavailable for binding in human hemoglobin. A critique of that study, however, indicated that only the β chain of hemoglobin covalently bound all of the *s*-triazine metabolites. The specific amino acid, the β-125 cysteine (Cys-125), of hemoglobin was studied with simetryn sulfoxide only, and not with atrazine (US EPA, 1989c).

Rats fed diets containing 500 ppm atrazine increased the cyclic AMP content of the liver from 7.7 to 12.4 pmoles per 10 mg wet weight (Messner *et al.*, 1979). Following a single intraperitoneal injection of 50 to 250 mg/kg atrazine, cAMP increased from 125 to 275% of control. Corresponding increases in blood glucose and decreases in liver glycogen were observed. *In vitro* studies indicated an inhibition of phosphodiesterase activity, but it could not be repeated *in vivo*. Atrazine potentiated the effect of glucagon on isolated hepatocytes

In a published two-generation study of hexobarbital sleeping time (Ugazio *et al.*, 1991), F_o male and female rats received 450 ppm (~22.5 mg/kg/day) atrazine starting 60 days prior to mating. F_1 rats also received 450 ppm (~45 mg/kg/day) from 21 to 100 days of age. A statistically significant ~25% shortening of hexobarbital sleeping time was noted in weanling animals of both sexes, with lesser decreases seen thereafter in males only. It was stated that the hexobarbital sleeping time alteration and further *in vitro* assays pointed to a monooxygenase induction. At weaning the induction also involved glutathione S-transferase, a phase II enzyme. The authors suggested that the inducing properties of atrazine may be transferred to offspring via the placental and/or mammary route, without evidence, and did not consider the possibility of pre-weanling consumption of maternal feedstuffs.

Epidemiological Studies

Kettles *et al.*, 1997: The incidence of breast cancer in females was examined in an ecological epidemiological study in Kentucky, on a county-by-county (n=120) basis. Exposure was estimated using four equally-weighted surrogates: water contamination data (groundwater and surface water), acres of corn planted and (total) pesticide use. Females in each county were classified into one of three groups, low (n=596,686), medium (n=972,001) or high (n=331,541). The basis for using these surrogates was that leaching and run-off had resulted in a large number of detections of triazines, the major use of triazines in Kentucky was on corn and that ≥1 million lbs of atrazine were used annually, making it the most heavily used pesticide in Kentucky. Breast cancer data were obtained from a statewide registry and the cancer data were controlled for age, race, income, education and delayed childbearing. The results, using the 1993/4 breast cancer rates, indicated an increased risk of breast cancer with increased triazine exposure. When the OR (odds ratio) for low exposure was set at 1.00, for medium exposure, the OR was 1.14 ($p<0.0001$) and for high exposure, OR=1.20 ($p<0.0001$). The authors stated that "the strength of association between the exposure and outcome is in the range of (OR=)1.0 to 2.0, a level that has been demonstrated for other established breast cancer risk factors." However, when the 1991/2 breast cancer data were considered, a dose response between

incidence and exposure was *not* obtained (OR=1.00, 1.09 and 1.07 at the three levels, respectively). Furthermore, lower incidences of breast cancer were often associated with the (4) components of the exposure estimate when these were considered individually, *e.g.* the highest numbers for acres of corn planted or amount of pesticide used gave OR values of 0.87 and 0.91 *vs.* 1.00 for low exposure, respectively. Thus, it is unclear whether an increased risk of breast cancer in humans really is associated with exposure to triazine herbicides. As the study authors suggest, perhaps a population-based, case-control study would help to resolve this issue.

Munger *et al.*, 1992: This study evaluated birth defects in women living in a part of southern Iowa where all of the drinking water was supplied from a single source, the Rathbun reservoir, over the period 1983-1989. Rathbun was known to be contaminated, during this period, with atrazine, cyanazine and metolachlor. Rathbun communities were compared with two populations: all other IA communities of similar size and communities in southern IA matched by population size. The excess of birth defects (53, total) was similar for both comparison groups. Using the first comparison group, the relative risk (RR) values were 2.51 (all birth defects), 2.96 (urogenital), 3.24 (limb reduction) and 3.38 (all cardiac). Using the second comparison group, the RR values were 2.36 (all birth defects), 2.68 (urogenital), 2.81 (limb reduction) and 3.59 (all cardiac). For major cardiac defects, the corresponding RR values were 5.71 (2.95-11.08, 95%C.I.) and 4.65 (2.15-10.22). Thus, cardiac effects were the most common birth defects. Although atrazine showed cardiotoxicity in chronic dog studies (Section III.D), whereas cyanazine (Gammon *et al.*, 1997) and metolachlor (DPR Toxicology Summaries) did not, this epidemiological study has several possible biases and confounding factors. It would therefore be premature to conclude that it lends support to the possible importance of cardiotoxicity of atrazine for human health chronic risk assessment.

Endocrine Effects

Summary: an endocrine mechanism has been proposed for the induction of mammary tumors in the female SD rat by triazine herbicides, including atrazine. On the one hand, there is some evidence for genotoxicity resulting from atrazine and/or unidentified derivative(s) of atrazine, which could result in a non-threshold mechanism. On the other, there is also some evidence that atrazine disrupts estrus in the SD rat (but not the F344 rat), perhaps resulting from a disruption in the hypothalamic control of the pituitary gland, which could have a threshold mechanism. This in turn could result in a multitude of endocrine systems being affected by atrazine, as has been observed in the SD rat. It has been shown that atrazine disrupts ovarian function in both SD and Long-Evans hooded rats. Since the SD strain (but not the F344) exhibits mammary tumors induced by atrazine, it is possible that mammary tumors and endocrine disruption are linked. However, it is not known whether atrazine results in an increased incidence of mammary tumors in the Long-Evans rat. Furthermore, the endocrine effects in this strain included decreased blood estradiol, elevated progesterone and prolonged diestrus, which are opposite those which favor an elevated incidence of mammary tumors. In a study using the SD rat, atrazine disrupted estrous cycles, causing prolonged estrous and diestrous. After ovariectomy, a large fall in the luteinizing hormone (LH) peak resulted from atrazine administration. This reduction was reversed by GnRH suggesting that atrazine may have blocked GnRH release/synthesis in the hypothalamus. The suppression of the LH peak would stimulate the release of estrogens in the (intact) SD rat (which would stimulate the growth of mammary tumors). Recent work has shown that triazine herbicides (and some degradates) induce the aromatase which converts androgens to estrogens, in human placenta carcinoma cells. It has also been shown that atrazine-induced mammary tumors are abolished by ovariectomy, but so too are mammary tumors in undosed SD rats. By

monitoring the stage of estrus in intact rats dosed with atrazine over two years, regardless of atrazine dosing, it is clear that rats which develop tumors usually spend an abnormally long time in estrus prior to carcinoma palpation. Recent experiments have shown a suppression of LH and prolactin surges in the blood of dosed lactating Wistar rats. There was also an apparent dose-related delay in puberty in males, along with prostatitis, but similar effects were found in diet-restricted control males. Increases in blood estradiol and estrone along with a fall in intratesticular testosterone were observed and these effects were unaffected by food consumption. In conclusion, there is considerable evidence to support an endocrine mechanism for mammary carcinoma induction in the SD rat, and such a mechanism would be anticipated to show a practical threshold, but the precise mechanism is unclear.

In a series of reports and literature publications, evidence for estrogenic effects of atrazine has been presented. The increased incidence and/or decreased latency of mammary tumors in rats is evident only in the female, Sprague-Dawley (Mayhew, 1986; Thakur, 1991a, 1992a) and not in the Fischer (F344) strain (Thakur, 1991b, 1992b). There is no reliable evidence for an oncogenic effect aside from these mammary tumors in the female SD rat.

Eldridge, 1994a: Atrazine administration by gavage at $\leq$300 mg/kg/day for 14-23 days, resulted in an increase in estrus duration and a corresponding fall in the percentage of the estrus cycle in diestrus, in the SD rat. In addition, there was a dose-related fall in plasma estradiol, a reduction in ovarian and uterine weight and an increase in plasma progesterone. In the F344 rat, none of these effects was observed.

Tezak *et al.*, 1992: Studies of hormonal effects of triazines and other pesticides have indicated activity in several areas *e.g.* the 2-methoxy analog of atrazine (atratone) affected specific regions in the hypothalamus, pituitary, and prostate of the male rat. The addition *in vitro* of atrazine or 2-methylthio-4,6-*bis*-isopropylamino-*s*-triazine (prometryne) inhibited the binding of 5-α-dihydrotestosterone to receptors of the hypothalamus, pituitary, and prostate of male rats. Similar results were obtained *in vivo*. A daily injection of atrazine or desethylatrazine to pregnant or lactating rats influenced the pituitary-gonadal axis in the offspring of both sexes. Atrazine, prometryne, and also lindane significantly inhibited the formation of specific estradiol-receptor complexes in the rat uterus cytosol, both *in vivo* and *in vitro* (Tezak *et al.*, 1992). Rats fed 50 ppm atrazine for 15 days had increased serum thyroid hormones T_3 and T_4 (Ghina *et al.*, 1979) and 100 ppm for 90 days altered the circadian rhythms of nucleic acids and total protein in thyroid and adrenal glands (Nicolau *et al.*, 1980, 1982).

Eldridge, 1993a,b: The reduced latency of mammary tumor development in the SD rat correlated with endocrine disruption in the form of changes in estrogen function (Eldridge, 1993a; McConnell, 1995). The F344 rat, exposed to atrazine in the diet, showed neither an increase in mammary tumors (Thakur, 1991b; 1992b) nor a disruption in hormone balance (Eldridge, 1993b; McConnell, 1995). However, it is not possible to conclude that humans will not be affected by triazines because of interspecies differences in estrus cycles (Eldridge, 1993a) because the mechanism of the possible hormonal effect is unknown. For example, Eldridge *et al.*, 1994b, found that a direct action of atrazine at the estrogen receptor appeared to be unlikely since no attainable concentration would inhibit the binding of ^{3}H-estradiol to rat uterine cytosol, *in vitro*. However, pre-incubation with 0.1 mM atrazine resulted in binding inhibition. The estimated IC_{50} for atrazine, simazine and DACT (the common metabolite) was *ca.* 10^{-5}M. Furthermore, atrazine inhibited both estrogen-dependent and estrogen-independent protein synthesis in rat uterus, suggesting that atrazine may be acting, at least in part, as an estrogen antagonist (rather than an agonist).

Safe *et al.*, 1995: Meanwhile, Safe *et al.*, 1995 have determined that atrazine (and simazine) did not compete with TCDD for the rat liver cytosolic A_h receptor, or with ^{3}H-estradiol at the E_2 receptor in SD rat uterus, human breast cancer cells and cloned yeast cells. Details are not available to establish whether pre-incubation of cells/tissues with atrazine occurred. In another study (Connor *et al.*, 1996), dosing of SD-rats with the two triazines at 50 to 300 mg/kg/day, by gavage, for 3 days resulted in no effects on a range of E_2-induced phenomena. There were, however, slight decreases in the binding of progesterone and in uterine peroxidase activity, which could both have been treatment-related effects. A model of the SD rat estrus cycle is being developed (Gearhart *et al.*, 1996; Cooper, 1995). At about one year of age, ovulation ceases, with persistent estrus and prolonged exposure to estradiol. Atrazine lengthens cycles and causes persistent estrus. Its site of action is thought to be between the hypothalamus and the pituitary. Tennant *et al.*, 1993 showed hypothalamic changes in the SD rat exposed to dietary DACT (a triazine metabolite) at 1000 ppm, which may be related to prolonged estrus.

Paech *et al.*, 1997: It remains possible that atrazine causes endocrine disruption through an interaction at a feedback receptor for estrogens in the pituitary/hypothalamus. Paech *et al.*, 1997 have demonstrated the existence of two types of estrogen receptor, ERα and ERβ, in the rat and human, by making use of three antagonists and two agonists. The ERs have different tissue distributions, the former being the predominant receptor in mammary tissue and the latter in the uterus. Estrogen agonists stimulate DNA transcription through binding at the ERα and antagonists inhibit transcription. At the ERβ, agonists (at the ERα) act as inhibitors of transcription, while antagonists stimulate transcription (the exact opposite of ERα). So far, the ER is the only known steroid receptor with different receptor subtypes. It is not yet known whether the negative feedback receptor resembles one of these subtypes. Similarly, possible effects of triazines on GnRH (gonadotrophin releasing hormone) related systems remain to be explored.

Stevens *et al.*, 1994: Other reports in the literature have concluded that triazines result in mammary tumors in the SD rat by altering hormone levels over time (Stevens *et al.*, 1994). Risk assessment should therefore be based on the hormonal imbalance caused by atrazine rather than on the tumors, according to Stevens, 1994. Furthermore, Tennant *et al.*, 1994, have shown that atrazine dosing of ovariectomized SD rats on days 14-23 of estrus, followed by injections of estradiol, resulted in a dose-dependent reduction in uterine weight. The binding of a tritiated progesterone ligand to uterine tissue, *in vitro*, was inhibited by atrazine dose-dependently. In tissues from rats which were *not* pretreated with estradiol, less inhibition of binding was reported, but the inhibition of binding was nonetheless statistically significant. Atrazine and simazine showed much greater activity in these assays than DACT. This suggests that the hypothalamic effects caused by DACT reported above (Tennant *et al.*, 1993) may also be more pronounced with these herbicides than with DACT.

Morseth, 1996a: The effects of atrazine were studied at 2.5, 5, 40 and 200 mg/kg/day, by oral gavage, on hormone levels in ovariectomized SD rats (90/group) which were implanted with estradiol-releasing devices (Morseth, 1996a). Prior to ovariectomy, atrazine caused a dose-dependent disturbance in estrus cycling, particularly a prolongation of diestrus and of estrus. The incidence of rats with diestrus blocks (of $\geq$4 consecutive days of diestrus) was increased at 40 and 200 mg/kg/day, from 21/90 (23%) in controls to 36/90 (40%) and 51/90 (57%), respectively ($p<0.05$ and $p<0.001$ at each dose, Fisher's exact test). Similarly, the number of rats with diestrus blocks of $\geq$7 consecutive days of diestrus was increased from 10 (11%) in controls to 21 (23%) and 34 (38%), also $p<0.05$ and $p<0.001$, respectively (Fisher's exact test). Ovariectomized rats had remarkable, dose-dependent decrements in the luteinizing hormone (LH) peak as a result of atrazine administration, at 40 and particularly, at 200 mg/kg/day. The suppression of the LH peak caused by atrazine was reversed by GnRH. This reversal was interpreted as being due to an effect of atrazine on hypothalamic control of GnRH synthesis

and/or release. There was also a reduction of peak prolactin levels at the HDT and a possible delay in the timing of the peak. It was concluded that atrazine leads to a delay in ovulation (and associated prolonged estrus) by disturbing the surges of release of LH and prolactin. The LOEL and NOEL values for hormonal disruption were 40 and 5 mg/kg/day, respectively.

Morseth, 1996b: In an analogous study to the oral gavage one of Morseth, 1996a, groups of 90 female SD rats were fed on atrazine at levels of 0, 25, 50 and 400 ppm for 6 months (Morseth, 1996b). Rats were ovariectomized 10 days before sacrifice and received estradiol capsules 7 days later. This ensured that sufficient estradiol was available to induce an LH surge (in undosed rats). There was a large increase in prolonged or constant estrous in the final 3 months, along with a significant fall in body weight, at 400 ppm. The LH pulse was blocked at this dose. The NOEL for the effects described was 50 ppm or 3.65 mg/kg/day. In a preliminary study from the same laboratory (Morseth, 1990), using smaller sample sizes of SD rats with an intact ovary, there was a dose-dependent reduction in the blood levels of progesterone and estradiol following the administration of atrazine or DACT.

Cooper *et al.*, 1996: The effects of atrazine on ovarian function in SD and Long-Evans hooded rats have been studied (Cooper *et al.*, 1996). Doses of 75, 150 and 300 mg/kg/day were given by oral gavage to females for 21 days. Blood samples were taken daily and clinical signs monitored. Atrazine disrupted estrus cyclicity in both strains. At the mid-dose, atrazine increased the number of days in diestrus for both strains, without a change in the number of days in estrus, unlike earlier results mentioned above. There was a corresponding increase in blood progesterone level and a decrease in estradiol level, which when combined with prolonged diestrus is known as pseudopregnancy. At the HDT, SD rats showed a similar effect, correlated with a slight increase in the level of both hormones. However, LE-hooded rats displayed a reduction in the level of both hormones at this dose, a condition known as anestrus.

Morseth, 1998: A study was undertaken to elucidate the mechanism for the increased incidence of mammary tumors induced by atrazine in female SD rats (Morseth, 1998). Ovariectomized SD rats (80/group) were fed atrazine in the diet for 2 yrs. at 0, 25, 50, 70 and 400 ppm, and limited autopsies were undertaken. Interim sacrifice was undertaken at 1 yr. on 20 animals per dose, with autopsy, to also evaluate the impact of ovarian hormones on mammary tumors. Because it is possible that there is more than one mechanism, for early and late onset carcinomas for example (see Mayhew, 1986; Thakur, 1991a), early and late effects have sometimes been considered separately. Ovary-intact rats were dosed identically and vaginal smears were collected daily for 14 consecutive days every month. The stage of estrus was therefore ascertained for each rat. It should be noted that the normal estrus cycle for the SD rat lasts for 4 days, of which the period in estrus is $\leq$24 hours.

No mammary tumors were found at 12-mon. in ovariectomized rats, in either dosed or untreated control animals. The same absence of mammary tumors was found at 24 months in ovariectomized rats. In contrast, rats with an intact ovary had mammary tumors, primarily carcinomas, which increased with dosage in 2/22, 1/20, 0/23, 2/22 and 5/23 rats at 0 to 400 ppm, at 12 months. Table 24 shows the incidence of mammary carcinomas which were palpated in the first year of study. The percentage of days in estrus over each 14-day period per month was established, over the first 46 weeks of the study, up to and beyond the time of first palpation. Considering the 12 rats which developed mammary carcinomas for which these data are available (2 at 0 ppm; 1 at 25 ppm; 2 at 70 ppm; 7 at 400 ppm), 10 showed 100% of days in estrus during the 14-day period at the time of palpation, the other two being 93% and 86%. The majority (8) of these ten individuals had several weeks of 100% estrus leading up to the time of first palpation. The (two) rats which did not have several weeks of 100% estrus

leading up to the time of first palpation were both dosed at 400 ppm and were the two earliest palpations in the study, 14 and 18 weeks, dying at 19 and 26 weeks, respectively.

At 2 yrs., ovary-intact rats dosed with atrazine showed a dose-related increase in mammary tumors, predominantly carcinomas (Table 25), similar to that shown in SD females by Mayhew, 1986. There was a dose-related increase in group mean percentage of days in estrus, driven mostly by 400 ppm rats ($p<0.01$, Kruskal-Wallis test). In both dosed rats and controls, there was an increased percentage of time spent by carcinoma-bearing rats in estrus compared with rats without carcinomas, during the 17 to 26 week ($p<0.01$, Mann-Whitney test) period as well as the 1 to 46 week ($p<0.001$) period (Table 26). However, rats without carcinomas spent slightly more time in estrus, especially during the 17-26 week period, as dose was increased ($p=0.05$, Kruskal-Wallis) whereas carcinoma-bearing rats did not. Subsequently, all of the rats spent a large proportion of their time in estrus, which is part of the normal reproductive aging in the SD rat. A comparison was made of controls *vs.* 400 ppm rats: rats at 400 ppm spent a longer percentage of time in estrus than controls, over 17-26 weeks ($p<0.001$, Mann-Whitney test) and 1-46 weeks ($p<0.01$); rats without carcinomas appeared to be responsible for this finding since they spent more time in estrus, over 17-26 weeks ($p<0.01$) and 1-46 weeks ($p<0.05$) whereas carcinoma-bearing rats did not spend a greater percentage of days in estrus. In comparing controls with all other rats combined, there were no significant differences in the percentage of time in estrus, regardless of whether rats were separated into carcinoma-bearing or not. Finally, a comparison was made of 400 ppm rats *vs.* all others, combined: a larger percentage of time was spent in estrus over weeks 17-26 ($p<0.001$) and weeks 1-46 ($p<0.01$) for rats at 400 ppm. This was largely a result of the rats without carcinomas ($p<0.01$, for each interval, Mann-Whitney test) rather than the rats with carcinomas (n.s.).

McShane & Wise, 1996: One of the most sensitive toxicological endpoints of atrazine in mammals is a reduction in body weight and body weight gain. In the paper cited here, the effects of caloric restriction on reproductive lifespan were studied, in the undosed SD rat, along with hormone measurements in an attempt to explain the findings. Dietary restriction to 60% of control resulted in a 24% reduction in body weight between 4 and 18 months. There was a slight delay in the onset of puberty but a large increase in reproductive lifespan. Persistent estrus was delayed by 4 months. In ovariectomized rats, mean blood LH levels decreased with age ($p<0.01$) but increased with caloric restriction ($p<0.05$). This increase was determined to be an increase in mean LH pulse amplitude rather than a decrease in intervals between pulses. It was attributed to be due to an effect on the hypothalamic/pituitary axis. Atrazine, despite causing a drop in food intake and body weight, caused effects on reproductive aging (earlier persistent estrus, reduced LH) which are precisely opposite the effects of caloric restriction, described above. This suggests that the intrinsic effects of atrazine on these endocrine parameters, resulting in earlier onset of reproductive aging in the female SD rat, may be even greater than those described above, since atrazine overcame the confounding effect of reduced food intake and body weight.

Sanderson *et al.*, 2000a,b: Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes were studied. Three triazine herbicides (atrazine, simazine, propazine) increased aromatase activity in human H295R (adrenocortical carcinoma) cells, *in vitro*, concentration-dependently. The aromatase enzyme is responsible for converting androgens to estrogens through the aromatization of the A ring in the steroid structure. The enzyme was assayed in two ways, firstly by the measurement of [^3H_2O] from [^3H]-androstenedione and secondly, by estrone production using a RIA. The positive control inducer was 8Br-cAMP. In addition to the measurements of enzyme activity, the active compounds also caused an increase in the levels of mRNA for CYP19, the cytochrome P450 enzyme responsible for the reaction, as measured using a RT-PCR assay. The authors did not exclude the possibility that mRNA for CYP19 was, at least in part, stabilized rather than induced. A SOT poster (Sanderson, 2000b) presented this

work plus unpublished data showing the effects of triazines on two other cell lines, the effects of several triazine degradates and the estrogenic and anti-estrogenic activity of the triazines and degradates on vitellogenin production (in carp hepatocytes). Triazines had no effect on aromatase in MCF-7 cells (human mammary carcinoma cell line) but neither did 8Br-cAMP, the positive control activator. All the triazines activated aromatase in JEG-3 cells (human placental choriocarcinoma cell line), with the same structure-activity and concentration response profiles as for H295R cells. The stimulation was not as great as for the latter cells, but this may have been because the basal level of the enzyme was much greater (~10-fold) in the JEG-3 cells. In the aromatase assay systems described, the monodealkylated metabolites of atrazine (DEA and DIPA) were active but the didealkylated (DACT) and hydroxy metabolites were inactive. The hydroxylated degradate of atrazine itself was not studied.

The triazines and their metabolites were unable to induce the synthesis of vitellogenin production in male carp hepatocytes and nor were they able to antagonize the vitellogenin production induced by estradiol. This suggests that the triazines are inactive at the E_2-receptor, but it should be remembered that there are (at least) two types of the E_2-receptor, with different pharmacological properties.

Laws *et al.* 2000: Sexual maturity in female Wistar rats was also affected by atrazine (the protocol for the experiments performed followed the EDSTAC recommendations). Irregular estrous cycles were observed for the entire dosing period (22 – 41 days). Vaginal opening was significantly delayed (3 - 4 days) at 50 or 100 mg/kg/day and indefinitely in 9 out of 16 rats at 200 mg/kg/day. There was also a significant reduction in uterine weight (at 100 or 200 mg/kg/day). Kidney and pituitary weights were reduced at 200 mg/kg/day. The effects on vaginal opening and estrous cycling disappeared on the discontinuation of dosing.

Stoker *et al.*, 1999: Suckling-induced prolactin secretion into the blood was completely inhibited by atrazine at 100 mg/kg/day (two 50 mg/kg doses/day), by oral gavage, in the Wistar rat. The LOEL and NOEL values were 25 and 12.5 mg/kg/day, respectively. The standard was the dopamine agonist, bromocriptine, which completely eliminated prolactin release at $\geq$ 0.208 mg/kg/day, but was without effects at 0.104 mg/kg/day. Prostate inflammation was studied in offspring that were exposed *via* the milk of dams treated (twice daily) during days 1 - 4 of lactation. Measures of inflammation were the induction of myeloperoxidase and histopathology. Males examined at 90 days showed no significant abnormalities but at 120 days, the incidence of prostate inflammation had increased significantly using both measures. LOEL and NOEL values were identical to those for the inhibition of prolactin secretion, 25 and 12.5 mg/kg/day, respectively. Moreover, the inflammation was eliminated by the co-administration of ovine prolactin (s.c. 0.3 mg/kg) along with atrazine at 25 or 50 mg/kg/day. However, the link between reduced prolactin secretion in the dam by atrazine (or bromocriptine) and prostate inflammation in offspring was clouded by the observation that the serum and pituitary levels of prolactin in males at 120 days were not reduced by atrazine or bromocriptine. It is also noted below (Stoker *et al.*, 2000b) that apparent effects of atrazine on various markers for male pubertal development in the Wistar rat could be mimicked by reducing dietary intake in undosed rats.

Stoker *et al.*, 2000a: Sexual maturity in male Wistar rats was delayed by atrazine, administered by oral gavage (at 50, 100 and 200 mg/kg/day) for 31 days, commencing on postnatal day 23. At the HDT, serum PRL and LH were suppressed by atrazine and there was a significant reduction in serum testosterone along with a significant increase in serum estradiol.

Stoker *et al.* 2000b: The effects of atrazine on puberty were assessed in male Wistar rats, dosed by gavage from PND 23 to PND 53 or 54 at 0, 12.5, 25, 50, 100, 150 or 200 mg/kg/day. Pair-fed (undosed) rats' diets were limited to match the food consumption of the 200 mg/kg/day

group. Body weights were reduced dose-dependently above100 mg/kg/day. There was a 17% reduction at 200 mg/kg/day, 11% at 150 mg/kg/day, 6.8% at 100 mg/kg/day and 15% in pair-fed controls. Ventral prostate and seminal vesicle weights were reduced dose-dependently and preputial separation was increased dose-dependently. However, because the pair-fed controls had values in between the 150 and 200 mg/kg/day groups, for each of these (3) endpoints, plus body weight, it is difficult to conclude that male pubertal development was delayed as a direct result of atrazine administration. Rather, it would appear that these (3) effects were all secondary to reduced dietary intake and/or body weight. The authors also measured the levels of various steroid hormones in the blood and testes at PND 45 and 53. There was a dose-dependent increase in estradiol and estrone on PND 53, with no difference between the controls and the pair-fed controls. It thus appears that these effects were a direct result of atrazine rather than being secondary to reduced dietary intake. A corresponding decrease in serum and intra-testicular testosterone was reported on PND 45 and 53, once again with the pair-fed control group having similar values to the free-feeding controls. The NOEL for the estradiol/estrone effects was 50 mg/kg/day, based on ~30% increase at the LOEL, 100 mg/kg/day. Aromatase induction was not measured to account for these hormone effects of atrazine. Pituitary hormone levels were also measured. On PND 53, serum LH showed a dose-dependent decrease, with a reduction of 47% at 200 mg/kg/day. No values were statistically significant but the trend was significant ($p<0.01$). Pair-fed rats were the same as controls. Thyroid hormone levels also showed an alteration which appeared to be compound-related rather than being secondary to body weight reduction. Serum TSH (T4) did not vary but T3 concentration increased, by up to 64% at the HDT ($p<0.05$). In conclusion, the effects of atrazine on male puberty development in the Wistar rat, as measured by organ weights or preputial separation, appear to be largely secondary to body weight loss or reduced nutritional status. However, the hormone effects, with a NOEL of 50 mg/kg/day, for example, for elevated estradiol and estrone levels, could present a plausible basis for some of the endocrine effects that have been reported for triazines in other studies.

Cooper *et al.*, 2000a: Disruption of ovarian cycling in Long-Evans, hooded rats was observed as repetitive pseudopregnancy and prolonged diestrus. The triazines were administered by oral gavage for 21 days, commencing at 90 days of age. Atrazine, simazine (75, 150 and 300 mg/kg/day) and cyanazine (18.8, 37.5 and 75 mg/kg/day) all caused these effects. So, too, did the (chlorinated) metabolites DEA, DIPA and DACT (75, 150 and 300 mg/kg/day). The efficacy of these metabolites was greater than the parent triazines. [It should be noted that DACT was inactive on the aromatase assay, Sanderson *et al.*, 2000b]

Cooper *et al.*, 2000b: Studies were conducted on Long-Evans (LE) and Sprague-Dawley (SD) females dosed 1, 3, or 21 days at 0, 50, 100, 200, or 300 mg/kg/day. Atrazine effects on levels of five hormones (LH, FSH, prolactin, TSH, and estradiol) in serum and anterior pituitary gland samples were evaluated. Ovariectomized rats were implanted with estrogen-filled silastic capsules 3 days before sacrifice. Only LH and prolactin gave treatment responses (a marked inhibition of surges in serum), therefore only the latter two hormones were assayed in an analogous experiment using daily dose levels of 0, 75, 150, or 300 mg/kg/day for a 21-day exposure. These groups showed significant reductions in surge levels of LH and prolactin in both rat strains at the expected diurnal peak surge time at 150 and 300 mg/kg/day. In addition, LH was significantly reduced at 75 mg/kg/day in LE rats. Pituitary samples were assayed for these two hormones in the 21-day groups. There were no treatment-related changes in LH levels, but prolactin was elevated at 75 to 300 mg/kg/day in both strains. Following 3-day exposure, pituitary prolactin levels were consistently reduced at 6 hr at all dose levels tested (50 to 300 mg/kg/day) in LE rats, whereas there was no treatment effect in SD rats. The numbers of oocytes shed were unaffected at any dose level in LE rats. When normally cycling, LE rats were monitored for estrous cycling by daily vaginal smears for 3 weeks after a single proestrus dose of atrazine: 7/9 of the 300 mg/kg females became pseudopregnant, whereas none of the

lower dose groups had abnormal cycling. To identify whether atrazine affects pituitary function directly, LE rats were hypophysectomized and autotransplants of pituitary tissue were placed within the kidney capsule. Blood prolactin levels were unchanged under these circumstances. To evaluate effects of GnRH, ovariectomized LE rats were implanted with estrogen-filled silastic capsules, then dosed with 300 mg/kg atrazine for 3 days and then given 50 ng/kg GnRH via catheter. Atrazine blocked LH secretion, but the combination of atrazine and GnRH led to peak LH concentrations similar to control rats. An *in vitro* perfusion study evaluated a possible direct action of atrazine on pituitary LH and prolactin secretion. Portions of anterior pituitary were taken from ovariectomized, estrogen-primed SD rats, and tissues were maintained in perfusion chambers and exposed to 0 or 100 μM atrazine. LH and prolactin secretion into the medium gradually declined over a 2 hr period. Subsequently, the pituitary was perfused with 30-min pulses of GnRH (85 nM) plus thyroid releasing hormone (TRH, 100 nM), resulting in a large (~5-fold) increase in LH and prolactin secretion. There was no difference between atrazine and control secretion of LH or prolactin *i.e.* atrazine had no effect on the release of these hormones by the isolated pituitary. In a parallel *in vivo* study, females were treated for 3 days by gavage with 0, 100, or 200 mg/kg/day atrazine prior to sacrifice. Pituitary gland samples, subjected to the *in vitro* perfusion assay, were found not to have decreases in LH or prolactin secretion peaks upon challenge by releasing hormones. Instead, there was an initial increase in prolactin release in atrazine-treated rats, consistent with elevated pituitary prolactin levels from atrazine treatment. This result also supports the concept that atrazine does not directly affect the pituitary. Instead, it may interfere with secretion of releasing hormones from the hypothalamus.

It is concluded that the ovary plays an important role in the development of mammary tumors in the SD rat, whether these tumors are the result of atrazine exposure or are background/ spontaneous in nature. Mammary carcinogens which are genotoxic or estrogenic, such as 3-methylcholanthrene (MCA) and estradiol (Li & Li, 1990), are known to cause mammary tumors even in the absence of an ovary in rodents; it is also known that high levels of prolactin enhance the formation of mammary carcinomas by DMBA in the rat (Ip *et al.*, 1980). Recent work points to a possible mechanism for mammary carcinoma formation which is linked to the induction of aromatase, resulting in an increased level of serum estradiol.

Table 24. Incidence of Mammary Carcinomas which were Palpated in the First Year of a Two Year study in Ovary-Intact Sprague-Dawley Rats on Dietary Atrazine.[a/]

	DOSE ppm[b/]				
	0	**25**	**50**	**70**	**400**
Rats at Risk [c/]	80	80	80	80	80
Carcinoma	3 (3.8%)	2 (2.5%)	0 (0%)	4 (5.0%)	8 (10%)

a/ from Morseth, 1998
b/ equivalent to dosages of 1.5, 3.1, 4.2 and 24.4 mg/kg/day at 25, 50, 70 and 400 ppm, respectively.
c/ combined rats scheduled for interim and final sacrifice.

Table 25. Incidence of Mammary Tumors in Ovary-Intact Sprague-Dawley Rats on Dietary Atrazine for Two Years.[a/]

	DOSE ppm[b/]				
	0	**25**	**50**	**70**	**400**
Rats at Risk [c/]	60	61	60	61	62
Carcinoma	12++ (20%)	18 (30%)	22* (37%)	12 (20%)	27** (44%)
Fibroadenoma	16 (27%)	24 (39%)	31** (52%)	27* (44%)	24 (39%)
Combined	24+d/ (40%)	33e/ (54%)	45***e/ (75%)	34f/ (56%)	42**e/ (68%)

a/ from Morseth, 1998
b/ equivalent to estimated dosages of 1.5, 3.1, 4.2 and 24.4 mg/kg/day at 25, 50, 70 and 400 ppm.
c/ includes all rats dying or being sacrificed from day 178 onwards (the time of the first mammary carcinoma), but excluding interim sacrifice animals.
d/ four rats had both carcinoma(s) and fibroadenoma(s).
e/ nine rats had both carcinoma(s) and fibroadenoma(s).
f/ five rats had both carcinoma(s) and fibroadenoma(s).
* significantly greater than control, p<0.05 (Fisher's exact test); ** p<0.01 (Fisher's exact test);
*** p<0.001 (Fisher's exact test)
\+ significant trend, p<0.05 (Peto's trend test); ++ p<0.01 (Peto's trend test).

Table 26. Mean Percentage of Time in Estrus for Rats with and without Mammary Carcinomas in a 2-yr Study in Ovary-Intact SD Rats on Dietary Atrazine.[a/]

Time interval	DOSE ppm				
	0	**25**	**50**	**70**	**400**
Weeks 17-26 [b/]					
w/ carcinoma (n)	59.4% (10)	62.4% (17)	52.5% (20)	64.1% (12)	66.8% (22)
w/o carcinoma (n)	45.6% (48)	47.3% (43)	49.6% (37)	45.9% (46)	63.5%** (35)
Weeks 1-46 [b/]					
w/ carcinoma (n)	63.0% (10)	62.7% (17)	55.9% (20)	56.7% (12)	64.2% (22)
w/o carcinoma (n)	51.9% (48)	53.1% (43)	49.3% (37)	48.3% (46)	59.1% (35)

a/ from Morseth, 1998.
b/ does not include rats which died prior to week 46: n=1 (controls), n=3 (400 ppm).
** p<0.01 (Student's t-test).

IV. RISK ASSESSMENT

A. HAZARD IDENTIFICATION

A human health risk assessment of atrazine has been conducted to evaluate the significance of the toxicity demonstrated in various animal studies.

Acute Toxicity

The LD_{50} and LC_{50} values for technical and formulated atrazine, and for atrazine metabolism or breakdown products are summarized in Table 2. The clinical signs of acute toxicity included decreased activity, constricted pupils, epistaxis, nasal discharge, piloerection, polyuria, salivation, muscle weakness, prostration, facial swelling, weight loss, ataxia, tremors, ptosis and dyspnea.

Acute toxicity tests are characteristically conducted to determine LD_{50} values, and as such, use relatively high dose levels in order to produce lethality. Consequently, the studies using atrazine did not establish NOELs for more sensitive toxicological endpoints. Therefore, it was necessary to use a teratology study (Table 22) to assess the risk associated with potential acute human exposure to atrazine. In such a study, using the New Zealand rabbit (Arthur, 1984), maternal toxicity was reported as an increased incidence of blood on vulva/ cage and of abortions at 75 mg/kg/day. Developmental toxicity was noted as increased skeletal variations (unossified bones), post-implantation loss and resorptions and decreased fetal body weight, with a LOEL of 75 mg/kg/day and a NOEL of 5 mg/kg/day. The single case of abortion at 5 mg/kg/day was not statistically significant or clearly compound-related and so a NOEL of 5 mg/kg/day was selected for acute risk assessment purposes.

A NOEL value of 1 mg/kg, from the same study, has previously been used to prepare the one-day and 10-day health advisory values in the *US EPA Drinking Water Criteria Document for Atrazine* (US EPA, 1990b). This NOEL was based on reduced maternal body weight gain, which did not become evident until after 7 to 12 daily administrations of atrazine and, therefore, is not considered by DPR to be an acute effect. More recently, however, US EPA established a NOEL of 5 mg/kg/day for this study (US EPA, IRIS, 1997).

Chronic Toxicity

Various systemic toxic effects were identified in subchronic/chronic studies of atrazine or metabolites in dogs, rats, or mice (Table 3,18 and 27). Non-neoplastic changes, typically associated with symptoms of generalized toxicity, included decreased food consumption and body weight, hematological abnormalities, and degenerative, hyperplastic or inflammatory changes in mammary gland, kidney, urinary bladder, heart, skeletal muscle, prostate or eye. Alterations in estrus cycles and reproductive hormone levels were also detected. The risk after potential chronic human exposure to atrazine for non-neoplastic changes was assessed based on two studies, each of which gave a similar NOEL or BMD.

The first study evaluated was a 1-year dog dietary study, with a NOEL for cardiac toxicity of 0.48 mg/kg/day (O'Connor, 1987). The possible significance of the cardiotoxicity of atrazine is exemplified in Table 27: not only atrazine but also three metabolites caused effects in chronic or sub-chronic studies, in both sexes of dog, on the heart weight, the PII wave (reduced amplitude) and atrial fibrillation. Secondly, extramedullary hematopoiesis (EMH) was observed in the spleen of females but not males, in a chronic rat study (Mayhew, 1986). EMH was recorded in a number of undosed rats, thus making the LOEL and NOEL unclear. A

benchmark-dose approach was therefore used for determination of the 95% lower confidence limit (0.47 mg/kg/day) on a dose corresponding to a 10% increase (ED_{10}) in EMH in exposed rats above controls (2.57 mg/kg/day). The importance of increased EMH in the rat spleen could be considerable. It represents a possible effect on the immune system, particularly since it was detected in combination with hyperplasia in the bone marrow. Possible effects on the immune system of the rat and mouse were also reported in studies described in Section III.I. Furthermore, both EMH and bone marrow hyperplasia were also reported in a sub-chronic study in the rat (Tisdel, 1977) and in a rat chronic toxicity study of cyanazine, the closely related triazine herbicide. There are presently no FIFRA testing requirements for immunotoxicity studies. Therefore, any endpoints that are indicators of possible immunotoxicity should be carefully evaluated.

The dog, chronic NOEL of 0.48 mg/kg/day (rounded to 0.5 mg/kg/day), was used for assessing the chronic risks from possible human exposure to atrazine. U.S. EPA concluded that the chronic NOEL for atrazine risk assessment was 70 ppm, equivalent to 2.86 mg/kg/day, based on decreased body weight gain in a 2-year rat chronic study using atrazine (U.S. EPA, 1994).

Table 27. Summary of Cardiotoxicity of Dietary Atrazine and Metabolites in the Dog.

Chemical	Duration	Endpoint	LOEL mg/kg/day	NOEL mg/kg/day
Atrazine [1/]	1-yr.	⇓PII wave (M+F); ⇑⇓ abs. heart wt.; atrial dilation	4.97	0.48
DIPA [2/]	14-wk.	⇓PII wave (M+F); ⇓ abs. heart wt. (M+F)	18	3.8
DEA [3/]	13-wk.	⇓Body wt. (M+F) ⇓PII wave (M+F); ⇑PII wave duration; ⇓ abs. heart wt.; atrial fibrillation; tachycardia (M+F); atrial inflammation	3.7 28.8	0.5 3.7
DACT [4/]	1-yr.	Clinical signs, incl. death ⇑(M+F); ⇑ rel. heart wt. (M); atrial enlargement; LDH ⇑; EKG: atrial fibrillation	21.5	3.62

References: 1/ O'Connor *et al.*, 1987. 2/ Thompson *et al.*, 1992. 3/ Rudzki *et al.*, 1992. 4/ Thompson *et al.*, 1990.

Oncogenicity

Atrazine-related neoplastic changes detected in a FIFRA-guideline oncogenicity study consisted of an increased incidence of malignant and/or benign mammary tumors in female Sprague-Dawley rats and an increased incidence of benign testicular interstitial cell tumors in male Sprague-Dawley rats (Mayhew, 1986). In females, there was a dose-related increase in the incidence of all mammary tumors combined in atrazine-treated groups, reaching statistical significance at the three highest dose levels of 2.86, 21.8 and 45.3 mg/kg/day. The incidence of mammary adenocarcinoma also increased with dose and reached statistical significance at these dose levels (Table 5). Increased incidence of mammary tumors was observed in an interim sacrifice at 12 months (Table 6) as well as at 13-months (Table 7) in which no control animals had mammary tumors, but the high dose group had an adenocarcinoma incidence of 50% (the intermediate dose groups were not examined histologically). In subsequent studies of atrazine oncogenicity, female Sprague-Dawley rats also indicated an earlier onset time for mammary carcinomas (Thakur, 1991a; 1992a). However, increased cancer incidence was not detected in female Fischer-344 rats (Thakur, 1991b; 1992b). In male rats, the incidence of

mammary gland tumors was not increased, although a statistically significant 3-fold increase in the incidence of mammary hyperplasia was present in the high dose group. Benign testicular interstitial cell tumors were also increased in the highest dose group. The increase was statistically significant, but may have been secondary to the increased survival in that group.

In various other non-FIFRA-guideline long-term studies of atrazine toxicity, increases in mammary, uterine, or lymphoid tumors were reported, but the studies had deficiencies which precluded their regulatory use. For example, there was a significant increase in mammary tumors in male F344 rats at the HDT (Pinter *et al.*, 1990). No carcinogenic effects were present in mice. A chronic toxicity study of the atrazine metabolite, hydroxyatrazine, revealed no increase in oncogenicity in SD rats.

Atrazine predominantly caused an increase in malignant tumors (adenocarcinomas) and other triazine pesticides have also been found to cause an increase in mammary adenocarcinomas in the female SD rat. Also in common with other triazines, the increased level of tumors was accompanied by body weight loss and reduced food intake; in the untreated rat a reduction of mammary tumor incidence accompanied body weight loss and reduced food intake (Turnbull *et al.*, 1985; Boorman *et al.*, 1990; Ip, 1991). It is thus possible that the intrinsic oncogenicity of atrazine is even greater than that measured from the data of Mayhew, 1986, with female SD rats *i.e.* if the food consumption of untreated rats had been restricted to the amount consumed by the high-dose group, the level of mammary tumors in the controls would probably have been lower and thus, in the dosed rats, the proportion of rats with tumors would probably have been even higher relative to the control group. Dietary restriction of female SD rats dosed with the genotoxic carcinogens N-methyl-N-nitrosourea (Beth *et al.*, 1987; Chevalier *et al.*, 1993) or 7,12-dimethylbenz[*a*]anthracene (Klurfeld *et al.*, 1989; Kritchevsky *et al.*, 1989) resulted in a reduced incidence of mammary tumors compared with free-feeding rats. The additional cancers resulting from these carcinogens were abolished at 30% and 40% dietary restriction. It therefore seems probable that the mechanism by which atrazine results in mammary tumors is different from those of these compounds.

It has been hypothesized that triazine herbicides, including atrazine, cause mammary tumors through an estrogen (or endocrine) mechanism (Ciba-Geigy, 1992d). Such a receptor-based mechanism, in the absence of genotoxicity, might justify the use of a threshold model for assessing risks to humans. However, the exact mechanism for the induction of mammary tumors in the female SD rat by atrazine, remains unclear. There is some evidence for genotoxicity resulting from atrazine and/or unidentified derivative(s) of atrazine (Tables 19-21). However, there is also evidence that atrazine disrupts estrus in the SD rat (but not the F344 rat), and this could result from a disruption in the hypothalamic control of the pituitary gland (Cooper, 1995). This in turn could result in a multitude of endocrine systems being affected by atrazine, as has been observed in the SD rat. Cooper *et al.* (1996) have shown that atrazine can disrupt ovarian function in both the SD and Long-Evans hooded rats. Since the SD strain (but not the F344) exhibits mammary tumors induced by atrazine, it is possible that mammary tumors and endocrine disruption are linked. However, it is not known whether atrazine results in an increased incidence of mammary tumors in the Long-Evans rat. Furthermore, the endocrine effects reported by Cooper *et al.*, 1996 resulted in conditions of decreased blood estradiol, elevated progesterone and prolonged diestrus, which are opposite those which favor an elevated incidence of mammary tumors. Therefore, although there is substantial evidence for an endocrine mechanism for oncogenicity, further scientific experiments are needed to identify the exact mechanism. Thus, the default non-threshold, low-dose extrapolation was also used in this risk assessment to address potential oncogenicity of atrazine in humans.

A multistage model, MSTAGE, (Crouch, 1985) was used to extrapolate the dosage response relationship obtained from the experimental animal data at high dose levels to a low dose range which is generally more typical of potential human exposures. Both the maximum likelihood estimate (MLE, Q_1) and the 95% upper confidence limit (UB, Q_1*) of the multistage model were presented as estimates of carcinogenic potency (Appendix II). The potential human oncogenic risk at a low dose range was calculated by multiplying the potency by the potential exposure. The potency values were estimated by fitting the Global 86 model to the incidence of all mammary gland tumors identified in the FIFRA-acceptable rat oncogenicity study (Mayhew, 1986), as shown in Table 28.

The human Q_1* value determined in this way for combined (malignant plus benign) mammary tumors was 0.12 $(mg/kg/day)^{-1}$. U.S. EPA has published (U.S. EPA, 1994) an equivalent Q_1* value of 0.22 $(mg/kg/day)^{-1}$ for atrazine oncogenicity in the same study. However, a conversion factor of $(\text{human body weight/rat body weight})^{2/3}$ was used by U.S. EPA to calculate the human Q_1* value from the rat Q_1*. It is DPR policy to use the 3/4 power instead of the 2/3 power as the animal-to-human dose equivalence, a policy which is to be adopted by U.S. EPA (U.S. EPA, 1992g). A large part of the difference in Q_1* values between DPR and U.S. EPA can be accounted for by this policy difference: multiplying the rat Q_1* of 0.0345 by $(\text{human body weight/rat body weight})^{2/3}$ increases the human Q_1* from 0.12 to 0.18 $(mg/kg/day)^{-1}$. The remainder of the difference can probably be accounted for by the use of default daily dosage levels by U.S. EPA instead of mean, measured dietary chemical intake by DPR. For the triazine herbicide cyanazine, these two factors accounted for the entire difference between U.S. EPA and DPR Q_1* values for humans (Gammon *et al.*, 1997).

Table 28. The MLE and 95% Upper CL for Mammary Gland Tumors in Female Sprague-Dawley Rats on Dietary Atrazine for 2 years (Mayhew,1986).

Tumor Type	Maximum Likelihood Estimate (Q_1) $(mg/kg/day)^{-1}$		95% Upper Confidence Limit (Q_1*) $(mg/kg/day)^{-1}$	
	animal	**human[a]**	**animal**	**human[a]**
Adenocarcinoma[b]	0.0108	0.037	0.0160	0.054
Malignant +benign[c]	0.0189	0.064	0.0345	0.12

a/ The human equivalent Q_1 and Q_1* were calculated by:
Q_1 human = Q_1 animal x (human body weight/animal body weight where the (female) body weight = 55 kg, rat body weight = 0.414 kg.
Q_1 human = 0.0189 x $(55/0.414)^{1/4}$ = 0.064; Q_1* human = 0.0345 x $(55/0.414)^{1/4}$ = 0.12

b/ calculated using data from Table 5: rats with adenocarcinoma(s) /rats at risk.

c/ calculated using data from Table 5: rats with adenocarcinoma(s), carcinosarcoma, fibroadenoma or adenoma /rats at risk. This includes all animals with mammary tumors which originated in the mammary glands.

Using the Morseth (1998) study, cancer potency factors for mammary tumors were estimated by both the MSTAGE model and the Weibull (time-to-tumor) model (Appendix III and IV), in intact SD rats. The MSTAGE model provided rat Q_1 and Q_1* values which were very similar to those obtained from the Mayhew(1986) study for carcinoma(s), but were higher for combined mammary tumors (malignant + benign). Using the Weibull method, the Q_1 and Q_1* values were (50%) higher for carcinoma(s) than the MSTAGE calculation from the Morseth (1998) data, but lower using combined (M+B) tumors. This suggests that atrazine caused the carcinomas, but not the fibroadenomas, to occur earlier. This is consistent with an earlier study in which interim sacrifice SD rats exhibited adenocarcinomas but not fibroadenomas at the HDT and neither tumor in the control animals (Tables 6, 7; Mayhew, 1986).

B. EXPOSURE ASSESSMENT

1. Occupational Exposure (Volume II)

A summary of data from a worker exposure study (Honeycutt *et al*.1996) is presented in Table 29. These values are from Volume 2, prepared by the WH&S Branch of DPR (Sanborn & Mehler, 1999). The principal crop uses of atrazine in California are sorghum and corn, for animal feed (Table 1). Applications are made from the ground and these are not considered seasonal. Uses on forests and rights-of-way have been removed from the label. There are currently no residential uses of atrazine in California.

The ADD value of 1.8 to 6.1 μg/kg/day, AADD values of 0.07 to 0.3 (commercial applicator) or 0.02 to 0.05 μg/kg/day (farmer) and LADD values of 0.04 to 0.1 (commercial applicator) or 0.008 to 0.03 μg/kg/day (farmer) are probably the most appropriate for risk assessment purposes (Section IV.C). These values are from recent studies which reflect current use practices on field corn and sorghum in California and are among the most health-protective obtained.

Table 29. Potential Occupational Exposure to Atrazine [a/]

Work Task	Daily Exposure (mg/person)	ADD[b/] (μg/kg/day)	AADD[c/] (μg/kg/day)	LADD[d/] (μg/kg/day)
Mixer/Loader/Tender Applicator[g/]				
(field corn)				
commercial app.[e/] 15 d/yr.	2.8	1.8 - 6.1[f/]	0.07 - 0.3	0.04 - 0.1
farmer, 3 d/yr.[e/]	2.8	1.8 - 6.1[f/]	0.02 - 0.05	0.008 - 0.03

a/ Numbers are from Table 7, 9,12 or 13 of Volume 2 (Sanborn and Mehler, 1999).
b/ Absorbed Daily Dosage (ADD) = Daily dermal exposure x absorption (5.6%)/kg b. wt.
c/ Annual Average Daily Dosage = ADD (8 h exposure) x d./yr.(15, or 3, farmer / 365 d).
d/ Lifetime Average Daily Dosage (LADD) = AADD x 40 yr. exposure / 75 yr. lifetime.
e/ Long-sleeved shirt, pants, chemical-resistant nitrile gloves
f/ 95%UB = 8.6 - >91 μg/kg/day (passive dosimetry); >13 - 28 μg/kg/day (urine)
g/ Honeycutt *et al*.1996**.**

2. Dietary Exposure

a. Introduction

DPR evaluates the risk of exposure to any active ingredient in the diet by considering two processes: (1) use of residue levels detected in foods to evaluate the risk from total exposure, and (2) use of tolerance levels to evaluate the risk from exposure to individual commodities (Section VI. Tolerance Assessment). For the evaluation of risk to detected residues, the total exposure in the diet is determined for all label-approved raw agricultural commodities, processed forms and animal products (meat and milk) that have established US EPA tolerances. These may be established for the parent compound and associated metabolites.

DPR considers these metabolites and other degradation products that may be of toxicological concern in the dietary assessment.

b. Residue Data

1) General

The sources for residue data for dietary exposure assessment include DPR and federal monitoring programs, field trials, and survey studies. Residue data obtained from the monitoring programs are preferred because they represent a realistic estimate of potential exposure. In the absence of data, surrogate data from the same crop as defined by the US EPA, or theoretical residues equal to US EPA tolerances are used. Residue levels that exceed established tolerances (over-tolerance) are not utilized in the dietary exposure assessment, because they are illegal in the channels-of-trade and are infrequent. However, in a separate risk assessment activity, DPR does evaluate the potential risk from consuming commodities with residues above tolerance levels using an expedited acute risk assessment process.

DPR currently has two major sampling programs: (1) priority pesticide, and (2) marketplace surveillance. The priority pesticide program focuses on pesticides of health concern as determined by DPR Enforcement and Medical Toxicology Branches. Samples are collected from fields known to have been treated with the specific pesticides. For the market place surveillance program, samples are collected at the wholesale and retail outlets, and at the point of entry for imported foods. The sampling strategies for both priority pesticide and marketplace surveillance are similar and are weighted toward such factors as pattern of pesticide use; relative number and volume of pesticides typically used to produce a commodity; relative dietary importance of the commodity; past monitoring results; and the extent of local pesticide use.

The US Food and Drug Administration (FDA) has three monitoring programs for determining residues in food: (1) regulatory monitoring and (2) total diet study, and (3) incidence/level monitoring. For regulatory monitoring , surveillance samples are collected from individual lots of domestic and imported foods at the source of the production or at the wholesale level. In contrast to the regulatory monitoring program, the total diet study monitors residue levels in the form that a commodity is commonly eaten or found in a prepared meal. The incidence/level monitoring program is designed to address specific concerns about pesticides residues in particular foods.

The US Department of Agriculture (USDA) is responsible for the Pesticide Data Program (PDP), a nationwide cooperative monitoring program. The PDP is designed to collect objective, comprehensive pesticide residue data for risk assessments. Several states, including California, collect samples at produce markets and chain store distribution centers close to the consumer level. The pesticide and produce combinations are selected based on the toxicity of the pesticide as well as the need for residue to determine exposure. In addition, USDA is responsible for the National Residue Program which provides data for potential pesticides in meat and poultry. These residues in farm animals can occur from direct application, or consumption of commodities or by-products in their feed.

<u>Acute Exposure</u>

Estimates of potential acute dietary exposure used the highest measured residue values at or below the tolerance for each commodity. The default procedure for acute dietary exposure assumed that "below detection limit" residues were equal to Minimum Detection Level (MDL) for each commodity. In the absence of any residue determinations for a particular commodity, the

DPR acute dietary exposure assessments utilize surrogate data from the same crop group as defined by the US EPA, or theoretical residues equal to US EPA tolerances. The actual residue values reported are presented in Table 30.

The following assumptions are used to estimate potential acute dietary exposure from measured residues: 1) the residue does not change over time, 2) the concentration of residue does not decrease when the raw agricultural commodity (RAC) is washed, 3) processing of RACs into various food forms does not reduce the residue, and 4) all foods that are consumed will contain the highest reported residue.

Chronic Exposure

Estimates of potential chronic dietary exposure used the average of measured and "below detection limit" residues for each commodity. The default procedure assumed that "below detection limit" residues were equal to one-half (50%) of the MDL for each commodity. In the absence of any residue determination for a particular commodity, the DPR chronic dietary exposure assessments utilize surrogate data from the same crop group as defined by the US EPA. The actual residue values reported are presented in Table 30.

The following assumptions were used to estimate potential chronic dietary exposures from measured residues: 1) the residue level does not change over time, 2) residues are not reduced by washing the RAC, 3) processing the RAC's into various food forms does not reduce the residue levels, 4) individuals will consume foods that contain the average calculated residue, and 5) exposures to a commodity at all reported residue levels do occur, i.e. a commodity with the average calculated residue is consumed every day at an annual average level (dosage).

2. (Atrazine) Residue Data

Atrazine (40 CFR #180.220) acute tolerance assessment, acute dietary exposure assessment, chronic non-oncogenic and oncogenic dietary exposure assessment were started in 1993 and completed in May, 2000 (TAS, 1993a,b). All available atrazine raw agricultural commodity (RAC) residue data for atrazine and chlorometabolites were evaluated (Table 30). The USEPA and the registrants are considering revising the CFR 180.220 tolerance of being characterized as atrazine alone to one of atrazine plus significant chlorometabolites. Chemicals measured in registrant submitted field residue studies include: G-30027 (atrazine) CAS # 1912-24-9; G-30033 (2-amino-4-chloro-6-isopropylamino-*s*-triazine), desethylatrazine (DEA) CAS # 6190-65-4; G-28279 (2-amino-4-chloro-6-ethylamino-*s*-triazine), desisopropylatrazine (DIPA) CAS # 1007-28-9; G-28273 (2,4 diamino-6-chloro-*s*-triazine) and diaminochlorotriazine (DACT) CAS # 3397-62-4. Atrazine parent compound and the chlorotriazine-containing metabolites are considered to be of possible toxicological concern (US EPA, 1994). The impact has been to give limited usefulness to previous FDA, USDA, and DPR atrazine monitoring programs, since they were not designed to detect chlorometabolites. The FDA minimum detection level (MDL) for atrazine parent material is 0.01 ppm (FDA, 1988-92). The USDA Food Safety Inspection Service (FSIS) meat monitoring atrazine parent material MDL is 0.005 ppm (USDA, 1991-92). The DPR atrazine parent material MDL was 0.1 ppm prior to 1988, and 0.05 ppm subsequently (DPR, 1988-91). The USDA Food Safety Inspection Service (FSIS) meat monitoring and DPR RAC residue monitoring programs do not currently characterize any atrazine chlorometabolites. The FDA does not routinely monitor for atrazine chlorometabolites and they are not recoverable using FDA methods in fatty food such as milk, eggs, and cheese (FDA , 1991). Therefore all RAC residue data used for atrazine and its chlorometabolites were obtained from Novartis (registrant- supplied field residue data) or the US EPA tolerance was used. The only exception to these sources came from the water residue values which used DPR's special California well water monitoring data obtained from wells known to have been contaminated

with atrazine (DPR, 1994 Table 30). The MCL for atrazine is 0.003 ppm (3 ppb) and the well water samples have shown a high value of 2.73 ppb (found in one of 16 non-municipal wells). The residue of 0.51 ppb is the mean value in the (16) atrazine-contaminated wells. Table 34 contains a summary of relevant margin of exposure data from conducting acute and chronic dietary exposure assessments.

c. Consumption data

1. General

For acute and chronic dietary exposure analysis, it is generally assumed that 100% of the crop is treated with the pesticide. However, it is also considered unrealistic to assume that an individual would consume a crop which has been all (100%) treated with a pesticide, containing averaged residues, for up to 70 years. If current and supportable crop treatment data are available from Pesticide Use Reports, it is reasonable to adjust the 100% treated assumption using more realistic pesticide treatment and use patterns. This method has been employed for two commodities, wheat and grain corn, that have atrazine tolerances. These commodities have very well detailed atrazine use histories at both the state (DPR Pesticide Use Report) and federal (USDA Ag Field Crops Summary annuals) levels. Very conservative assumptions were made when setting the percentage of crop treated adjustment factors for the chronic dietary exposure section. Three years of atrazine use and acreage harvested data on wheat and corn were evaluated based on state and federal data.

2. Specific database used in the analysis

The California wheat acreage harvested in 1990, 1991 and 1992 was 619,000, 442,000 and 605,000 acres, respectively. The harvested California wheat acreage amounts to an average of approximately 1% of the total annual U.S. wheat harvest. Atrazine was applied to 20 acres of California wheat in 1990 and 60 acres in 1991. The United States wheat acreages (12 major production states) harvested were 59 million acres, 57 million acres and 56 million acres, for 1990, 1991 and 1992, respectively. Based on USDA Agriculture Marketing Statistics division information, atrazine was applied to 1% or less of the wheat acreage in the 12 major producing states. Based on this U.S. wheat use information, a 10% crop adjustment factor was used for wheat in the chronic dietary residue TAS file. The actual use data indicates that 99% of the wheat crop in California is not treated, however the 10% adjustment value is conservative and takes into consideration less defined use patterns found in minor wheat production states.

Table 30. Summary of Atrazine Residues used in the Dietary Exposure Assessment

Raw Agricultural Commodity	Source[3/]	Tolerance (ppm)	Residues (ppm) Acute	Chronic	N	Comments
Beef: [1/]	Reg-h					
Meat byproducts		0.02N[5/]	0.02	0.02	-	US EPA
Organmeats		0.02N	0.02	0.02		tolerance
Dried		0.02 N	0.001	0.001		
(Boneless) Fat		0.02 N	0.001	0.001		
Kidney		0.02 N	0.004	0.004		
Liver		0.02N	0.02	0.02		
(Boneless) Lean		0.02 N	0.001	0.001		
Cane sugar	Reg-f	0.25	0.002	0.002	8	Combined MDLs
Chicken: [2/]	Reg-h					
Byproducts		0.02N	0.006	0.006		
Giblets (liver)		0.02N	0.006	0.006		
(Boneless) Fat		0.02N	0.0003	0.0003		
(Boneless) Lean		0.02N	0.0003	0.0003		
Giblets (excl. liver)		0.02N	0.006	0.006		
Corn:	Reg-f					
pop		0.25	0.04	0.02	6	Combined MDLs
sweet			0.06	0.022	24	
grain-endosperm			0.04	0.02	6	
grain-bran			0.04	0.02	6	
grain-oil			0.02	0.02	6	
Eggs:	Reg-h					
whole, white, yolk		0.02N	0.005	0.005	-	US EPA tolerance
	EPA	0.05	0.05	0.025	-	
Guava	Reg-f	0.25	0.20	0.10	6	US EPA tolerance
Macadamia nut						Combined MDLs
Milk	Reg-f					
solids		0.02N	0.005	0.005	4	Extrapolated
sugar (lactose)			0.005	0.005		residues
Molasses	Reg-m		0.02	0.0055		
Sorghum, grain	Reg-f	0.25	0.12	0.105	2	Combined res.
Sugar, cane	Reg-f	0.25	0.002	0.002	8	refined, 0.5 LOD
Water	DPR					
tap, non-food		0.003[4/]	0.00273	0.000631	16	DPR California
bottled			0.00273	0.000631		well
commercial			0.0001	0.00005		
Wheat,						MDL
germ oil	Reg-f	0.25	0.04	0.02	16	
rough, bran	Reg-f		0.04	0.02		Combined MDLs
flour	Reg-m		0.02	0.02		

1/ goat, horse, sheep, pork and veal anticipated residues are based on beef data
2/ turkey and other poultry anticipated residues are based on chicken data.
MDL = Minimum Detection Level
3/ Reg-f = Registrant field study; Reg-h = Registrant high value; Reg-m = Registrant mixed data
4/ US EPA Maximum Contaminant Level (MCL) for atrazine in drinking water.
5/ N = Negligible

The California corn grain acreage harvested in 1990, 1991 and 1992 was 160,000, 115,000 and 145,000 acres, respectively. The total harvested California grain corn acreage constitutes less than 1% of the total annual U.S. grain corn production. Atrazine was applied to 19,377 acres of California grain corn in 1990 (12%) and 2,658 acres in 1991 (2%). The United States grain corn acreage (17 major production states) harvested during 1990 was 74 million acres, in 1991, 69 million and for 1992 it was 71 million acres. Based on USDA Agriculture Marketing Statistics division data, atrazine was applied to 64% of the 1990 acreage, 66% of the 1991 acreage, and to 69% of the 1992 grain corn acreage in the 17 major production states. Based on the U.S. grain corn use information, a 70% crop adjustment could be used for grain corn. The actual adjustment factor used in the chronic dietary TAS file was 80%. The reason for this choice is that, while U.S. corn acreage has been relatively steady, atrazine usage appears to be increasing. It is not known if the increase is actual or just annual variation about the actual figure. The use data indicate that at least 30% of the grain corn crop is not treated with atrazine. However, the 80% adjustment value is conservative and also takes into consideration less well defined use patterns found in the minor grain corn production states.

d. Exposure (TAS) Analysis

1) Acute (daily) EX-4

Acute dietary exposure analysis was conducted using the Exposure-4 software program developed by Technical Assessment Systems, Inc. (TAS). The Exposure-4 program estimates the distribution of user-day (consumer-day) exposure for the overall U.S. population and specific subgroups (TAS, 1990b). A user-day is any day in which a food from the commodity list of the residue file is consumed. The consumption analysis uses individual food consumption data as reported in the 1987-88 USDA Nationwide Food Consumption Survey (USDA, 1987-88). Based on the 95th percentile of user-days exposures for all specific population subgroups, the potential acute dietary exposure to atrazine from all labeled uses ranged from 0.234 to 0.857 μg/kg/day. Infants (< 1 yr.), especially non-nursing ones, had the highest potential acute dietary exposure (Table 31). Seniors (>55 yrs.) had the lowest potential exposure. The complete dietary exposure analysis is presented in Appendix VI.

Table 31. Potential acute and chronic dietary exposure to atrazine in all commodities with U.S. EPA tolerances and in drinking water.

Population subgroup	ACUTE[a/] (μg/kg-day)	CHRONIC [b/] (μg/kg-d)	CHRONIC [c/] adj. (μg/kg-d)
US Pop. all seasons	0.438	0.107	0.070
Western Region	0.446	0.105	0.070
Hispanics	0.492	0.108	0.076
Non-Hispanic Whites	0.427	0.107	0.069
Non-Hispanic Blacks	0.476	0.104	0.069
Non-Hispanic Other	0.454	0.112	0.073
Infants (nursing)	0.407	**0.046**	**0.038**
Infants (non-nursing)	**0.857**[d/]	0.230	**0.203**
Children (1-6 yrs)	0.733	**0.286**	0.201
Children (7-12 yrs)	0.475	0.175	0.115
Females (13-19 yrs, not preg. or nursing)	0.282	0.092	0.057
Females (13+ yrs, preg. not nursing)	0.288	0.092	0.061
Females (13+ yrs, nursing)	0.354	0.097	0.061
Females (20+ yrs, not preg. or nursing)	0.244	0.071	0.045
Males (13-19 yrs)	0.338	0.114	0.071
Males (20+ yrs)	0.250	0.074	0.045
Seniors (55+ yrs)	**0.234**[d/]	0.069	0.044
Pacific Region	0.433	0.103	0.069
All Infants	0.831	0.177	0.156
Females (13-50 yrs.)	0.260	0.076	0.047
Males/Females, 16+ yrs.	0.251	-------	-------

a/ 95th. percentile of dietary exposure based on crops with residues given in Table 30, including drinking water at 2.73 ppb.
b/ Annual average dietary exposure, including water at 0.51 ppb.
c/ Annual average dietary exposure, including water at 0.51 ppb, adjusted for percentage of crop treated.
d/ bolded values give the range

2) Subchronic (seasonal)

It is not anticipated that seasonal, dietary exposure will be an issue for atrazine.

3) Chronic (annual) EX-1

The potential chronic dietary and drinking water exposure was calculated using the Exposure-1™ software program (TAS, 1992b). The food consumption data for the chronic analysis was also based on the 1987-88 USDA Nationwide Food Consumption Survey (USDA, 1987-8). The program estimates the annual average exposure for specific population subgroups. In addition to calculations of theoretical dietary exposure assuming that 100% of each registered crop was treated with atrazine, calculations were made adjusting for percentage of crop-treated in California (Appendix VI).

All potential dietary exposure was pooled by combining atrazine residues in all commodities on which atrazine use is registered (Table 30). The mean potential annual dietary exposure ranged from 0.046 (nursing infants) to 0.286 μg/kg-day (Children, 1-6 yr.), based on 100% of crop treated. Percentage of crop-treated adjustment factors were 80% for corn; 10% for sorghum and wheat. The equivalent mean potential chronic dietary exposure levels, adjusted for percentage of crop-treated, were 0.038 (nursing infants) and 0.203 μg/kg-day (non-nursing infants) (Table 31). Thus, the

theoretical chronic dietary exposure to atrazine using residue data adjusted for percentage of crop-treated was reduced by 17% (infants) and 30% (children, 1-6 yrs.) of the exposure calculated for 100% crop-treated. The complete dietary exposure analysis is in Appendix VI.

4) Combined Occupational/Dietary

The potential acute combined exposure level from occupational and dietary sources has been calculated for the sub-population, Males/Females, 16+ yrs. (the most likely group to include workers) *i.e.* 6.1 (ADD) + 0.251 (Dietary), totaling 6.4 μg/kg/day. Occupational exposure accounts for the major proportion (95%) of total exposure. At the 95%UB of acute, occupational exposure, combined exposure would increase by only about 0.2%, remaining at 91 μg/kg/day.

For potential combined chronic (annual) exposure, the acute occupational exposure was amortized over 3 or 15 days per year (AADD), to give 0.02 - 0.05 (farmer) or 0.07 - 0.3 μg/kg/day (commercial applicator) (Table 29), and added to the chronic (annual) adjusted dietary exposure, 0.04 μg/kg/day (Males, 20+ yrs.). Thus, the combined chronic (high end) exposure estimates are 0.09 μg/kg/day for the farmer and 0.3 μg/kg/day for the commercial applicator.

For potential combined chronic (lifetime) exposure, the chronic occupational exposure (0.05 or 0.3 μg/kg/day) was amortized over 40 years out of 75 years lifetime (LADD), to give 0.03 or 0.1 μg/kg/day, and was added to the chronic (annual) dietary exposure, 0.04 μg/kg/day (Males, 20+ yrs.). Thus, the chronic (lifetime) combined exposure estimates are 0.07 μg/kg/day for the farmer and 0.1(4) μg/kg/day for the commercial applicator.

C. RISK CHARACTERIZATION

The risk characterization process consists of calculating a margin of exposure (MOE) by dividing the critical acute or chronic NOEL value for a specific toxicological endpoint (Section IV A) by an estimate of human exposure (Section IV B). The probability of excess cancer risk in a lifetime was calculated by multiplying the LADD values (occupational) and/or the chronic annual average dietary exposure, by the cancer potency factors. Additionally, the cancer risk was calculated for combined occupational and dietary exposure, through the consumption of potential crop residues, with the inclusion of potential drinking water residues. An excess cancer risk of 1 in a million is generally considered to be a negligible risk for any particular pesticide.

Occupational Exposure

The estimates of occupational exposure, following atrazine application to sorghum or corn, are given as the ADD, AADD and LADD (Table 29). These estimates were used to calculate the acute and chronic MOE, as well as the probability of excess cancer risk in a lifetime, respectively (Tables 32, 33). Based on a recently conducted study on field corn, which reflects current label-required application methods (study 3, Table 29), the acute MOE, based on the mean ADD, for farmers and commercial applicators was 820 to 2,800. Based on the 95$^{th.}$ percentile of the ADD, the MOE was 55 to 580. The annual (chronic) MOE, based on the mean AADD, was 1,700 to 7,100 for commercial applicators and 10,000 to 25,000 for farmers. The probability of excess cancer risk in a lifetime was 2.6E-06 to 6.4E-06 (MLE) and 4.8E-06 to 1.2E-05 (95%UB) for commercial applicators. For farmers, the equivalent figures for excess lifetime cancer risk were 5.1E-07 to 1.9E-06 (MLE) and 9.6E-07 to 3.6E-06 (95%UB).

Table 32. Margins of exposure from potential occupational exposure to atrazine.

WORKER TASK (sorghum or corn)	ADD	ACUTE MOE [a,b/] MEAN	ACUTE MOE [a,b/] 95th. Percentile	AADD	CHRONIC MOE[c/]
M/L/T/A [e/] (field corn)					
commercial app.	1.8 to	820 - 2,800	55 - 580[d/]	0.07-0.3	1,700 - 7,100
farmer	6.1	820 - 2,800	55 - 580[d/]	0.02-.05	10,000 - 25,000

a/ MOE = NOEL (of 5 mg/kg/day, from Arthur, 1984) /ADD.
b/ Mean ADD (4.6 μg/kg/d), 95th. Percentile (28 μg/kg/d), Table 29, from Vol. 2, Table 4.
c/ MOE = NOEL (of 0.5 mg/kg/day, from O'Connor *et al.*, 1987) /AADD.
d/ based on 95th. percentile ADD values ranging from 8.6 to 91 μg/kg/day (Table 29).
e/ Honeycutt *et al.*1996

Table 33. Oncogenic Risks for Workers Exposed to Atrazine.

Work Task	Daily Exposure (mg/person	LADD [b/] (μg/kg/day)	Increased Lifetime Risk [a/] MLE [c/]	Increased Lifetime Risk [a/] UB [d/]
Mixer/Loader/Tender/ Applicator [e/] (field corn)				
commercial app.,15 d/y	2.8	0.04-0.1	2.6E-06 - 6.4E-06	4.8E-06 - 1.2E-05
farmer, 3 d/yr.	2.8	0.008-0.03	5.1E-07 - 1.9E-06	9.6E-07 - 3.6E-06

a/ based on the product of LADD values (Table 29) and human cancer potency factor (Q_1 or MLE and Q_1* or 95% confidence interval, UB, in Table 28),) derived using incidence data for all rats showing mammary tumors originating in the mammary glands, in the female SD rat (Mayhew, 1986).
b/ Lifetime Average Daily Dosage
c/ increased lifetime risk calculated at human equivalent MLE of 0.064 $(mg/kg/day)^{-1}$
d/ increased lifetime risk calculated at human equivalent UB of 0.12 $(mg/kg/day)^{-1}$
e/ numbers are from Table 29, taken from Tables 3 and 4 of Volume 2.

Table 34. Margins of Exposure (MOEs) for Potential Dietary Exposure to Atrazine in all Commodities with U.S. EPA Tolerances + Drinking water

Population subgroup	ACUTE[a/] MOE	CHRONIC [b/] MOE	CHRONIC [c/] adj. (μg/kg-d)
US Pop. all seasons	11,400	4,690	7,180
Western Region	11,200	4,760	7,190
Hispanics	10,200	4,610	6,580
Non-Hispanic Whites	11,700	4,680	7,240
Non-Hispanic Blacks	10,500	4,800	7,280
Non-Hispanic Other	11,000	4,460	6,870
Infants (nursing)	12,300	**10,900**	**13,100**
Infants (non-nursing)	**5,830**[d/]	2,170	**2,460**
Children (1-6 yrs)	6,820	**1,750**	2,490
Children (7-12 yrs)	10,500	2,860	4,360
Females (13-19 yrs, not preg. or nursing)	17,700	5,450	8,710
Females (13+ yrs, preg. not nursing)	17,300	5,420	8,150
Females (13+ yrs, nursing)	14,100	5,150	8,210
Females (20+ yrs, not preg. or nursing)	20,500	7,060	11,200
Males (13-19 yrs)	14,800	4,390	7,000
Males (20+ yrs)	20,000	6,720	11,100
Seniors (55+ yrs)	**21,300**[d/]	7,260	11,300
Pacific Region	11,600	4,850	7,280
All Infants	6,020	2,820	3,210
Females (13-50 yrs.)	19,200	6,570	10,600
Males/Females, 16+ yrs.	19,900	-------	-------

a/ MOE=NOEL/95$^{th.}$ percentile of acute dietary exposure (Table 31); based on an acute NOEL of 5 mg/kg/day (from Arthur, 1984).
b/ MOE = NOEL / annual average dietary exposure, all crops (Table 31); based on a chronic NOEL of 0.5 mg/kg/day (from O'Connor, 1987).
c/ as for Chronic MOE (above) except that the "Percent crop treated" factor was used
d/ bolded values give the range

Dietary Exposure

Acute Exposure

The MOEs for all population subgroups ranged from 5,830 for (non-nursing) infants to 21,300 for seniors. The MOEs are listed in Table 34.

Chronic Exposure

The MOEs for these subgroups ranged from 1,750 for children (1-6 yrs.) to 10,900 for (nursing) infants. Using residue data adjusted for percent of crop treated, the equivalent MOEs were 2,460 for non-nursing infants and 13,100, for nursing infants, respectively.

Lifetime Exposure

The excess oncogenic risks from dietary exposure to the US population (all seasons) were 4.5E-06 to 8.4E-06 for Q_1 and Q_1*, respectively, using percent of crop treated.

Combined Occupational and Dietary Exposure

The acute MOE for the male (16+ yrs.) of 700 was reduced to 675 (*i.e.* 3.5% less) when potential dietary exposure was added to potential occupational exposure. For 95%UB acute occupational exposure, the combined MOE would remain at 55. For chronic combined exposure, the MOE fell to 5,000 (farmer) and 1,500 (commercial applicator). For the US population (all seasons), the excess oncogenic risk for combined exposure, for the farmer, was increased to 4.8E-06 (MLE) to 1.2E-05 (UB), using percent crop-treated or to 7.2E-06 (MLE) to 1.7E-05 (UB), assuming that 100% of each crop was treated. For the commercial applicator, the excess oncogenic risk for combined exposure was increased to 6.8E-06 (MLE) to 2.0E-05 (UB) using percent crop treated adjustment or 9.2E-06 (MLE) to 2.4E-05 (UB) for 100% of crop-treated.

V. RISK APPRAISAL

A. Introduction

Risk assessment is the process which is used to evaluate the potential for exposure and the likelihood that the toxic effects of a substance will occur in humans under specific exposure conditions. Every risk assessment has inherent limitations and uncertainties in the application of existing data to estimate the potential risk to human health. Therefore, certain *a priori* assumptions are incorporated into the hazard identification, dose-response assessment and exposure assessment processes. These, in turn, result in uncertainty in the risk characterization, which integrates all of the information in these three processes. Qualitatively, risk assessment for all chemicals has similar types of uncertainty. However, the degree or magnitude of the uncertainty varies depending on the availability and quality of the data and the exposure scenarios being assessed. Varying degrees of uncertainty are involved in the estimation of these parameters, affecting the accuracy of the risk characterization. Specific areas of uncertainty associated with this risk assessment for atrazine are delineated in the following discussion.

B. Hazard Identification

Acute toxicity tests measure the effects of a chemical after a single or brief period of exposure. Developmental toxicity studies are a special case in the battery of such tests. Typically, daily dosages are administered to pregnant animals during the period of organogenesis of the fetus. In the absence of data to the contrary, it is generally assumed that a reported developmental effect, such as a malformation or anomaly, can result from a single dose on a particular day during this dosing period (U.S. EPA, 1991a). Atrazine is not removed from the body within 24 hours; metabolites were still detected in urine 48 h after an oral dose to humans (Davidson, 1988). It is therefore possible that an effect could occur after repeated dosing and result from an accumulation of chemical above a critical threshold. In such a case, the acute NOEL value would underestimate the "true" NOEL and the “true” MOE. The NOEL value used to determine the acute MOE values for atrazine was derived from such a study, using New Zealand white rabbits. The maternal NOEL was based on an increased incidence of blood on the vulva or cage and the NOEL for developmental toxicity was based on decreases in live litter size and mean fetal weight, increased resorptions and an increased incidence of skeletal variations (Arthur, 1984). Moreover, perhaps partly because of the dose selection used, the NOEL in this study (5 mg/kg/day) was 15-fold below the LOEL (75 mg/kg/day) and it is quite possible that the “true” NOEL is closer to 75 than to 5 mg/kg/day. It should be noted, however, that the next lowest acute NOEL was 25 mg/kg/day for developmental and maternal toxicity in the rat.

In the evaluation of chronic toxicity the most sensitive non-cancer toxicological endpoint in rodents and dogs was loss of body weight and body weight gain, along with cardiotoxicity in the dog. An increased incidence of EMH in the spleen was also noted in the female SD rat, with a BMD the same as the NOEL for cardiotoxicity in the dog. However, increased EMH could have been secondary to an increased incidence of mammary carcinomas, which has been analyzed separately. Furthermore, in an analysis of historical control data for F-344 rats in 90-day studies, EMH was observed in the spleen in 100% (159/159) of rats of both sexes (Dixon *et al.*, 1995). It is therefore possible that the incidence of EMH in the atrazine chronic study is merely a reflection of the natural aging process in the SD rat, having limited toxicological significance. The NOEL for cardiotoxicity in the dog (O’Connor *et al.*, 1987) was used to assess risk arising from chronic exposure. In this study, perhaps partly as a result of the dose selection, the NOEL (15 ppm, equivalent to 0.5 mg/kg/day) was 10-fold below the LOEL (150 ppm). The toxicity at 1,000 ppm was clear-cut, but the assessment of toxicity at 150 ppm was compromised by a male death; one of the three remaining males at this dose showed cardiotoxicity qualitatively similar to

that at 1,000 ppm. Therefore, it is possible that the NOEL is closer to 150 ppm than it is to 15 ppm. It should be noted that cardiotoxicity in the form of similar (atrial) effects was also observed in sub-chronic toxicity studies with the atrazine metabolites DIPA and DEA (Thompson *et al.*, 1992; Rudski *et al.*, 1992). The NOEL dose for both compounds was 100 ppm and the LOEL values were 500 ppm (DIPA) and 1,000 ppm (DEA).

Oncogenicity was assessed using a linear multi-stage model which assumes a non-threshold mechanism. It is possible that mammary tumors resulting from atrazine exposure in the female rat arose from an estrogenic (receptor-mediated) effect (Stevens *et al.*, 1994; Tennant *et al.*, 1994), which might be expected to show a threshold. Ovariectomy has been shown to eliminate mammary tumors in the SD rat which arise both "spontaneously" and as a result of atrazine administration (Morseth, 1998). This is unlike other known (genotoxic or estrogenic) mammary gland carcinogens, which continue to increase the incidence of mammary tumors in ovariectomized rats (Geschickler & Byrnes, 1942; Li & Li, 1995). It has been noted that triazine herbicides induce aromatase in human placental cells. It is possible that the ovary is the source of the aromatase in SD rats (resulting in estrogen biosynthesis), since the mammalian ovary is known to contain mixed function oxidases and various cytochrome systems (Thomas, 1996). In order to confirm this, the induction of aromatase would need to be demonstrated in the ovary of the SD rat (and presumably, not be induced in the F-344 rat). Such a mechanism would reduce the relevance of the linearized multi-stage model for quantifying mammary tumors induced by atrazine for human risk assessment purposes; instead, a MOE approach could be used exclusively. Other risk assessments of simazine and atrazine have been conducted, by the British Government for the EU (UKPD, 1996), the Australian Government (NRA, 1997) and by IARC (1999). All of these bodies have concluded that mammary tumors in the SD rat are formed *via* a secondary, hormone-mediated mechanism, of little or no toxicological relevance to human health risk assessment. Each authority has determined that atrazine is unlikely to be a human carcinogen or has inadequate evidence of carcinogenicity.

Atrazine showed some positive responses in genotoxicity assays, but atrazine and many of its metabolites were negative in the standard battery of genotoxicity assays. However, a potentially genotoxic degradate of atrazine, nitroso-atrazine, was identified in soil which had been incubated with atrazine and sodium nitrite (Zwickenpflug & Richter, 1994). The genotoxicity of nitroso compounds probably occurs through the formation of a carbonium ion (Reddy & Hayes, 1989). Triazine herbicides which were incubated in human gastric juice containing sodium nitrite, *in vitro*, were also transformed into nitroso-derivatives (Cova *et al.*, 1996). In a non-FIFRA guideline study, nitroso-atrazine was not carcinogenic in either female Swiss mice or female Wistar rats, despite being fed at doses which caused toxic effects (Weisenburger *et al.*, 1990). Furthermore, genotoxic nitroso compounds are usually highly reactive and induce tumors close to the site of their production or first contact with an organism *e.g.* the lung for tobacco nitroso compounds. It is thus difficult to rationalize why atrazine-induced tumors, if they were a result of nitroso-atrazine formation, would be specific to the mammary glands, and of one sex of one strain of rat. In Sasaki et al., 2000 the effects of nine *N*-nitroso compounds were reviewed. All were carcinogenic at multiple sites in both sexes of both rats and mice (and several other animal species also, in some cases). The most common sites for cancer were stomach, colon, lung, liver, kidney, bladder. None of them caused mammary tumors of any sort in any species. Using the COMET assay in the mouse *in vivo*, all of these *N*-nitroso compounds caused DNA damage in stomach, colon, liver and lung. Eight of them also caused DNA damage in the bladder and a different eight also caused DNA damage in the kidney. None of these *N*-nitroso compounds resulted in DNA damage in mammary gland tissue. Thus, there was excellent concordance between the sites of genetic damage in a short-term test (COMET) and the sites of cancer in chronic studies.

Current analytical methods for residues of atrazine and atrazine degradates in water and crops do not measure nitroso-triazines; therefore, potential human exposure is not known. The

relationship between potentially genotoxic degradates of atrazine and the expression of oncogenicity is a subject which awaits further study.

C. Exposure Assessment

Occupational Exposure

Occupational exposure studies using atrazine 4L formulations on corn and sorghum probably provide a good reflection of total use since these are the major use crops in California. Novartis has 3 registered products in California (Aatrex 4L, Aatrex Nine-O and BICEP II Magnum) and Drexel has one (Drexel Atrazine 4L), indicating that 4L is the most preponderant formulation type. In addition, the occupational exposure studies used to estimate probable exposure are fairly recent and therefore reflect current use rates and appropriate engineering controls. The sources of error are therefore probably few and are likely to be related to the use of atrazine on other, less frequently treated crops for which labels have been discontinued in California, such as forest trees and rights-of-way. However, it should be noted that current labels require the M/L/A to wear, in addition to the PPE used in the studies [coveralls (or long-sleeved shirt and pants), waterproof gloves], chemical-resistant footwear and socks. For the M/L, protective eyewear is also required. It is therefore reasonable to assume that occupational exposure would actually be lower for workers wearing the appropriate PPE than the exposure numbers determined (Sanborn and Mehler, 1999). Because dermal absorption is much more complete after application of a pesticide to the face than to most other body parts (Feldmann & Maibach, 1974), this omission could have been significant under field conditions. The atrazine 4L label does *not* currently require a closed system or closed cab to reduce occupational exposure.

Human dermal penetration data were available; absorption was measured using the 4L formulation containing [^{14}C]-labeled atrazine. The value of 5.6% used in calculations was the mean absorption at the low application dose; it was based on recovery of ^{14}C in urine plus feces. Because dermal absorption at a higher dose was only 1.2%, reflecting the inverse relationship between dose applied and percentage absorbed that normally applies to the dermal absorption of pesticides (Feldmann & Maibach, 1974), 5.6% may be an overestimate of dermal penetration under practical occupational exposure conditions. However, depending on the method used for measuring dermal penetration in humans, it is possible that (apparent) absorption through the skin could overestimate actual absorption. This is because volatilization of a pesticide will be greater over a greater surface area (of skin). Such loss will not necessarily be reported as such for a pesticide but may be assumed to have penetrated the skin. It may also be relevant to note that the field workers would likely have perspired more than the laboratory subjects; the effect of this on relative dermal penetration is unknown, but is probably compound and/or formulation-dependent.

An assumption, which would tend to increase the occupational exposure estimates, was the use of a maximum number of loads per day. On the other hand, a factor which would reduce occupational exposure was the use of a mean application rate (1.5 lb. a.i./A) rather than the maximum label rate (2.5 lb. a.i./A). The information on application rates was obtained from the California pesticide usage reports, 1995-1997. The use of geometric (rather than arithmetic) mean values for ADD, AADD and LADD will tend to underestimate exposure.

Residential Exposure

There are currently no registrations in California for Home and Garden uses of atrazine. Thus exposure from residential uses of atrazine is irrelevant.

Dietary Exposure

Residues of atrazine are rarely detected at harvest in any RACs. Therefore, the default residue values used for calculating possible dietary exposure are probably a “worst-case” situation. Furthermore, because the tolerance for atrazine includes (3) chlorotriazine degradates, default residue values for these have been included also in the calculations of possible dietary exposure. In practice, the actual MOE values for dietary exposure are thus likely to be considerably higher than those calculated. The residues in drinking water, which were used for calculating MOE values and excess cancer risk, were probably upper bounds on likely actual exposure. The acute drinking water residue was the highest detected in a municipal well; the chronic value was the mean of 16 wells which were all contaminated with atrazine *i.e.* wells which were free of atrazine were not included.

D. Risk Characterization

For the reasons described in Sections B and C, it is likely that the characterization of risk represents a "worst-case scenario."

E. Issues Related to the Food Quality Protection Act

Introduction

The Food Quality Protection Act of 1996 mandated USA EPA to "upgrade its risk assessment process as part of the tolerance setting procedures" (US EPA, 1997a,b). The changes to risk assessment were based in part on recommendations from the National Academy of Sciences report, "Pesticides in the Diets of Infants and Children" (NRC, 1993). The act required an explicit determination that tolerances were safe to children. US EPA was required to use an extra 10-fold safety factor to take into account both pre-/post natal developmental toxicity and the completeness of the database, unless US EPA determined, based on reliable data, that a different margin would be safe. In addition, US EPA must consider available information on: 1/ aggregate exposure from all non-occupational sources; 2/ effects of cumulative exposure to the pesticide plus others with common mechanisms of toxicity; 3/ effects of *in utero* exposure; 4/ the potential for endocrine disrupting effects.

Aggregate Exposure(s)

This refers to the possibility that an individual might be exposed to a particular chemical by more than one route. In the case of atrazine, exposure is likely to be almost entirely *via* the dermal route for occupational exposure and through the oral route for dietary exposure. Combined exposure (occupational and dietary) has been calculated in Section B.2.d.4. and it is noted that for a 16+ yrs. person, occupational exposure would account for *ca.* 96% of the total exposure. There are no home and garden registrations for atrazine in California.

Cumulative Exposure(s)

There is a possibility that an individual could be exposed to multiple chemicals sharing the same mechanism of toxicity. An effort is to be made under FQPA to attempt to combine these "cumulative exposure(s)" to related chemicals. In the case of atrazine, such multiple chemical

exposure(s) are difficult to estimate because only one other chlorotriazine, cyanazine, has had a risk assessment completed by DPR. This compound is being phased out and, for some of its former uses, it is being replaced by another triazine, propazine. However, a risk assessment has not yet been commenced for propazine, or for the most heavily used triazine in California, simazine. It is therefore difficult, at the present time, to anticipate the exposure(s) and risk from "cumulative exposure(s)" to the triazine class of pesticides.

Pre-/Post Natal Sensitivity

Four developmental toxicity studies (three rat, one rabbit) failed to show fetal or embryonic toxicity at doses of atrazine less than those affecting dams. No evidence was forthcoming from these experiments that there was an increase in sensitivity among fetal/embryonic animals compared with adults. It is therefore unlikely that an additional factor will be required to protect against increased pre-/post natal sensitivity to atrazine. However, the developmental toxicity of four atrazine degradates was also studied in the rat. For two of these, developmental toxicity was observed below doses which caused maternal toxicity: desisopropylatrazine caused fused sternebrae in the fetus (with a LOEL of 25 mg/kg/day, NOEL of 5 mg/kg/day) whereas in dams, a loss of mean body weight was reported (with a LOEL of 100 mg/kg/day, NOEL of 25 mg/kg/day). Similarly, diaminochlorotriazine caused incomplete skeletal ossification in the fetus (with a LOEL of 25 mg/kg/day, NOEL of 2.5 mg/kg/day) whereas in dams, a loss of mean body weight and food intake were reported (with a LOEL of 75 mg/kg/day, NOEL of 25 mg/kg/day).

In a 2-generation (SD) rat reproductive toxicity study, reduced food intake, body weight and body weight gain were reported in adults (with a LOEL of 25 mg/kg/day, NOEL of 2.5 mg/kg/day) with no effects on reproduction (NOEL$\geq$25 mg/kg/day). It is therefore unlikely that atrazine has adverse effects on reproduction.

Endocrine Effects

Endocrine effects caused by a pesticide are also to be addressed under FQPA. The main hormonal systems under consideration are male and female reproductive hormones and thyroid hormones. Several literature and supplementary reports have found effects which could represent endocrine effects: atrazine inhibited the binding of [^{3}H]-5α-dihydro-testosterone to receptors in the hypothalamus, pituitary and prostate of male rats, *in vitro* and *in vivo*; numerous reports showed premature reproductive senescence, along with other effects which could have arisen through endocrine mechanism(s), in female SD rats; triazine herbicides induced the enzyme aromatase in human placental carcinoma cells, *in vitro*, and an analogous effect could account for the reduced testosterone and increased estradiol levels which have been reported in the blood of male Wistar rats after atrazine dosing; administration of atrazine to the rat for 15 days resulted in increased levels of thyroid hormones, T_3 and T_4 (thyroxine) in the blood. It is difficult to know the role that this information might have in risk assessment because the mechanisms for these effects are not fully understood. In conclusion, DPR does not believe that the exact mechanism of atrazine-induced mammary tumors has yet been resolved. However, the preponderance of evidence is that an endocrine mechanism exists. Such mechanism(s) could include aromatase induction, an effect on the hypothalamic/pituitary axis and/or an effect on an endocrine hormone receptor. Although there is limited evidence for each of these three mechanisms, critical experimental evidence is lacking.

VI. TOLERANCE ASSESSMENT

A. BACKGROUND

A tolerance is the maximum, legal amount of a pesticide residue that is allowed on a raw or processed agricultural commodity, or in an animal tissue used for human consumption. The U.S. EPA tolerance program was developed as an enforcement mechanism to identify illegal residue concentrations resulting from potential non-compliance with the product label requirements (e.g. improper application rates or methods, inadequate pre-harvest intervals, direct or indirect application to unapproved commodities). Tolerances are enforced by FDA, USDA, and state enforcement agencies (e.g. Pesticide Enforcement Branch of DPR).

Currently, pesticide tolerances are generally set at levels that are not expected to produce deleterious health effects in humans from chronic dietary exposure. Typical data requirements for establishing a specific tolerance include 1) toxicology data for the parent compound, major metabolism or degradation products and impurities, 2) product chemistry, 3) analytical method(s) that are readily available, accurate and precise, 4) measured residues in crops used for animal feeds, 5) measured residues in animal tissues (e.g. meat, milk, eggs) from direct or indirect (feed) applications, 6) measured residue levels from field studies. The minimum requirements for the field study include: 1) an application rate at or above the highest rate on the product label, 2) the greatest number of allowable repeat applications, 3) the shortest pre-harvest interval listed on the product label. Generally the registrant of a pesticide requests a commodity-specific tolerance, which is equal to the highest measured residue, or some multiple of that value, from the field trial using the specific pesticide.

Assembly Bill 2161 (Bronzan and Jones, 1989) requires the DPR to "conduct an assessment of dietary risks associated with the consumption of produce and processed food treated with pesticides". In the situation where "any pesticide use represents a dietary risk that is deleterious to the health of humans, the DPR shall prohibit or take action to modify that use or modify the tolerance....." As part of the tolerance assessment, a theoretical dietary exposure for a specific commodity and specific population subgroups can be calculated from the product of the tolerance and the daily consumption rate.

ACUTE EXPOSURE

An acute dietary exposure assessment using the residue level equal to the tolerance was conducted for each individual label-approved commodity. The TAS Exposure-4 software program (TAS, 1990a) and the USDA consumption data base (USDA) were used in this assessment. The acute tolerance assessment does not routinely address multiple commodities at the tolerance levels since the probability of consuming multiple commodities at the tolerance decreases as the number of commodities included in the assessment increases.

Results

The RAC accounting for the highest dietary exposure to atrazine is sweet corn. Based on the 95th percentile of the theoretical consumption rate estimate (exposure dosage) of 2.472 μg/kg-bw/day, the lowest acute MOE was 2,020, for non-nursing infants consuming sweet corn. All MOEs were considered adequate for the protection of human health. Margins of exposure for other RACs and population subgroups are given in Table 35.

CHRONIC EXPOSURE

A theoretical chronic exposure assessment using residues equal to the established tolerances for individual (or combinations of) commodities was not conducted, because it is highly improbable that an individual would chronically consume single or multiple commodities with pesticide residues at the tolerance levels. Support for this conclusion comes from the DPR pesticide monitoring programs which indicate that less than one percent of all sampled commodities have residues at or above the established tolerances (*e.g.* DPR, 1992b).

Table 35. Atrazine - Acute Tolerance Assessment Summary.

Commodity (RAC)	Consumption estimate (% days)	Tolerance (ppm) N=Negligible	Acute Tolerance Contribution (μg/kg-bw/day)	Theoretical MOE 95th. percentile (range)[1/]
Beef (fat)	11 - 95%	0.02N	0.008 - 0.033	150,000[c/] - 599,000[e/]
Beef (lean	10 - 76%	0.02N	0.047 - 0.137	36,400[a/] - 106,000[e/]
Cane sugar	38 - 100%	0.25	0.116 - 0.438	11,400[b/] - 43,300[d/]
Corn, grain	38 - 100%	0.25	0.330 - 2.085	2,400[c/] - 15,200[d/]
Corn, sweet	<1 - 17%	0.25	0.635 - 2.472	2,020[a/] - 7,870[f/]
Guava	<1 - 2%	0.05	0.003 - 0.051	98,300[g/] - >1 million[h/]
Milk	27 - 100%	0.02N	0.211 - 2.069	2,420[a/] - 23,700[d/]
Poultry (fat)	<1 - 44%	0.02N	0.006 - 0.022	229,000[a/] - 871,000[e/]
Poultry(lean)	<1 - 44%	0.02N	0.056 - 0.159	31,500[a/] - 88,800[f/]
Wheat	9 - 99%	0.25	0.570 - 1.816	2,750[b/] - 8,780[d/]

1/ Population Subgroups:
a/ Non-nursing infants
b/ Children (1-6 Years)
c/ All infants
d/ Seniors (55+ Years)
e/ Females (13+ Years/Nursing)
f/ Females (13+ Years/Pregnant/Not Nursing)
g/ Western U.S.
h/ Males = (13-19 Years)

Comment: Pineapple tolerance for atrazine was cancelled in 1998, therefore it was not evaluated.

VII. CONCLUSIONS

The toxicological risk from potential exposure to technical atrazine, as present in herbicide formulations, was evaluated for agricultural workers, and for anticipated dietary exposure, including water. The potential risk from consumption of water and foods containing the highest legal residues of atrazine (tolerances) was also assessed.

A margin of exposure or MOE of at least 100 is generally considered adequate to protect people from the toxic effects of a chemical when the NOEL is based on toxicology data from animal studies. In the case of atrazine, MOE values were calculated using currently available toxicity data from animal studies and an occupational exposure study, from application to corn. Mean, short-term worker exposure data resulted in MOE values above 100 for both farmers and commercial applicators when calculated using toxicity to the rabbit fetus as the toxicological endpoint. An estimated 95$^{th.}$ percentile of acute exposure gave an MOE value of 55 *i.e.* below 100, for these workers. Long-term occupational exposure data resulted in MOE values above 100 for both farmers and commercial applicators when calculated using cardiotoxicity in a dog chronic study as the toxicological endpoint. For a genotoxic carcinogen, an excess lifetime cancer risk of below 10^{-6} (1 in 1,000,000) is generally considered negligible for the carcinogenic effects of a chemical. Based on an increased incidence of mammary tumors in the female SD rat, this risk was 4.8 to 12 in a million *i.e.* greater than 10^{-5} but less than 10^{-4} for commercial applicators and 0.96 to 3.6 in a million *i.e.* greater than 10^{-6} but less than 10^{-5} for farmers. Recent studies have indicated that, instead of causing cancer by a genotoxic mechanism, atrazine may induce aromatase, the enzyme which converts androgen hormones to estrogens. Such a mechanism would be anticipated to exhibit a threshold, thus making the linearized, low-dose cancer risk calculations inappropriate. Instead, the MOE calculations (above) for long term toxicity would be used to include cancer. Because the aromatase mechanism would qualify as an endocrine effect within the meaning of FQPA (1996), an additional $\leq$10-fold margin of exposure would need to be considered if the NOEL for this effect were being used for assessing chronic risk from atrazine exposure. However, there is no evidence that cardiotoxicity in the dog resulting from atrazine administration has an endocrine basis. Therefore, this endpoint would not be considered under FQPA to require an extra 10-fold uncertainty factor.

Based on the available toxicity and residue data, DPR concluded that the MOE values for potential acute (daily) dietary exposure of 5,830 to 21,300 and chronic (annual) dietary exposure of 2,460 to 13,100, for all commodities for which U.S. EPA tolerances have been established plus drinking water, were above 100 for all population subgroups studied. The excess lifetime cancer risk for the general population was 4.5 to 8.4 in a million *i.e.* greater than 10^{-6} but less than $10,^{-5}$ when adjusted for the percentage of crop treated. As has been described, it is possible that a threshold (hormonal) mechanism may explain the increased incidence of mammary tumors, thus making the linearized cancer risk calculations inappropriate. In addition, because most of the residue data used for assessing dietary risk comprised non-detects *i.e.* LOD values, it is likely that the exposure calculations are overestimates, giving rise to overestimates of dietary risk.

The consumption of crops with residues at tolerance resulted in (acute) MOE values above 2000, for all population subgroups. The lowest MOE, of 2020, was for non-nursing infants eating sweet corn with residues of 0.25 ppm.

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IX. APPENDIX I

Table A1. Historical Control Mammary Tumor Data for Female SD Rats in 2-Year Studies at EPL Inc.[a/]

Tumor Type	Mayhew, 1986[b/]	Study A (1982-84)[b/]	Study B (1982-84)[b/]	Study C (1982-84)[c/]	Study D (1981-84)[c/]
Adenocarcinoma	15/66 (23%)	3/37 (8.1%)	16/65 (25%)	3/43 (7.0%)	2/34 (5.9%)
Carcinosarcoma	0/66	0/37	0/65	0/43	0/34
Adenoma	1/66 (1.5%)	0/37	0/65	0/43	4/34 (12%)
Fibroadenoma	29/66 (44%)	17/37 (46%)	41/65 (63%)	25/43 (58%)	23/34 (68%)

a/ it is unclear whether interim sacrifice data were included in the historical control data. However, they were not included in the data calculated for Mayhew, 1986.
b/ includes terminal sacrifice (24 mos.) plus rats killed *in extremis*, 12 to 24 mos.
c/ includes terminal sacrifice (24 mos.) plus rats killed *in extremis*, 18 to 24 mos.

APPENDIX II

Global 86: file for mstage calculation
Atrazine, Female Rats w/ Adenocarcinomas, Mammary Glands, from Mayhew (1986).
5 0 0 2 1 0 1 1 -1
66 64 68 65 64
0.0 0.41 2.86 21.8 45.3
15 15 26 27 35
1E-6 2

```
      DATE: 10/05/1995     GLOBAL 86 (MAY 1986)      TIME: 11:30:28
     BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM
 CLEMENT ASSOCIATES, 1201 GAINES STREET, RUSTON, LA 71270; (318) 255-4800

  Atrazine, Female Rats with Adenocarcinomas in Mammary Glands

      POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
                            #RESPONSES              #RESPONSES
   GROUP    DOSE       OBSERVED/#ANIMALS     PREDICTED
     1      .000000          15/ 66                17.78
     2      .410000          15/ 64                17.45
     3      2.86000          26/ 68                19.83
     4      21.8000          27/ 65                27.50
     5      45.3000          35/ 64                35.38

      CHI-SQUARE GOODNESS OF FIT STATISTIC IS 3.7981
      P-VALUE FOR THE MONTE CARLO TEST IS .2250000000
   FORM OF PROBABILITY FUNCTION:
      P(DOSE) = 1 - exp( -Q0 - Q1 * D - Q2 * D^2 )

         MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS
      ------------------------------------------------------------
                  Q( 0) =    .313817451014
                  Q( 1) =   1.083682206760E-02
                  Q( 2) =    .000000000000

      MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -205.503875790
         CALCULATIONS ARE BASED UPON EXTRA RISK

      GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK
      ********************************************************
                       LOWER BOUND    CONFIDENCE   COEFFICIENTS FOR
   RISK       MLE DOSE       ON DOSE      LIMIT SIZE   CONFIDENCE LIMIT
   ----       --------     -----------    ----------   ----------------
 1.00000E-06   9.22780E-05    6.23365E-05      95.0%     Q( 0) =   .28148
                                                        Q( 1) =  1.60420E-02
                                                        Q( 2) =   .00000
 NORMAL COMPLETION!
```

ATRAZINE 8-15-01

Atrazine, Female Rats with All Mammary Gland Tumors from Mayhew (1986).
5 0 0 2 1 0 1 1 -1
66 64 68 65 64
0.0 0.41 2.86 21.8 45.3
35 39 47 47 56
1E-6 2

DATE: 09/28/1995 GLOBAL 86 (MAY 1986) TIME: 16:30:07
BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM
CLEMENT ASSOCIATES, 1201 GAINES STREET, RUSTON, LA 71270; (318) 255-4800

Atrazine: Female Rats with All Tumors Combined in Mammary Glands

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	35/ 66	39.52
2	.410000	39/ 64	38.52
3	2.86000	47/ 68	42.15
4	21.8000	47/ 65	47.79
5	45.3000	56/ 64	55.99

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 2.8196
P-VALUE FOR THE MONTE CARLO TEST IS .2650000000
FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2 - ... - Q 6 * D^ 6)
MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .913392754030
Q(1) = 1.887028645769E-02
Q(2) = .000000000000

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -194.364638618
CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK
****

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-06	5.29934E-05	2.89769E-05	95.0%	Q(0) = .84152
				Q(1) = 3.45103E-02
				Q(2) = .00000

NORMAL COMPLETION!

APPENDIX III

Table B1. The MLE and 95% Upper CL for Mammary Gland Tumors in Female Sprague-Dawley Rats Dosed with Atrazine for 2 years.[a/]

Tumor type	Maximum Likelihood Estimate Q_1 in $(mg/kg/d)^{-1}$	95% Upper Confidence Limit Q_1* in $(mg/kg/d)^{-1}$
GLOBAL 86 (MSTAGE)		
Carcinoma	0.012	0.021
Combined (M* + B)	0.13	0.20
WEIBULL 82 (Time-to Tumor)		
Carcinoma	0.018	0.032
Combined (M* + B)	0.045	0.074

a/ data from Morseth, 1998

APPENDIX IV

Morseth, 1998: Weibull File

160554 controls mam.type score: 1=no adenocarcinoma
Morseth98 wk died 2=carc incidental to death
intact female SD rat, Group 6 3=carc cause of death
Groups 7 to 10 follow Score #2: fib. or carc. cause of death = 3; incidental=2

RAT #	day died	Type	Score #1	Score #2	Comment , cause of death
62258	367	carc.	2	2	s.s.
62259	367	no	1	1	s.s.
62260	367	no	1	1	s.s.
62261	367	no	1	1	s.s.
62262	367	no	1	1	s.s.
62263	367	no	1	1	s.s.
62264	367	no	1	1	s.s.
62265	367	no	1	1	s.s.
62266	367	no	1	1	s.s.
62267	367	no	1	1	s.s.
62268	369	no	1	1	s.s.
62269	369	no	1	1	s.s.
62270	369	no	1	1	s.s.
62271	369	no	1	1	s.s.
62272	369	no	1	1	s.s.
62273	369	no	1	1	s.s.
62274	369	no	1	1	s.s.
62275	369	no	1	1	s.s.
62276	369	no	1	1	s.s.
62277	369	no	1	1	s.s.
62278	731	no	1		s.s.
62279	141	no	1		unknown
62280	479	no	1		mesothelioma
62281	555	no	1		pituitary neoplasm
62282	731	fibroads 2	1	2	s.s.
62283	681	carcinoma	3	3	
62284	582	fibroad	1	2	pituitary neoplasm
62285	731	no	1		pit. adenoma s.s.
62286	627	no	1		pit. neoplasm
62287	733	carcinoma	2	2	s.s.
62288	674	fibroad	1	3	fib. 9x9x4.5 cm
62289	550	fibroad	1	2	pit. adenoma
62290	577	fibroads 2	1	3	fib. 5x4x2.5 cm
62291	731	fibroad	1	2	s.s.
62292	547	fibroad	1	3	fib. 9x8x4 cm.
62293	674	fib + carc	3	3	
62294	225	carcs. 2	3	3	

62295	731	no	1		s.s.
62296	731	no	1		s.s.
62297	514	no	1		s.s.
62298	558	no	1		pit. neoplasm
62299	733	no	1		s.s.
62300	731	no	1		s.s.
62301	733	no	1		s.s.
62302	731	no	1		s.s.
62303	733	no	1		s.s.
62304	731	lipoma	1		s.s.
62305	387	no	1		pit. neoplasm
62306	541	no	1		pit. neoplasm
62307	707	fib + carc	2	2	pit. neoplasm
62308	454	no	1		pit. neoplasm
62309	605	fib + carc	2	2	pit. neoplasm
62310	593	no	1		pit. neoplasm
62311	632	no	1		pit. neoplasm
62312	731	no	1		s.s.
62313	485	no	1		pit. neoplasm
62314	632	fib + carc	3	3	mammary neoplasms
62315	717	no	1		pit. neoplasm
62316	676	fib.	1	2	pit. neoplasm
62317	736	no	1		s.s.
62318	716	no	1		pit. neoplasm
62319	731	no	1		s.s.
62320	547	carc	2	2	pit. neoplasm
62321	615	fib.	1	2	pit.neoplasm
62322	736	fib.	1	2	s.s.
62323	731	carc. (2)	2	2	s.s.
62324	731	no	1		s.s.
62325	736	carc.	2	2	s.s.
62326	731	no	1		s.s.
62327	612	no	1		pit. neoplasm
62328	675	no	1		pit. neoplasm
62329	736	fib. (3)	1	2	s.s.
62330	737	no	1		s.s.
62331	526	no	1		pit. neoplasm
62332	731	no	1		s.s.
62333	737	no	1		s.s.
62334	597	fib.	1	2	pit. neoplasm
62335	485	carc (2)	3	3	
62336	731	no	1		s.s.
62337	473	no	1		pit. neoplasm
0					

Group 7 (25 ppm)

RAT #	day died	Type	Score		Comment, cause of death
62338	367	no	1		s.s.
62339	367	no	1		s.s.

62340	367	carc.(1)	2	2	s.s.
62341	367	no	1		s.s.
62342	367	no	1		s.s.
62343	367	no	1		s.s.
62344	367	no	1		s.s.
62345	367	no	1		s.s.
62346	367	fib.	1	2	s.s.
62347	367	no	1		s.s.
62348	369	no	1		s.s.
62349	369	no	1		s.s.
62350	369	no	1		s.s.
62351	369	no	1		s.s.
62352	369	no	1		s.s.
62353	369	no	1		s.s.
62354	369	no	1		s.s.
62355	369	no	1		s.s.
62356	369	no	1		s.s.
62357	369	no	1		s.s.
62358	398	carc.	3	3	basosquamous carc.
62359	733	fib.2;carc.2	2	2	s.s.
62360	681	fib.2	1	3	s. extremis
62361	317	no	1		pit. neoplasm
62362	733	no	1		s.s.
62363	541	no	1		pit. neoplasm
62364	663	fib.	1	3	s. extremis
62365	716	fib.2; carc.	2	2	pit. neoplasm
62366	701	carc.2	3	3	
62367	400	no	1		unknown cause of death
62368	716	carc	2	2	pit. neoplasm
62369	731	fib.; carc.	2	2	s.s.
62370	731	fib.	1	2	s.s. 2? cos 6x5x2 cm.
62371	507	fib.; carc.	3	3	
62372	733	no	1		s.s.
62373	632	fib.	1	3	11x8x4 cm. neoplasm
62374	652	no	1		unknown c.o.d.
62375	674	carc. (2)	3	3	9x8x5.5 cm. & 4x3.5x1.5 cm.
62376	527	carc.	2	2	pit. neoplasm
62377	717	fib. (2)	1	2	2? pit. neoplasm
62378	565	fib.	1	2	2? pit. neoplasm
62379	396	fib.	1	3	11x7.5x5 cm. neoplasm
62380	731	fib.; carc.	2	2	s.s. fib.12x7x6; carc. 3x1.5x1 cm.
62381	619	no	1		pit. neoplasm
62382	623	fib.	1	2	2? pit. neoplasm 3x2.5x1 cm
62383	733	fib.	1	2	2? s.s. 3x3x1.5 cm.
62384	619	no	1		pit. neoplasm
62385	367	fib.; carc 3	3	3	also pit. adenoma

62386	731	fib.	1	2	2? s.s.
62387	449	no	1		pit. neoplasm
62388	516	no	1		pit. neoplasm
62389	731	no	1		s.s.
62390	590	no	1		pit. neoplasm
62391	507	no	1		pit. neoplasm
62392	736	fib.2; carc	2	2	s.s.
62393	485	no	1		pit. neoplasm
62394	701	no	1		pit. neoplasm
62395	716	no	1		pit. neoplasm
62396	731	fib.2	1	2	s.s.
62397	507	fib.	1	2	pit. neoplasm
62398	676	no	1		pit. neoplasm
62399	392	no	1		pit. neoplasm
62400	555	no	1		pit. neoplasm
62401	570	fib.	1	2	pit. neoplasm
62402	736	carc.3	2	2	s.s.
62403	732	carc.2	3	3	6x5x2 & 2.5x2x1 cm.
62404	736	no	1		s.s.
62405	731	no	1		s.s.
62406	612	carc.	3	3	6x5x3.5 cm.
62407	737	fib.; carc.	2	2	s.s.
62408	654	no	1		unknown
62409	640	no	1		pit. neoplasm
62410	492	no	1		pit. neoplasm
62411	656	fib.2	1	2	p i t . sm; 5x5x2 cm. fib. neoplasm. 5x5x2 cm fib.)
62412	536	no	1		pit. neoplasm
62413	717	no	1		pit. neoplasm
62414	610	fib.; carc.	2	2	p i t . sm; 6x5x3.5 cm fib. neoplasm;
62415	737	no	1		s.s.
62416	731	carc.	2	2	s.s.; 1.5x1.5x1.5 cm. carc.
62417	731	fib.	1	2	s.s.; 5x3.5x2.5 cm. fib.

Group 8 (50 ppm)

RAT #	day died	type	score #1	score #2	Comment, cause of death
62418	367	no	1		s.s.; interim
62419	367	no	1		s.s.; int.
62420	367	no	1		s.s.; int.
62421	367	no	1		s.s.; int.
62422	367	fib.	1	2	s.s.; int.
62423	367	no	1		s.s.; int.
62424	367	no	1		s.s.; int.

62425	367	no	1		s.s.; int.
62426	367	no	1		s.s.; int.
62427	367	no	1		s.s.; int.
62428	369	no	1		s.s.; int.
62429	369	no	1		s.s.; int.
62430	369	no	1		s.s.; int.
62431	369	no	1		s.s.; int.
62432	369	no	1		s.s.; int.
62433	369	no	1		s.s.; int.
62434	369	no	1		s.s.; int.
62435	369	no	1		s.s.; int.
62436	369	fib.	1	2	s.s.; int. 2.5x2x1 cm. fib.
62437	369	no	1		s.s.; int.
62438	526	carc.	2	2	pit. neoplasm; carc. 3.5x3x2.5 cm.
62439	731	no	1		s.s.
62440	731	fib.2;carc.2	2	2	s.s.
62441	561	no	1		pit. neoplasm
62442	731	no	1		s.s.
62443	731	fib.2	1	2	s.s.; 10x5x5 & 5x3x2.5 cm.
62444	550	no	1		pit. neoplasm
62445	731	carc.	3	3	8x7x40 cm.
62446	674	carc.	2	2	pit neoplasm; carc.7.5x4.5x3.5 cm.
62447	716	carc.	2	2	unknown
62448	701	fib.2	1	3	5x5x3 & 9x6x4 cm.
62449	548	fib.2	1	3	12x11x3 cm. + small one
62450	478	no	1		pit. neoplasm
62451	387	fib.	1	3	6.5x5x4.5 cm.
62452	733	fib.2	1	2	s.s. 6.5x5.5x3 & 4x3x1.5 cm.
62453	655	fib.;carc.2	2	2	pit. neoplasm. 2.5x2x1.5 & 2x1x1
62454	653	no	1		pit. neoplasm
62455	733	carc.	2	2	s.s.; 2.5x2x1 cm.
62456	717	fib.3	1	2	pit. neoplasm
62457	625	fib.2	1	3	7.5x5.5x5.5 & 8x5.5x4 cm.
62458	457	no	1		pit. neoplasm
62459	733	no	1		s.s.
62460	731	fib.	1	2	s.s.; 5x4.5x2 cm.
62461	736	no	1		s.s.
62462	693	fib.2;carc.2	2	2	pit. neoplasm
62463	401	no	1		uterine lymphoma
62464	716	fib. & ad.	2	2	unknown
62465	487	carc.	3	3	7.5x5x4 cm.
62466	547	carc.2	3	3	4x3x2 & 5x3x2.5 cm.
62467	288	no	1		unknown
62468	538	carc.	3	3	pit. neoplasm & carc. 3.5x2.5x1 cm
62469	26				accident
62470	711	fib.	1	3	9x7.5x3 cm.
62471	731	missing			s.s.

62472	605	fib.	1	2	pit. neoplasm; fib. 1x1x0.5 cm.
62473	711	fib.	1	3	8x7x3 cm.
62474	736	carc.	2	2	s.s.; 1.5x1x0.6 cm.
62475	640	carc.	3	3	3.5x2x1.5 cm.
62476	617	fib.4;carc.2	3	3	
62477	467	no	1		endometrial stromal sarcoma
62478	310	no	1		pit. neoplasm
62479	675	fib.;carc.2	3	3	carc. 3.6x2.6x1 & 2x1.8x1 cm.
62480	619	fib.	1	3	13x10x9 cm.
62481	526	sarcoma	1		myxosarcoma 12.5x10x10 cm
62482	711	fib.	1	2	pit. neoplasm; 7.5x5.5x5 cm
62483	640	fib.;carc.	3	3	
62484	673	fib.	1	2	unknown 4x4x2 cm.
62485	736	fib.2	1	2	s.s. 1.5x1.5.0.5 & 6x3.5x1.5 cm.
62486	619	fib.	1	3	11x4x4.5 cm.
62487	731	fib.4;carc.2	2	2	s.s.
62488	367	no	1		pit. neoplasm
62489	471	fib.	1	3	12.5x11x3.5
62490	736	fib.3;carc.	2	2	s.s.
62491	701	fib.;carc.2	3	3	
62492	737	fib.	1	2	s.s.
62493	568	fib.	1	2	pit. neoplasm
62494	492	fib.	1	3	4.5x3x2.5 cm.
62495	593	carc.	2	2	pit. neoplasm; carc. 3.5x3x2 cm.
62496	737	fib.	1	2	s.s. 4.5x2.3x1.2 cm.
62497	500	no	1		pit. neoplasm

Group 9 70 ppm

RAT #	**day died**	**Type**	**Score #1**	**Score #2**	**Comment, cause of death**
62498	367	no	1		s.s. int.
62499	367	no	1		s.s. int.
62500	367	no	1		s.s. int.
62501	367	no	1		s.s. int.
62502	367	no	1		s.s. int.
62503	367	carc.	2	2	s.s. int. 5.5x5.5x3 cm.
62504	261	no	1		pit. neoplasm
62505	367	no	1		s.s. int.
62506	367	no	1		s.s. int.
62507	367	carc.	2	2	s.s. int. 3x2.5x1.5 cm.
62508	367	no	1		s.s. int.
62509	369	fib.	1	2	s.s. int.
62510	369	no	1		s.s. int.
62511	369	no	1		s.s. int.
62512	369	no	1		s.s. int.
62513	369	no	1		s.s. int.
62514	369	no	1		s.s. int.
62515	369	no	1		s.s. int.

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62516	369	no	1		s.s. int.
62517	369	fib.	1	2	s.s. int. 2.5x2.5x0.5 cm.
62518	369	no	1		s.s. int.
62519	572	fib.	1	3	8x6x4 cm.
62520	733	fib.4	1	2	s.s.
62521	443	no	1		unknown
62522	733	no	1		s.s.
62523	733	fib.	1	2	s.s.
62524	682	fib.	1	2	histiocytic sarcoma (abdomen)
62525	731	fib.	1	2	s.s.
62526	681	fib.	1	3	12x12x4 cm.
62527	681	fib.	1	3	11x8.5x5 cm.
62528	674	fib.	1	2	pit. neoplasm; fib. 4x3.5x1.5 cm.
62529	632	fib.2	1	3	8.5x7x3 & 2x1.5x1.5 cm.
62530	656	fib.;carc.2	2	2	pit. neoplasm
62531	731	fib.; carc.	2	2	s.s.
62532	731	no	1		s.s.
62533	393	carc.	2	2	pit. neoplasm; carc. 3x2x1 cm.
62534	593	fib.	1	3	10x9x4.5 cm.
62535	461	no	1		pit. neoplasm
62536	597	carc.	2	2	pit. neoplasm; carc. 2x2x1 cm.
62357	733	no	1		s.s.
62538	225	no	1		unknown
62539	731	carc.	2	2	s.s.; carc. 1.4x1x0.8 cm.
62540	736	no	1		s.s.
62541	674	fib.3	1	3	8 . 5 x 8 . 5 x 5 . 5 ; 1; 2.5x2x1 cm 2.5x2.5x1 & 2.5x2x1
62542	716	fib.	1	2	pit. neoplasm; fib. 2.5x2x1.3 cm.
62543	382	no	1		pit. neoplasm
62544	489	no	1		unknown
62545	593	no	1		histiocytic sarcoma (subcutaneous)
62546	573	fib.3	1	2	p i t . sm; fib.<4x4x1.5 cm. neoplasm; f i b . 4 x 4 x 1 . 5 cm.
62547	555	fib. + carc.	2	2	p i t . n e o p l a s m ; 2 & 2x1.5x1 5x3.5x2&
62548	396	no	1		pit. neoplasm
62549	484	fib.	1	2	pit. neoplasm; fib. 3x1.5x1 cm.
62550	736	carc.	2	2	s.s.
62551	736	no	1		s.s.
62552	731	no	1		s.s.
62553	673	fib.	1	2	p i t . sm; fib. 6x6x2.5 cm neoplasm
62554	554	no	1		pit. neoplasm
62555	547	fib. + carc.	3	3	
62556	627	no	1		pit. neoplasm

62557	674	no	1		pit. neoplasm
62558	619	fib.	1	3	11x9x7.5 cm.
62559	731	fib.	1	2	s.s.; fib. 9x6x4 cm.
62560	632	carc.	2	2	pit. neoplasm; carc. 2x2x1.5 cm.
62561	710	no	1		unknown
62562	736	no	1		s.s.
62563	737	no	1		s.s.
62564	382	no	1		pit. neoplasm
62565	561	no	1		pit. neoplasm
62566	556	carc.	2	2	pit. neoplasm; carc. 5x4.5x3 cm.
62567	568	no	1		pit. neoplasm
62568	731	fib.2	1	2	s.s.; fib. 4.5x3x1 & 4x2x1 cm.
62569	547	no	1		pit neoplasm
62570	737	no	1		s.s.
62571	731	fib.	1	2	s.s.; fib 3x2.5x2 cm.
62572	597	carc.	2	2	pit. neoplasm; carc. 5x2.5x1.5 cm.
62573	731	fib.	1	2	s.s.; fib. 8.5x6x2 cm.
62574	717	fib.	1	2	pit neoplasm; fib. 2.8x1.8x0.8 cm.
62575	635	no	1		pit. neoplasm
62576	632	fib.+carc.2	3	3	
62577	598	fib.	1	2	unknown, but not fib. 3x3x3 cm.

Group 10 400 ppm

RAT #	day died	type	Score #1	Score #2	Comment, cause of death
62578	129	carc.	3	3	3.5x2x1 cm.
62579	367	no	1		s.s.
62580	367	carc.	2	2	s.s. 3.5x3x1.5 cm.
62581	367	no	1		s.s.
62582	367	no	1		s.s. pit. neoplasm
62583	367	no	1		s.s. lge. galctocele
62584	367	no	1		s.s.
62585	367	no	1		s.s.
62586	367	no	1		s.s.
62587	367	no	1		s.s.
62588	367	no	1		s.s.
62589	369	fib. + carc.	2	2	s.s. fib. 6x2x1 cm.
62590	369	no	1		s.s.
62591	369	no	1		s.s.
62592	369	no	1		s.s.
62593	369	no	1		s.s.
62594	369	no	1		s.s. pit. neoplasm
62595	369	no	1		s.s.
62596	369	no	1		s.s.

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62597	369	no	1		s.s.
62598	369	no	1		s.s.
62599	499	carc.	2	2	pit. neoplasm; carc. 5.5x4.5x4 cm.
62600	731	fib.2; carc	2	2	s.s. 3.5x3x1; 7x6.5x3.5; 1x1x.5 cm.
62601	733	carc.	2	2	s.s. 2.5x2x1 cm.
62602	733	fib.; carc.2	2	2	s.s. 2.5x1.5x1; 6x4x3; 5x3.5x2 cm.
62603	733	no	1		s.s.
62604	547	fib.	1	3	10x9x5 cm.
62605	434	no	1		pit. neoplasm
62606	632	fib.4	1	3	
62607	284	carc.	3	3	4.5x3x2.5 cm.
62608	660	no	1		pit. neoplasm
62609	611	fib.	1	2	pit. neoplasm
62610	396	carc.2	3	3	6x5.5x4 & 2x2x1 cm.
62611	548	fib.	1	2	dermatitis fib. 6x2.5x2.5 cm.
62612	459	fib.+carc.	3	3	7x6x4 & 4x3x2 cm.
62613	568	carc.	3	3	3.5x3.5x2.5 cm.
62614	178	carc.	3	3	5.5x4x1.5 cm.
62615	717	fib.	1	2	pit. neoplasm; fib. 6x3x1 cm.
62616	733	fib.	1	2	s.s. fib. 4x3.5x2 cm.
62617	676	fib.2	1	2	pit. neoplasm; fib. 4.3x3.3x1.2 cm
62618	561	fib.	1	2	pit. neoplasm; fib. 1.5x1x1 cm.
62619	479	carc.	3	3	4.5x3x2 cm.
62620	418	no	1		pit. neoplasm; lge galactocele
62621	498	no	1		pit. neoplasm; mod. galactocele
62622	736	carc.2	2	2	s.s.; carcs.6x4x1.5 & 3x3x1.5 cm.
62623	370	carc.	2	2	pit. neoplasm
62624	494	carc.	2	2	unknown; carc.1.5x1x.5 cm.
62625	459	no	1		pit. neoplasm
62626	572	no	1		pit. neoplasm
62627	635	fib.+carc.	2	2	pit. neoplasm; 1.7x1.5x1 & 1.5x1x1
62628	484	no	1		pit. neoplasm
62629	554	carc.	3	3	pit. neoplasm also; rc.5x3.5x3.5 carc.5x5x3.5
62630	736	fib.	1	2	s.s.; fib. 9x7x2.3 cm.
62631	656	no	1		pit. neoplasm
62632	674	fib2+carc2	3	3	
62633	681	fib.+carc.	3	3	9x7x4.5 & 7.5x6x4 cm.
62634	660	carc.	3	3	5x4.5x1 cm.
62635	736	no	1		s.s.
62636	677	fib.2+carc	2	2	pit. neoplasm; 1 cm. (x2) 3x2.5x1 cm two)

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62637	731	no	1		s.s.
62638	492	carc.	3	3	3.5x2.5x1.5 cm.
62639	736	fib.	1	2	s.s. fib.3.5x3x1.5 cm.
62640	473	carc.2	2	2	pit. neoplasm; carcs. v.small v small
62641	365	no	1		unknown
62642	601	fib.+carc.	2	2	pit. neoplasm; fib/carc 3x3x1.5 cm.
62643	681	fib.2+carc	3	3	4x3x2.5; 3x2x2; 12x9x6 cm.
62644	675	no	1		pit. neoplasm
62645	731	carc.	2	2	s.s. carc. 2.5x1.5x1 cm.
62646	640	no	1		pit. neoplasm
62647	556	fib.2	1	2	pit. neoplasm; fib.2x2x.7 & 7x5x2
62648	513	no	1		pit. neoplasm
62649	676	fib.2	1	3	11x9x3 & 8x6x2 cm.
62650	541	no	1		pit. neoplasm
62651	442	no	1		pit. neoplasm
62652	676	fib.2	1	2	pit. neoplasm
62653	737	carc.	2	2	s.s. carc/galac 5x3.5x1 cm.
62654	717	fib.	1	2	pit.neopla sm; fib.2x1.5x.6 cm.
62655	527	no	1		pit. neoplasm
62656	701	no	1		pit. neoplasm
62657	737	fib.2	1	2	s.s. fib. 11x6x3 & 3x1.7x1.2 cm.

APPENDIX V.

Toxicology Summaries

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH
SUMMARY OF TOXICOLOGY DATA

ATRAZINE

Chemical Code # 000045, Tolerance # 00220
SB 950 # 008
August 11, 1986
Revised 7/10/87, 11/20/87, 5/23/88, 2/9/89, 5/26/89, 11/29/89, 3/22/90, 6/30/90, 2/25/92, 10/18/93, 12/7/93, and 4/20/94, 10/4/96, 6/1/98, 5/20/99, 10/15/99, 01/05/00 and 7/27/01

I. DATA GAP STATUS

Chronic rat:	No data gap, no adverse effect
Chronic dog:	No data gap, possible adverse effect
Oncogenicity rat:	No data gap, possible adverse effect
Oncogenicity mouse:	No data gap, no adverse effect
Reproduction rat:	No data gap, no adverse effect
Teratogenicity rat:	No data gap, no adverse effect
Teratogenicity rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome Effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

** indicates acceptable study.
Bold faced DPR Document/Record Numbers indicate a possible adverse effect.

All record numbers identified as relevant in data indexed by DPR as of 01/04/00 have been reviewed. These include record numbers through 171843 (in Document No. 220-541), plus any relevant record numbers over 900000. This revision (7/27/01) adds summaries of three publications (pages 9 – 12).
Previous revisions by M. Silva, J. Gee, K. DiBiasio, and C. Aldous. Revision on 01/05/00 by Aldous. Revision on 7/27/01 by Aldous and Gee.
These pages contain summaries only. Individual worksheets may contain additional effects.
File name: T2001727

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II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

COMBINED, RAT

NOTE: Studies using Sprague-Dawley female rats indicate that atrazine increases mammary tumor incidence, or reduces the latency to appearance of mammary tumors. Submitted data indicate that dose levels that elicit tumors also block the surge of luteinizing hormone (LH), which is necessary to stimulate ovulation. Vaginal cytology evaluations show that the prevalence of persistent estrus is enhanced after about 3 months of exposure to 400 ppm atrazine. Record No. 156970, p. 19, makes reference to a study, evidently by Tyrey *et al.*, (1996), which reported that the failure of high dose atrazine group females to produce the LH surge is **not** due to a lack of responsiveness of the pituitary gland to gonadotropin releasing hormone (GnRH). There is no report as yet that atrazine effects on GnRH levels have been studied. Studies to date evaluating possible atrazine estrogenic effects have **not** indicated remarkable effects on estrogen function. In particular, the 2-year dietary study in Sprague-Dawley rats, DPR Record No. 160554, found no mammary tumors in ovariectomized rats up to the highest atrazine dose of 400 ppm, administered throughout the study.

The most plausible explanation for the increased mammary tumors in Sprague-Dawley rats is that a perturbation in the control processes regulating reproductive hormones occurs at the level of the hypothalamus. Since the primary locus (loci) of the alteration is (are) not known, it cannot be determined what analogous changes would be expected in humans. Information provided thus far does not establish that a threshold phenomenon exists for atrazine effects on reproductive hormonal changes or possible consequences thereof, including tumor development.

There are so many studies that were performed to evaluate the basis of the mammary tumors in Sprague-Dawley females that the portion of this Summary on chronic toxicity and oncogenicity in rats has been subdivided into sections, with explanatory comments for the larger sections. Aldous, 6/1/98.

Guideline "Combined" Rat Study (and associated records)

**** 064-078 044294-044308** Mayhew, D., "Twenty-four Month Combined Chronic Oral Toxicity and Oncogenicity Study in Rats Utilizing Atrazine Technical." (American Biogenics Corp. [Toxigenics], 5/29/86.) Atrazine Technical (95.8%, lot FL-0821575); fed at 0, 10, 70, 500, 1000 ppm in the diet to Sprague-Dawley rats. NOEL for non-neoplastic effects = 70 ppm (diminished weight gain in both sexes at 500 and 1000 ppm, irritability in males and pale appearance and probable anemia in females at those dosages. **POSITIVE FOR ONCOGENICITY**: Female mammary tumors included dosage-related increases in adenocarcinomas and fibroadenomas: statistically significant for pairwise comparisons at 70 ppm and above for carcinomas and at 500 ppm and above for all mammary tumors. Testicular interstitial cell tumors were increased in the 1000 ppm males. Complete and ACCEPTABLE for primary review. Additional data or re-arrangement of existing data may be required for risk assessment group evaluation. C. Aldous, 7/16/86. **NOTE**: Results of CDFA and DPR re-evaluations of these data follow:

Re-examination #1: The above study (Doc. #s -064 through -078) found no significant adverse non-oncogenic effects below

the LEL of 500 ppm. Weight gain decrements, reduced food consumption, and clinical signs were observed in a dose-related fashion at 500 and 1000 ppm, so that non-oncogenic NOEL was 70 ppm. Miscellaneous findings at 1000 ppm are given in the report and do not indicate significant chronic risks. Incidence of fibroadenomas and more particularly of adenocarcinomas in female mammary gland indicate dose-related responses that extend to the 70 ppm level and will require risk assessment procedures. It is not clear whether the apparent increase in testicular interstitial cell adenomas are true treatment effects, as indicated in the review (C. Aldous, July 86). There is no evidence for interstitial cell tumor induction at or below 500 ppm, indicating that such tumor risk is unlikely to be the basis for major oncogenic risk evaluation. Pituitary tumors were noted by investigators as increased in 1000 ppm females when differential mortality was considered, but such an increase was not apparent on inspection of incidence data. C. Aldous, 7/10/87.

Re-examination #2: [Purpose of this re-examination was to tabulate and discuss findings of potential concern]. There were no substantial differences from earlier conclusions: testicular interstitial cell tumors are possibly treatment-related in high dose males, as was prostate epithelial hyperplasia. An examination of evidences for a NOEL for anemia in females (including a re-examination of splenic extramedullary hematopoiesis and bone marrow myeloid hyperplasia) still supports a NOEL of 70 ppm. Several histopathology findings limited to 1000 ppm rats are included in this worksheet. Aldous, 12/2/93.

062 042305 (Experimental Pathology Labs) Pathology tables from interim report. Final study subsequently reviewed (See one-liner and comment on full study above: study in volumes 064-078, Rec. #s 044294-044308). J. Schreider, 4/18/86.

049 016884 (ToxiGenic, Inc., 1984) Twelve-month interim report. (See one-liner and comment on full study above: study in volumes 064-078, Rec. #s 044294-044308). J. Schreider, 5/09/85.

059 038012 Duplicate information of 049 016884.

220-407 138990 Hardisty, J., "Atrazine: Supplement to two-year chronic feeding/oncogenicity study in rats administered atrazine", EPL re-evaluation of female mammary tissue sections for the primary rat combined study in Record Nos. 044294-044308. Date of EPL report: Oct. 1, 1987. An individual EPL pathologist (G.I. Riley) performed this re-evaluation, whereas

U.S. EPA currently requires a "pathology working group" evaluation to serve this purpose. Summary tables, recorded in the DPR review of this record, confirm essential conclusions noted in the existing 1-liner of Record No. 044294. In the 24-month rats, 500 and 1000 ppm groups had increases of benign tumor lesions such as "fibroadenoma, NOS" and in "proliferative fibroadenoma". Malignant tumor data indicated treatment effects at 70 ppm upwards, with the greatest contribution from two noninvasive tumor lesions: "tubulopapillary carcinomas" and "compact tubular carcinomas" (from pp. 10 to 16). Combined data for the 12- and 13-month intervals indicated a treatment response at the 1000 ppm level for the latter tumor forms at that time interval. Aldous, 10/4/96.

Mechanistic Studies in Sprague-Dawley Females

SUMMARY: The chronic (12/24 month) study (Record No. 151475) reaffirmed the mammary tumor development at 400 ppm in intact females, but found no mammary tumors at all to date in ovariectomized rats. This does not eliminate the possibility of an estrogenic effect of atrazine, since the vestigial mammary tissue in ovariectomized rats would likely have required substantial hormonal influence to develop sufficiently to express hormonally-mediated tumors. Vaginal smear data available thus far are consistent with earlier studies in demonstrating enhanced estrus in females after about 3 months on treatment. Record No. 151472 provides the most definitive data available to date on effects of atrazine on the LH surge that precedes ovulation and completion of estrus, demonstrating an elimination of the surge at 400 ppm. At even higher atrazine levels, the magnitude of the prolactin peak was also reduced (LEL of 200 mg/kg/day and NOEL of 40 mg/kg/day for this effect: see the 4-week gavage exposure study in Record No. 146011, below). Very high dose levels of atrazine also led to reduced circulating estradiol levels (reduced estradiol at 200 to 300 mg/kg/day, LEL for this finding = 100 mg/kg/day: see Record No. 139110). It appears that the effect on the LH surge is the most important of the findings in this section. Aldous, 4/1/98.

220-511 160554 Morseth, S. L., "Chronic (12-24 month) study in rats with Atrazine Technical", Covance Laboratories Inc., 4/15/98. Study No. 2386-108. Eighty intact Crl:CD7BR females per group were dosed with 0, 25, 50, 70, or 400 ppm Atrazine Technical (97.1%) in a 2-yr study. Twenty/group were designated for 1-yr interim sacrifice. Parallel groups of ovariectomized rats of the same size and dose levels were treated on the same schedule. Primary focus was histopathology of reproductive tissues and pituitary. Vaginal cytology smear samples were

taken daily for 14 consecutive day periods every 4 weeks (see next paragraph). Apparent NOEL for non-neoplasia, excluding vaginal cytology data (not provided in this report) = 70 ppm (slightly reduced body weights at 400 ppm in ovariectomized and intact females). There was a decreased time-to-tumor for mammary carcinomas in 400 ppm intact rats. There were no mammary tumors in ovariectomized rats. DPR review compares these findings with those of other long-term studies using Sprague-Dawley females. Valid ancillary study. Aldous, 5/27/98.

220-539 170324 Thakur, A. K., "Addendum 1 to Study No. 2386: Chronic (12/24 month) study in rats with atrazine technical (EPA MRID No. 44544701)". These data are associated with DPR Document/Record No. 220-511 160554 (Covance Study No. 2386-108: see above). Study design and tumor data were previously reviewed under the latter record number. Eighty intact (i.e. not ovariectomized) Crl:CD7BR females per group were dosed with 0, 25, 50, 70, or 400 ppm Atrazine Technical (97.1%) in a 2-yr study. Of these rats, twenty/group were designated for 1-yr interim sacrifice. Vaginal cytology smear samples were taken for periods of 14 consecutive days, at intervals beginning every 4 weeks. The present study evaluated relationships between treatment levels, prevalence of estrus (as indicated by vaginal cytology), and carcinoma incidence. **General associations of mammary carcinomas and time in estrus:** Data revealed several important relationships, the strongest associations being between mammary carcinomas and vaginal cytological signs of estrus during weeks 17-26. This critical period marked a time of rapid change from normal estrous cycling to vaginal cytology signs of constant estrus. This was also the time of maximal difference in estrous state between control and 400 ppm rats. Control rats and dose levels of 25 through 70 ppm reached 50% of time in estrus at about week 22 of the study (about week 29 of age), compared to 400 ppm rats, which reached 50% of time in estrus at about study week 18. Some changes reflected this acceleration of age-related change in 400 ppm rats during study weeks 17-26 (the time period used for comparisons unless otherwise indicated): (1) 400 ppm rats averaged appreciably more time in estrus than controls and other groups, (2) rats that eventually bore mammary carcinomas averaged appreciably more time in estrus than rats in corresponding dose groups that did not have carcinomas, (3) 400 ppm rats with carcinomas had only marginally greater time in estrus than 400 ppm rats without carcinomas, (4) 400 ppm rats with carcinomas had only slightly greater time in estrus than rats in other treatment groups with carcinomas. There were no comparable associations of time in estrus and mammary fibroadenomas. **Associations of early-**

appearing mammary carcinomas (evident by week 53) and time in estrus. Seventeen rats (including 8 of the 400 ppm rats) had early-appearing carcinomas. All 17 rats reached or approached 100% of time in estrus by the time of the first palpation of a carcinoma. Two of these high dose rats had carcinomas palpable at weeks 14 and 18, respectively. These findings corresponded to the first periods in which these rats showed cytological signs of sustained estrus. Four of the other six high dose rats with carcinomas that were palpable within the first year had an increased percentage of days in estrus during weeks 17-26 compared to the study average: time in estrus was less than the study mean for the other two rats. The majority of rats in other groups that had carcinomas that were palpable within the first year had lower average time in estrus during this time period than the overall study average. Thus the limited numbers of rats with early-appearing carcinomas did not show a consistent association with increased time in estrus during weeks 17-26. Histopathology for this study and other studies had previously shown that the primary effect of chronic feeding of CD females with 400 ppm atrazine was increased incidence of early-onset mammary carcinomas. It is particularly interesting that there was no clear association between prolonged estrus (i.e., constant estrus over a period of several weeks), particularly during the timeframe of weeks 17-26, and early-onset tumors in this study. Some period of constant estrus, most commonly of several weeks duration, almost always preceded the appearance of a carcinoma, and usually was present at the time that the tumors first became palpable. The need for some estrogen influence to allow mammary carcinoma development is consistent with the histopathological findings of Record No. 160554, which reported no mammary tumors in ovariectomized rats up to the highest atrazine dose of 400 ppm, administered throughout the 2-year study. Since prolonged estrus is not a necessary condition for early-onset mammary carcinomas in 400 ppm atrazine rats, it is possible that the general increase of time in estrus *per se* is not the sole or primary cause of the increase in early carcinomas in 400 ppm rats, but instead the altered vaginal cytology may be a visible manifestation of a more central toxicological response. Aldous, 5/20/99 (edited to add Record Number and Document Number on 10/14/99).

NOTE: Interim results of estrous cycling data from Study No. 2386-108 were mailed directly to Medical Toxicology Branch on 5/4/98. Data were presented as plots showing prevalence of persistent estrus or persistent diestrus, evaluated over time periods of week 0 to week 38, in each of the above treatment groups. Data suggest that 400 ppm rats entered persistent estrus by week 21, whereas controls and treated groups through 70 ppm did not reach this incidence of persistent estrus until

about week 29. Given the large numbers of slides yet to be evaluated to complete the analyses, it will take some time before this report is completed. Aldous, 5/5/98.

220-458 151475 Interim report of Laboratory Study # CHV 2386-108. Aldous, 4/7/98. (See review of final report of histopathology data in Record No. 160554, above.)

220-457 151472 Morseth, S. L., "Evaluation of the luteinizing hormone (LH) surge in atrazine-exposed female Sprague-Dawley rats - 6-month report", Corning Hazleton Inc.,10/25/96. Laboratory Study # CHV 2386-111. Ninety Crl:CD7BR females per group were dosed in diet with 0, 25, 50, or 400 ppm Atrazine Technical, purity 97.1%, for 6 months. To assess estrous cycling, smear samples were taken daily for vaginal cytology for 14 consecutive days at 4 week intervals. Rats were ovariectomized 10 days before sacrifice. A capsule containing 4 mg estradiol per ml sesame oil was implanted 3 days before sacrifice. These measures ensured that sufficient estrogen was available to induce an LH surge regardless of treatment regimen. Blood was sampled after 6 months of treatment for prolactin and LH (study outcome parameters) and estradiol (to validate experimental technique) by RIA. Blood was sampled at intervals over a 12-hour period of the day during which surges of LH and prolactin are known to occur. Ten rats/group were bled 6 times over a 12-hour period on the day of sacrifice, providing sufficient blood for LH assays each time. The other 80 rats/group were sacrificed at intervals, to provide sufficient blood for prolactin and estradiol assays as well as LH. [Reproductive tissues were preserved for histopathology: to be presented in a later report. An additional report will present pituitary hormone level data, from tissues collected in this study]. There were modest but statistically significant body weight and food consumption decrements at 400 ppm. Vaginal cytology data showed an impressive increase in prolonged or constant estrus at 400 ppm during the last 3 months of the study. At most sampling intervals for vaginal cytology, there was no indication of effects at lower dose levels. **More thorough evaluation is requested on vaginal cytology at weeks 21-22, at which time it appears that intermediate dose groups show prolonged or constant estrus**. The pulse of luteinizing hormone, which normally appears late in the daylight period in ovariectomized rats under estradiol treatment, was effectively blocked at 400 ppm. NOEL for body weight and food consumption decrements and for lack of the normal luteinizing hormone surge in estradiol-primed rats was 50 ppm (3.65 mg/kg/day). There was no definitive NOEL for estrous cycling alterations, although sustained effects were limited to 400 ppm rats. Aldous, 4/7/98.

220-429 146011 Morseth, S. L., "Evaluation of the luteinizing hormone (LH) surge in atrazine-exposed female Sprague-Dawley rats", Corning Hazleton Incorporated, Vienna, VA (CHV), 1/25/96. CHV Study No. 2386-111. Ninety Crl:CD7(SD)BR females/group were dosed daily by gavage (in 0.5% aq. CMC) for 28-31 days prior to ovariectomies, and continuing for 10 days afterwards, with 0, 2.5, 5, 40, or 200 mg/kg/day atrazine. During weeks 2-4 of treatment (prior to ovariectomy), rats were evaluated for estrous cycle stage by vaginal smear analysis. Seven days after ovariectomy, rats were implanted with silastic capsule designed to provide estradiol (E2) levels $\geq$ 12 pg/ml. On the tenth day after ovariectomy, blood samples were taken at intervals (20-25 samples/interval) for assays of E2 (to verify implantation), LH, and prolactin. Prior to ovariectomy, estrous cycling was disturbed, most remarkably by prolonged periods of diestrus at 40 and 200 mg/kg/day. Duration of estrus was also prolonged at 200 mg/kg/day. Ovariectomized rats provided with E2-releasing implants had remarkable decrements in LH peak levels at 40 and especially at 200 mg/kg/day. There was also a reduction in peak prolactin levels at 200 mg/kg/day, with a possible delay in timing of prolactin peak levels. Data are consistent with the hypothesis that the primary atrazine toxic event leads to delays of ovulation by disturbing the surges of releases of LH and prolactin. The author suggests that data support a "threshold", however spacing of dose levels and high response variability do not allow a definitive decision on that matter. This is a valid ancillary study. Aldous, 10/4/96.

220-424 146005 Protocol for CHV Study No. 2386-111, for which the gavage portion has been reviewed (DPR Record No. 146011, above). No worksheet is needed. Aldous, 9/5/96.

220-427 146009 Morseth, S. L. Method validation for 220-429 146011, above. No worksheet is necessary. Aldous, 8/14/96.

220-428 146010 Morseth, S. L. "Evaluation of the luteinizing hormone surge in female Sprague-Dawley rats - Method validation", Corning Hazleton Inc., 1/18/96. Laboratory No. CHV 2386-110. Relates to 220-429 146011, above. One dose of atrazine (300 mg/kg/day) was tested in repeat-bled rats only. There was an apparent reduction in the LH surge. Prolactin levels did not return to baseline in the course of the day: a possible indication that the stress associated with repeat bleeding may lead to invalid prolactin data. [Note that prolactin was not assayed in repeat-bled rats in the primary study (Record No. 146001)]. No worksheet is necessary. Aldous, 8/14/96.

220-409 139110 Morseth, S. L., "Fourteen-day repeated dose oral toxicity/hormone study in female albino rats", Hazleton Laboratories America, Inc., March 6, 1990. HLA Study No. 483-268. Groups of 15 female Crl:CD7BR rats were dosed for at least 2 wk with 100, 200, or 300 mg/kg/day of atrazine (97%) or diaminochlorotriazine (DACT: 97.4%). High doses had been originally 400 mg/kg/day, but were reduced to 300 mg/kg/day for both test compounds on day 4 due to excessive toxicity. Two groups of 15 each served as controls: one received only corn starch suspension vehicle, and the other (a positive control for prolactin secretion) received an ip dose of metoclopramide 20 min before sacrifice. Rats were sacrificed at the time of first determination of diestrous stage after at least 14 daily treatments, cycle stage being determined by vaginal cytology. Serum collected at sacrifice was assayed for prolactin, LH, FSH, progesterone, and estradiol (E2). Higher dose levels were clearly toxic: the majority of atrazine rats at 200 to 300 mg/kg/day as well as DACT rats at 100 to 400 mg/kg/day had clinical signs of "thin" or "few or no feces". All DACT groups and 200 to 300 mg/kg/day atrazine rats had dose-related b.w. decrements. All atrazine and DACT groups had marked and dose-related decrements in thymus weights as the primary organ weight finding. Coincident with the above signs of general toxicity, there were possible hormone level changes, particularly decreases of LH, progesterone, and E2 in 200 to 300 mg/kg/day DACT rats. In general, the high variabilities evident in hormone levels, coupled with high general toxicity in groups appearing to manifest hormone level changes, makes this study of very limited value for assessing intrinsic effects of test articles on hormone controls. Newer, more refined studies are more valuable than this study. Aldous, 10/4/96.

220-425 146007 Pettersen, J. C. and J. C. Turnier, "1-year chronic toxicity study with atrazine technical in rats", Ciba-Geigy Corporation, Farmington, CT, Dec. 8, 1995. Lab Study # F-00171. The present study focused on histopathology of reproductive tissues (only mammary and pituitary gland tissues were systematically examined for this report) in female Crl:CD7(SD)BR rats dosed for up to 1 yr with 0, 15, 30, 50, 70, or 400 ppm atrazine. There were 55 rats per group, with interim sacrifices of 10/group at 3, 6, and 9 months, and 25/group maintained for 1 yr terminal sacrifice. Estrous cycle stage was evaluated by vaginal cytology at each interim time, and blood was sampled at these times for reproductive hormone assays: these data are to be reported separately. NOEL [excluding vaginal smear data] = 50 ppm (very slight b.w. decrement at 70 ppm). There was a significant decrement in body weight and modest reduction in food consumption at 400 ppm. There was a

significant increase in mammary tumor bearing rats at 400 ppm. Most tumors were adenocarcinomas. There were no other important histopathology effects, and lower dose groups were unaffected. Investigators' tumor onset time analyses found a significant trend toward reduced time to tumor for the categories of "benign" mammary tumors and for "all mammary tumors". Removing the high dose group from analyses eliminated significance in all cases. This is a valid ancillary study, confirming the capacity of atrazine to elicit mammary tumors within a 1-yr timeframe. Since there was no testing in the range just below 400 ppm, there is no information on dose-response at dose levels approaching the MTD. Aldous, 10/3/96.

Note: Initial examinations of the preliminary vaginal smear data appeared to show some increases cases of "= 3 days in estrus" at dose levels as low as 15 ppm (Aldous comments on 4/6/98 and 6/1/98). Subsequently more vaginal smear data have been submitted, and no coherent pattern has been evident below 400 ppm atrazine. Aldous, 1/5/00.

220-377 137872 Preliminary report of Lab Study No. F-00171 (Record No. 146007, above)

Mechanistic Studies in Male Rats (any strain)

Stoker, T. E., S. C. Laws, D. L. Guidici, and R. L. Cooper, "The effect of atrazine on puberty in male Wistar rats: An evaluation in the protocol for the assessment of pubertal development and thyroid function", *Toxicological Sciences* **58**, 50-59 (2000). National Health and Environmental Effects Research Laboratory, U. S. EPA, Research Triangle Park, NC. Male Wistar rats were dosed with atrazine by gavage at levels of 0, 12.5, 25, 50, 100, 150, or 200 mg/kg/day from PND day 23 to PND 53 or 54 (except for some which were killed earlier for LH receptor evaluations). Pair-fed rats' diets were limited to match the food consumption of the 200 mg/kg/day group. Measurements included body weights, organ weights (ventral prostate, seminal vesicle with coagulating gland), time to preputial separation, serum estradiol and estrone levels, intra-testicular testosterone at PND 45 and PND 53, testicular LH receptor density on PND 45 and PND 53, and PND 53 serum levels of TSH, T3, T4, LH, prolactin, plus pituitary levels of the latter two hormones. Body weights were reduced in dose-related fashion at 100 mg/kg/day upwards. Controls weighed 281 g at PND 53, compared to 200 mg/kg/day rat mean weight of 233 g and pair-fed group weight of 239 g. The most sensitive finding justified by the data in this report is reduction of ventral prostate weights, with a NOEL of 25 mg/kg/day. Lateral prostate and testicular weights were comparable to controls. Next in order of sensitivity is

elevated serum estradiol, with a NOEL of 50 mg/kg/day. At these NOEL's there was no alteration of body weights. Investigators discussed a proposed "NOAEL" of 12.5 mg/kg/day for preputial separation in this paper, however this NOAEL is not supported by the data (which are tabulated in the DPR review, and which do not show dose-response over a wide dosage range). Useful supplemental data. Aldous, 7/25/01.

Stoker, T. E., C. L. Robinette, and R. L. Cooper, "Maternal exposure of atrazine during lactation suppresses suckling-induced prolactin release and results in prostatitis in the adult offspring", *Toxicological Sciences* **52**, 68-79 (1999). National Health and Environmental Effects Research Laboratory, U. S. EPA, Research Triangle Park, NC. Most studies involved treatment of Wistar dams during lactation days 1-4 with 0, 6.25, 12.5, 25, or 50 mg/treatment/day (2 treatments/day, by gavage) of atrazine. The normal suckling-induced prolactin secretion into the blood, which occurs in lactating rats, was studied in dams fitted with indwelling cardiac catheters on day 3. Prolactin secretion was completely inhibited at 50 mg/kg/dose atrazine. Controls and 6.25 mg/kg/dose atrazine rats had normal suckling-induced increases of prolactin. About half of the dams in intermediate groups had inhibition of prolactin secretion. Bromocriptine, a dopamine agonist known to inhibit prolactin release, when administered sc on the same schedule as atrazine, completely eliminated prolactin release at 0.104 mg/kg/dose and above, but not at 0.052 mg/kg/dose. To assess prostate inflammation in male offspring which were exposed via milk of dams which were treated twice daily with atrazine as described above during lactation days 1-4, the young adult offspring were sacrificed at day 90 or 120 for assessment of inflammation of lateral prostates (using myeloperoxidase activity as in index of inflammation, and confirming by histopathology). There was no statistical change at 90 days, however at 120 days, atrazine doses of 12.5 mg/kg/dose significantly increased prostate inflammation incidence. Bromocriptine at dose levels of 0.104 mg/kg/dose and above also significantly increased inflammation incidence. The importance of prolactin in milk for normal neurological development in neonates was supported by a subsequent test in which dams received ovine prolactin (sc, 0.3 mg/kg) on the same schedule as atrazine treatments with 25 or 50 mg/kg/dose atrazine. The combination of prolactin and atrazine eliminated the prostate inflammation which was present with atrazine alone. To assess critical periods of exposure, additional dams were treated twice daily with 25-50 mg/kg/dose on lactation days 6-9 or 11-14, and inflammation in prostates of offspring was evaluated on day 120. Treatment in the latter periods had no significant influence on prostate inflammation.

It had previously been shown that in rats, milk-derived prolactin during the first days of lactation is critical for development of the tuberoinfundibular dopaminergic system. This system in adult rats, as in humans, provides an inhibitory control on pituitary release of prolactin. Abnormal development of this system (as by neonatal exposure to dopamine agonists such as bromocriptine) can lead to inadequately controlled prolactin secretion and an associated lateral prostate inflammation as rats mature. Investigators were uncertain whether a comparable effect occurs in humans, in which the tuberoinfundibular dopaminergic system is largely organized before birth. Although there were several results of this study which implicated altered early-neonatal prolactin levels as the cause of subsequent lateral prostate inflammation, assays of serum or pituitary prolactin in male offspring at 120 days postpartum did not demonstrate an alteration of prolactin levels with treatment with atrazine or with bromocriptine. This suggests that the prostate inflammation is not dependent on altered prolactin levels in the maturing adult. NOEL = 6.25 mg/kg/dose (= 12.5 mg/kg/day for a 3 or 4-day exposure), based on reduced suckling-induced prolactin secretion in dams, and on lateral prostate inflammation in male offspring at day 120. Useful supplemental data. Aldous, 7/23/01.

Cooper, R. L., T. E. Stoker, L. Tyrey, J. M. Goldman, and W. K. McElroy, "Atrazine disrupts the hypothalamic control of pituitary-ovarian function", *Toxicological Sciences* **53**, 297-307 (2000). National Health and Environmental Effects Research Laboratory, U. S. EPA, Research Triangle Park, NC. Most mechanistic studies involved Long-Evans (LE) and Sprague-Dawley (SD) females dosed 1, 3, or 21 days at 0, 50, 100, 200, or 300 mg/kg/day. **[1]**. **Hormone secretion effects (especially LH and prolactin)**: One series of experiments evaluated atrazine effects on levels of five hormones (LH, FSH, prolactin, TSH, and estradiol) in serum and anterior pituitary gland samples. These studies involved ovariectomized LE and SD rats implanted with estrogen-filled silastic capsules 3 days before sacrifice. Rats were treated at above doses for 1 or 3 days, respectively, then were terminated at 0, 1, 3, or 6 hr after final treatment, for the above hormone assays. Only LH and prolactin gave treatment responses, therefore only the latter two hormones were assayed in an analogous experiment using daily dose levels of 0, 75, 150, or 300 mg/kg/day for a 21-day exposure. **Serum hormone surges**: After a single dose, only the 300 mg/kg group of LE rats responded: these rats had marked inhibition of LH and prolactin surges evidenced by low LH and prolactin levels in serum samples at all post-treatment sampling times. After the 3-day treatment, LE rats had delayed response times for LH and prolactin surges at 50 mg/kg/day (spikes did not appear until

about 6 hours, compared to the control peaks at about 3 hours), and secreted amounts for both hormones were markedly reduced at all time periods examined at all higher dose levels. SD rats after the 3-day treatment had significantly reduced prolactin levels at hrs 1-6 at 300 mg/kg/day only, but no alterations at lower doses with prolactin and none at all with LH. The 21-day exposure groups showed significant reductions in surge levels of LH and prolactin in both rat strains at the expected diurnal peak surge time at 150 and 300 mg/kg/day. In addition, LH was significantly reduced at 75 mg/kg/day in LE rats. **Pituitary hormone levels**: Pituitary samples were assayed for these two hormones in the 21-day groups. There were no treatment responses for pituitary LH levels, however pituitary prolactin levels were elevated at 75 to 300 mg/kg/day in both strains after 21 days. Prolactin levels were also measured in pituitaries of LE and SD rats following 3-day exposure. In that case, pituitary prolactin levels were consistently reduced at 6 hr at all dose levels tested (50 to 300 mg/kg/day) in LE rats, whereas there was no treatment effect in SD rats. **[2] Ovulation and estrous cycling effects:** Numbers of oocytes shed were counted in LE females by flushing the oviducts of rats which were administered up to 300 mg/kg atrazine on the day of vaginal proestrus, then sacrificed on the day of the following estrus. There were no treatment effects at any dose tested. When normally cycling, similarly treated LE rats were monitored for estrous cycling by daily vaginal smears for 3 weeks after a single proestrus dose of atrazine, 7/9 of the 300 mg/kg females became pseudopregnant, whereas none of the lower dose groups (75 or 150 mg/kg) had abnormal cycling. **[3] Ectopic pituitary studies**: To identify whether atrazine affects pituitary function by direct interaction with the lactotrophs, LE rats were hypophysectomized and autotransplants of pituitary tissue were placed within the kidney capsule. After 4 wks recovery, rats were treated once with 300 mg/kg atrazine, and blood samples were taken hourly (tail vein) for 6 hr for prolactin assays. Prolactin levels were unchanged under these circumstances, however there were no associated controls for method validation. **[4] Exogenous gonadotropin-releasing hormone (GnRH) effects:** To evaluate effects of GnRH, ovariectomized LE rats were implanted with estrogen-filled silastic capsules, then dosed with 300 mg/kg atrazine for 3 days. Just after the third dose, rats were fitted with intra-cardiac catheters, and shortly thereafter given 50 ng/kg GnRH via catheter. Blood was sampled at time 0 and over the next 60 minutes to assay for LH. Atrazine alone blocked LH secretion, but the combination of atrazine and GnRH led to peak LH concentrations similar to control rats noted above. This suggests that the lack of an LH surge results from lack of stimulation of pituitary endocrine cells by hypothalamic

GnRH. **[5] Responsiveness of pituitary tissues in vitro to hypothalamic hormone stimulation:** An *in vitro* perifusion study evaluated possible direct action of atrazine on pituitary LH secretion. Portions of anterior pituitary were taken from ovariectomized, estrogen-primed SD rats, and tissues were maintained in perfusion chambers and exposed to 0 or 100 ìM atrazine. LH or prolactin levels in medium were measured for over 2 hr as hormone levels diminished. Subsequently, medium was treated twice for 30-min intervals (spaced 1 hr apart) with GnRH (85 nM) plus thyroid releasing hormone (TRH, 100 nM). There was no difference between atrazine and control treatment responses of LH or prolactin secretion peaks upon challenge by releasing hormones. In a parallel *in vivo* exposure study, in which females were treated for 3 days by gavage with 0, 100, or 200 mg/kg/day atrazine prior to sacrifice and collection of pituitary gland samples for a comparable *in vitro* perifusion study, there were similarly no decreases in LH or prolactin secretion peaks upon challenge by releasing hormones. Instead, investigators noted an initial increase in prolactin release in atrazine-treated rats, consistent with elevated pituitary prolactin levels from atrazine treatment reported in part [1], above. This result also supports the concept that atrazine does not directly affect the pituitary, but interacts via interfering with secretion of releasing hormones from the hypothalamus. Useful supplemental data. Aldous, 7/27/01.

Studies Contrasting Hormone Level and Reproductive Tissue Histopathology of Sprague-Dawley vs. F344 Female Rats

SUMMARY: Increased mammary tumor incidence and/or decreased time to mammary tumor developments have been consistently shown in Sprague-Dawley female rats, but not in F-344 rats. The following studies have focused on histopathology of reproductive organs in these strains, as well as vaginal cytology (to determine patterns of estrous cycling), and blood hormone levels. The following studies show that a pattern of prolonged or constant estrus, which is a normal feature of aging Sprague-Dawley females, is brought on prematurely in Sprague-Dawley females exposed to 400 ppm atrazine, but not at levels as low as 70 ppm. Record No. 137871 indicated increased estradiol levels in 400 ppm Sprague-Dawley females at 9 months into the study. Prolactin levels were reportedly elevated in that group at 9 months, however it should be noted that prolactin levels were not remarkably affected in long-term studies of ovariectomized rats implanted with estradiol capsules, except for a reduction of peak levels of prolactin at very high atrazine dose levels (see section on "**Recent Studies Relating the Surge of LH. . .**", above). Record No. 137871 reported a dose-related increase of numbers of antral follicles at 70 to 400 ppm in Sprague-Dawley

rats but not F-344 rats at 9 months; a finding consistent with ovaries not receiving hormonal signals to ovulate and move beyond estrus stage. The influences of reproductive hormones that appear to be eliciting the increased numbers or premature appearance of mammary tumors in Sprague-Dawley rats were considered by investigators not to be relevant to human physiological patterns (Record No. 128816). Aldous, 4/1/98.

220-167 112325 "Determination of Hormone Levels in Sprague-Dawley Rats Treated with Atrazine" (A. K. Thakur, Hazleton Washington, Inc., Study No. 483-278, 10/17/91) Atrazine technical, 97%, was fed in the diet at 0, 70 or 400 ppm to 70 female Sprague-Dawley rats per group. Ten/group were sacrificed at 1, 3, 9, 12, 15, 18 months and survivors at 24 months. Selected tissues were examined for histopathology: pituitary, mammary glands, uteri, and ovaries. The stage of estrus was determined prior to sacrifice. Samples of blood were taken but the data are not included in this report. The time to the appearance of tumors (mammary gland and pituitary) during the first year was shortened at 400 ppm. The overall incidence, however, over the two years was similar to controls and not statistically significant. Body weight gain was also lower at 400 ppm. Chronic NOEL = 70 ppm. The study confirms a **possible adverse effect for tumor induction.** Supplementary to the combined study in the rat. Gee, 2/24/92.

220-186 123799 Eldridge, J. C., Wetzel, L.T., Tisdel, M. O., and Luempert, L.G., "Determination of hormone levels in Sprague-Dawley rats treated with Atrazine Technical". Hormonal studies were performed at Bowman Gray School of Medicine, Winston-Salem, NC on samples from a study conducted at Hazleton Laboratories America, Inc. Date of hormonal studies report: April 8, 1993. (The histopathology aspect of the study using these same animals is reported as DPR Record No. 112325, HWA Study No. 483-278). Atrazine technical, 97%, was fed in the diet at 0, 70 or 400 ppm to 70 female Sprague-Dawley rats per group. Ten/group were sacrificed at 1, 3, 9, 12, 15, 18 months and survivors were sacrificed at 24 months. Prior to sacrifice, rats were examined daily by vaginal smears to provide a "10-day cytology index". Blood collected at sacrifice was assayed for hormones, including estradiol and progesterone. Based on vaginal cytology, 400 ppm females had an increased percentage of days in estrus over the first 18 months. Estradiol levels were elevated over other groups at months 3 and 9 (statistically significant only at 3 months). Data had substantial variability, but were generally consistent with the hypothesis that elevated estrogenic stimulation in early adult life of Sprague-Dawley rats influenced the elevated incidence of mammary

tumors (or earlier onset of such tumors). Useful ancillary data. Aldous, 10/18/93.

220-220 128813 (an earlier version of Record No. 123799, above: no review)

220-168 (3 parts) 112326 "Determination of Hormone Levels in Fischer-344 Rats Treated with Atrazine Technical." (A. K. Thakur, Hazleton Washington, No. 483-279, 11/8/91.) Atrazine technical, 97%, was fed in the diet at 0, 10, 70, 200, or 400 ppm to 70 Fischer-344 female rats per group. Ten per group were sacrificed at 1, 3, 9, 12, 15 and 18 months and surviving animals at 24 months. Selected tissues were examined microscopically - mammary glands, pituitary gland, ovaries, and uteri of all animals. There was weight gain depression, especially early in the study, at 200 and 400 ppm. No other effects were reported. The incidences of mammary and pituitary tumors were comparable across groups and no evidence of an effect on time-to-tumor was noted. Supplementary data. Gee, 2/24/92

220-187 123801 Eldridge, J. C., Wetzel, L.T., Tisdel, M. O., and Luempert, L.G., "Determination of hormone levels in Fischer-344 rats treated with Atrazine Technical" [Study was done in parallel with Record No. 123799, which employed Sprague-Dawley females]. Hormonal studies were performed at Bowman Gray School of Medicine, Winston-Salem, NC on samples from a study conducted at Hazleton Laboratories America, Inc. Date of hormonal studies report: April 8, 1993. (The histopathology aspect of the study using these same animals is reported as DPR Record No. 112326, HWA Study # 483-279). Atrazine technical, 97%, was fed in the diet at 0, 10, 70, 200, or 400 ppm to 70 female Fischer-344 rats per group. Ten/group were sacrificed at 1, 3, 9, 12, 15, 18 months and survivors were sacrificed at 24 months. Prior to sacrifice, rats were examined daily by vaginal smears to provide a "10-day cytology index". Blood collected at sacrifice was assayed for hormones, including estradiol and progesterone. The present study did not identify treatment-related changes in vaginal cytology to indicate changes in "percent of time in estrus", nor were there treatment-related changes in serum hormone levels nor deviations from any other of the normal estrous processes over time in these rats. Useful ancillary data, Aldous, 10/18/93. [Record # 220-221:145382 is an earlier version of this report, for which there is no separate DPR review].

220-376 137871 McConnell, R.F., "A histomorphologic reevaluation of the ovaries, uterus, vagina, mammary gland, and pituitary gland from Sprague-Dawley and Fischer-344 female rats

treated with atrazine", [re-examination of slides from Hazleton Washington, Inc. Study No. 483-278 for Sprague-Dawley rats, and No. 483-279 for F344 rats (original reports were reviewed under DPR Record Nos. 112325, 112326, 123799, and 123801)]. Date of present document is 3/10/95. The re-examination was conducted (1) to determine whether the animals in these studies were indeed sacrificed at proestrus, as intended (assessed by vaginal cytology and by histomorphology of reproductive tissues), and (2) to re-examine several estrogen-sensitive tissues in these two strains of rats in order to ascertain why treated Sprague-Dawley females had increased mammary tumor incidence. Results: most F344 rats were in proestrus, as desired, during the pivotal age span of 3 to 12 months on study, whereas most Sprague-Dawley rats had histomorphological features of both proestrus and "continuous estrus" during months 9-12. There was no clear atrazine effect on cycle stage at any sacrifice time in either strain. The major hormone level changes were limited to Sprague-Dawley rats at 9 months, when there was a modest increase in estradiol (E_2) levels, and a more remarkable increase in prolactin levels at 400 ppm. Mammary gland histomorphology changes, commonly associated with aging rats, appeared prematurely (most noticeably at 9 months) in 400 ppm Sprague-Dawley rats. These changes included enhanced degrees of age-associated changes such as acinar/lobular development, secretory activity, and duct ectasia with galactocele formation. The primary ovarian effect was a marked increase of numbers of antral follicles at 400 ppm and slight increase at 70 ppm in Sprague-Dawley rats at 9 months. The author did not observe vaginal epithelium changes due to atrazine that would be typical of potent exogenous estrogens (there were no positive controls for comparison). Investigators concluded that atrazine elicits histomorphologic changes in Sprague-Dawley rats by interference with ovarian function, perhaps through alteration of LH secretion (which was not a measured parameter in this study). Impairment of the LH surge necessary for ovulation could lead to earlier onset of mammary tumors through mediation of endogenous estrogens and prolactin in Sprague-Dawley females. Reproductive senescence in F344 rats follows a different pattern, and no such atrazine effect is seen. An EPA critique of this study and the Ciba response are contained in 220-375 137870, below. Aldous, 10/4/96.

220-188 123802 Thakur, A. K., "Strain-dependent responses to long-term atrazine feeding in female Sprague-Dawley and Fischer-344 rats: Statistical analyses of mammary and pituitary tumors, body weight gain, and survival" [discussion of several studies: no new data], April 22, 1993. This short discussion refers to the 1986 rat combined study (American Biogenics

Corporation, DPR Record No. 044294), and more particularly to four separate studies, two employing Sprague-Dawley rats, and two employing F-344 rats. As of the time of this submission, DPR had received only two of the four reports (one per rat strain, namely Record Nos. 112325 and 112326, with corresponding HWA Study Nos. 483-278 and 483-279, respectively). The remaining studies were subsequently received under DPR Record Nos. 128811 and 129145 (see DPR reviews of the latter studies, performed in 1994). The conclusions of this submission were that (1) there was an earlier onset of mammary tumors in 400 ppm S-D females but not in 70 ppm S-D females, (2) neither time of onset nor total incidence of tumors was affected at any dose tested in F-344 rats, (3) mortality was elevated in S-D 400 ppm females, suggesting that the MTD had been exceeded, however mortality was unaffected in 400 ppm F-344 females, and (4) body weight gain decrements in both strains suggested that 400 ppm had achieved the MTD. Aldous, 10/13/93 (amended by Aldous on 4/12/94).

NOTE: See a more recent evaluation of these data by Eldridge et al., below (Record No. 128816).

061 039984 (Hazleton Labs, 1961) J. Schreider, 4/18/86. Summary. See EPA one-liner. No adverse effect reported. EPA one-liner: "Too few animals to determine organ weight changes at sacrifice to set NOEL. Poor viability-infections. Core grade: Supplementary. (Two-year feeding; Hazleton Labs, 1961; MRID # 00059211)."

053 016905 Exact duplicate of 061 039984.

220-014 942528 Histopathology supplement to 039984, above.

220-219 128811 "Oncogenicity Study in Sprague-Dawley Rats with Atrazine Technical", (A. K. Thakur, Hazleton Washington, Inc., HWA Study No. 483-275, 1/27/92). Atrazine technical, purity 97%, was admixed with the feed at concentrations of 0, 70 or 400 ppm and fed to 60 female Sprague-Dawley rats/group for 104 weeks. Study was supplementary to earlier studies, and focused on tissues under female hormonal influence: primary microscopic examinations were on uterus, ovary, pituitary, and mammary gland. NOEL = 70 ppm (increased mortality; reduced body weight). A decrease in time of onset of mammary tumors was noted at 400 ppm, although total rats with mammary tumor neoplasia was not affected. This scientifically valid ancillary study provides additional useful information about the influence of atrazine on mammary tumor development, but does not affect the designation of the test article as having a "possible adverse effect". Kishiyama and Aldous, 4/20/94. **NOTE:** Data were re-examined to evaluate incidence of mammary tumors first

palpated within year 1 of study. Incidences of fibroadenomas arising during first year were 2, 1, and 4 for control, 70 ppm and 400 ppm groups, respectively. Respective mammary carcinoma incidences arising in the first year were 0, 1, and 6. Thus there was a treatment-related earlier onset for mammary carcinomas and for combined mammary tumor incidence at 400 ppm only. The effect on mammary carcinoma incidence is probably the most important outcome of the study. Aldous, 5/29/98.

220-291 129145 Thakur, A. K., "Oncogenicity study in Fischer-344 rats with Atrazine Technical", Hazleton Washington, Inc., HWA Study # 483-277, 2/18/92. Atrazine, 97%, was fed in diets of 60 rats/sex/group at dose levels of 0, 10, 70, 200, and 400 ppm for 2 years. Histopathology was evaluated on the standard battery of tissues. NOEL = 70 ppm (modest body weight decrements in both sexes). Study is **acceptable, with **no adverse effects**. Aldous, 4/11/94.

220-222 128816 Eldridge, J. C., Wetzel, L.T., and Tisdel, M. O., "The effects of atrazine on endocrine parameters in the female Sprague-Dawley rat: A threshold-mediated and strain-specific response for mammary tumor development", 9/22/93. This submission is an evaluation of the relationship of mammary tumors in Sprague-Dawley and F-344 rats to atrazine exposure, and influences of atrazine on hormonal status and hormonally-mediated events that eventually result in mammary tumors. Primary studies cited were DPR Record Nos. 112325, 112326, 128811, and 129145. Findings of cited records have been reviewed previously by DPR. Investigator's conclusions are that the earlier onset of mammary tumors in Sprague-Dawley rats was related to changes in estrogen function, and that the changes have a threshold (effects at 400 ppm but not at 70 ppm). Their evaluation further concluded that this mechanism would not apply to humans, since the processes of neuroendocrine changes with age in the two species are quite different. This reviewer agrees that it is probable that humans would also have a hormonal response to atrazine, but the mechanism of that response is not known, and the presence of possible thresholds and the associated dose levels cannot be determined at this time. Aldous, 4/20/94.

220-368 137861 Eldridge et al., "Short-term effects of chlorotriazines on estrus in female Sprague-Dawley and Fischer 344 rats", J. Toxicol. Environ. Health., 43:155-167 (1994). (Conducted at Bowman-Gray School of Medicine, Winston-Salem, NC). Virgin rats (10-13 wk old) of Sprague-Dawley and F344 strains were dosed by gavage with 0, 100, or 300 mg/kg/day atrazine or simazine for 14 to 23 days (dosing was stopped and

rats were sacrificed when vaginal smear data first indicated proestrus stage, after a minimum of 14 days of treatment). Simazine effects were generally small or absent compared to atrazine effects, so that this review addresses strictly atrazine effects. Body weights at sacrifice for Sprague-Dawley rats were reduced 13% and 27% compared to controls, suggesting dose levels exceeding "MTD" criteria. Both strains showed dose-related absolute and relative decreases in ovarian and uterine weights, and absolute and relative increases in adrenal weights. Plasma hormone level changes included a dose-related drop in estradiol levels (significant only in Sprague-Dawley rats), and a significant elevation in progesterone levels in 300 mg/kg/day Sprague-Dawley rats only. Mean estrous cycle duration was elevated in treated groups compared to concurrent controls, significant only in Sprague-Dawley rats. Sprague-Dawley rats had a dose-related and significant increase in the percentage of cycle in estrus and a decrease in the percentage of the cycle in diestrus. In contrast, F344 rats had a slightly opposite trend. These differences were consistent with longer term studies, in which Sprague-Dawley rats administered high doses of atrazine spent larger portions of the time in estrus than untreated rats, a phenomenon not seen in F344 rats. Aldous, 10/3/96.

Hormone Receptor Binding Studies

SUMMARY: Studies below show that atrazine is capable of binding weakly to estrogen receptors, and a few estrogen-mediated processes are altered by atrazine in *in vivo* studies. *In vitro* studies evaluating atrazine receptor binding typically have found little or no direct agonist or antagonist activity of atrazine. Several *in vitro* studies showed that atrazine did not alter estradiol-mediated effects on cell growth or activity (see especially Record No. 146008). Investigators concluded that apparent estrogen effects influenced by atrazine are not mediated by direct estrogen-receptor interactions. Aldous, 4/1/98.

220-371 137864 Tennant, M. K. et al., "Atrazine and simazine: possible antiestrogenic properties of chloro-s-triazines in rat uterus", J. Toxicol. Environ. Health. 43:183-196 (1994). At least some of these studies were performed at Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC. (1) **Effects on uterine weight**: Ovariectomized Sprague-Dawley rats were dosed with graded doses of atrazine, simazine, or the common metabolite, DACT, for 3 days. On days 2 and 3, half of each group was injected with estradiol. All rats were killed 24 hr after the final dose. Dose-related decreases in uterine weights were obtained in rats with estradiol treatment plus

atrazine. Without estradiol treatment, atrazine had no consistent effect on uterine weight. (2) **Thymidine uptake in uterine tissues:** Intact, immature female rats were gavaged with 0, 1, 10, 50, 100, or 300 mg/kg/day atrazine, simazine, or DACT for 2 days. On day 2, all animals received estradiol injections. After 24 hr, all animals were killed, and uterine slices were prepared for incubation with [3H]-thymidine. Radiolabel incorporation into slices was decreased at 50 to 300 mg/kg/day only (NOEL for this effect was 10 mg/kg/day for all 3 compounds). (3) **Uterine progesterone receptor binding**: Ovariectomized rats were dosed for 2 consecutive days with 50 or 300 mg/kg/day of atrazine, simazine, or DACT: each dose was followed by sc injections of estradiol. Parallel groups were treated with 0 or 300 mg/kg/day of respective triazines, without estradiol. Rats were killed 24 hr after the last dose, and uteri were dissected, homogenized, and supernatants were collected following centrifugation at 36,000 x g for 60 min. The synthetic progesterone ligand, [3H]-R-5020, was used to evaluate progesterone receptor binding. Net progesterone receptor binding was reduced significantly in atrazine and simazine groups subjected to estradiol pretreatment, and non-significantly in estradiol-pretreated DACT rats: in all cases, only at the 300 mg/kg dose level. In studies without estradiol pretreatment, lesser but nevertheless statistically significant reductions in progesterone receptor binding were observed. **Investigators' conclusions:** These studies suggested that the triazines display very low antagonistic potency against estradiol function. It was postulated that these triazines may operate through cellular interactions unrelated to these hormonal effects. Aldous, 7/31/95 (only highly reduced data were presented, hence no worksheet).

220-372 137866 Tennant, M. K. et al., "Chloro-s-triazine antagonism of estrogen action: Limited interaction with estrogen receptor binding" J. Toxicol. Environ. Health 43:197-211(1994). This is principally a report of estrogen binding studies as described in Record No. 137859 [Eldridge, J. C. et al., above (part 3)]. In this report, competition studies employing atrazine, simazine, and DACT are reported together. All three test substances led to the same conclusion: the triazines evidently bind only weakly to estrogen receptors, and other molecular interactions must explain part of triazine effects on target tissues. Aldous, 8/1/95 (only highly reduced data provided, hence no DPR worksheet).

220-426 146008 This record is a draft of the article: Connor, K., J. Howell, I. Chen, H. Liu, K. Berhane, C. Sciarretta, S. Safe, and T. Zacharewski, "Failure of Chloro-S-triazine-Derived

Compounds to Induce Estrogen Receptor-Mediated Responses in Vivo and in Vitro", Fundam. Appl. Toxicol. **30**, 93-101 (1996). Study was done at Dept. of Veterinary Physiology and Pharmacology, Texas A&M University, and Department of Pharmacology and Toxicology, Univ. of Western Ontario, London, Ontario, Canada. S-D female rats, 21 days old, received 50, 150, or 300 mg/kg/day atrazine or simazine for 3 days for in vivo studies and were sacrificed 20 hr after the third dose. Uterine weights were not significantly changed, however both compounds significantly reduced cytosolic progesterone receptor binding and uterine peroxidase activity. The triazines had no significant effect on estradiol (E2)-induced increases in uterine weights, however there were small (possibly treatment-related) decreases in cytosolic progesterone receptor binding and uterine peroxidase activity in triazine-treated E2-induced rats. In studies with MCF-7 human breast cancer cell line preparations, the triazines had no effect on cytosolic progesterone receptor-DNA complexing with or without E2 addition. Basal MCF-7 cell growth was not affected by the triazines, nor did triazines inhibit such growth when stimulated by E2. Also, when MCF-7 cells were transfected with an estrogen receptor chimera and with a luciferase reporter gene under conditions in which E2 demonstrated a many-fold luciferase activity response, there was no effect by either of the triazines over a wide dosage range. Co-treatment of triazines with an effective dose of E2 in this assay showed no influence of triazines on the luciferase response. An S. cerevisiae strain that is uracil dependent except when the URA3 gene is expressed (an estrogen receptor-dependent gene) underwent measurable growth in the presence of E2, but not with either of the triazines. Results were interpreted to indicate that triazine effects on hormonally-mediated tissues are not mediated by the estrogen receptor. Useful ancillary study (not relevant to data gaps). No adverse effects are indicated. Aldous, 10/3/96.

220-373 137867 Safe, S. et al., "Failure of atrazine and simazine to introduce estrogenic responses in MCF-7 human breast cancer cells". Studies were performed at Texas A&M Univ. and/or Univ. of Western Ontario, submission dated 3/13/95. The two triazines did not compete with TCDD for rat liver cytosolic Ah receptor. Triazines did not elicit estrogenic or antiestrogenic effects, such as estrogen-dependent cell growth. There was no effect on estrogen-dependent nuclear progesterone receptor levels. The triazines did not alter background measurements in the absence of estrogen, nor did they alter the pharmacologic effects of estrogen. Aldous, 8/1/95, (only highly reduced data provided, hence no DPR worksheet).

220-492 156971 McDougal, A., Wilson, C., and Safe S., "Induction of estradiol 2-hydroxylase activity in MCF-7 human breast cancer cells by pesticides and carcinogens", Environ. Toxicol. Pharmacol. (accepted for publication: no volume number was assigned: date of submission of registrants' cover page was 6/26/97). Study was performed at Texas A and M University. Atrazine and several other unrelated chemicals were incubated with MCF-7 cells for 2 or 48 hours in media containing [2-^{3}H]estradiol. Estradiol 2-hydroxylase activity was assayed by counting tritium in the supernatant after removal of residual [2-^{3}H]estradiol by charcoal adsorption. Atrazine, *p,p'*-DDE, and especially DMBA inhibited estradiol 2-hydroxylase activity, whereas several other test articles increased 2-hydroxylase activity over untreated controls. There was no apparent predictive value relating a chemical's effects on 2-hydroxylase to mammary oncogenicity potential. This is potentially useful information, but not applicable to data requirements. Aldous, 6/1/98.

220-217 128787 Wittliff, J. L., "Chronic toxicity study in rats: Influence of atrazine ingestion on steroid-receptor recognition", Hormone Receptor Laboratory, University of Louisville, 10/15/90. Rats from a reproduction study (see Record No. 063785) were maintained chronically at 0, 10, 50, or 500 ppm atrazine and evaluated for changes in various sex hormone receptor binding effects in mammary gland, pituitary gland, and uteri. Interim sacrifices were at weeks 8 (males and females) or 35 (females). Terminal sacrifices were at week 52 (males) or 104 (females). A subset of lifetime study females (10 controls and 10 high dose group) was taken off treatment at wk 65. Estrogen and progestin in vitro binding to cytosol receptors was measured, as well as prolactin binding to membrane receptors. The specific binding capacity (SBC) and binding constants (K_d) were compared for uterine tissues, and for "normal" and "tumor-type" tissues of mammary and pituitary glands as a function of atrazine exposure. The SBC's for estrogen and progestin were increased significantly in tumor tissues. This study did not identify atrazine effects. Standard deviations were often large compared to means, so that this study did not offer potential to identify subtle changes. Aldous, 10/3/96.

Chronic Study in F1 Treated Rats Derived from a Reproduction Study:

220-446 147021 Rudzki, M. W., G. C. McCormick and A. T. Arthur, "Atrazine Technical: Chronic toxicity study in rats", Study No. 852214, Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, Summit, NJ, 1/28/91. Crl:VAF/PlusJCD7(SD)BR rats were used in a reproduction study, (DPR Record No. 063785; Ciba-Geigy Study No. 852063), and F1a offspring from that study were randomly selected from respective groups for the present study. Initially there were 50/sex/group at 0, 10, 50, and 500 ppm in diet. Males of all groups were sacrificed at wk 8 (10/dose) or 52 wk (all remaining males). Females (excepting those assigned to a crossover study) were sacrificed at wk 8 (10/dose), or wk 35 (10/dose), or wk 104 (20/dose at 0 and 500 ppm, 30/dose in other groups). Control and 500 ppm females assigned to the crossover study (10/group) were maintained on original test diets for 65 wk, then switched from 0 to 500 ppm or vice versa for the rest of the 104 wk study. Primary parameters evaluated were histopathology of mammary gland (both sexes), testes, and pituitary (females only). Pituitary glands were also treated with immunocytochemical stains for prolactin, FSH, and LH. Other parameters evaluated included b.w., food consumption, hematology, clinical chemistry, and ophthalmology. Apparent NOEL for parameters evaluated = 50 ppm (body weight decrements in both sexes, and modest reductions in RBC parameters and elevations in cholesterol levels in females). Males had no indication of histopathologic change. There was an apparent increase in pituitary adenomas in high dose females, and also in females in the two crossover studies. This appears to have been due to unusually low incidence in 0 and 50 ppm females compared to historical control values, rather than a treatment effect. No adverse effects were identified, however this study did not provide major new information relating to previously identified oncogenicity of atrazine. Value of information was limited due to the small group sizes. Study is acceptabl as an ancillary study, given these limitations. Aldous, 10/3/96.

Unacceptable Studies, Not Usable for Hazard Assessment

014 942527-8 (1961, Hazleton Labs) No toxicity seen. UNACCEPTABLE study: dosages too low, inadequate histology, test article apparently not technical material, doses changed mid-study, etc. J. Christopher, 3/5/85.

061 039985 (1981, IBT) Summary only. Appears to be the study classified as Core Supplementary data in a 1983 Dynamac report (p. 2 of EPA 1-liners). J. Schreider, 4/18/86.

053 016906 Exact duplicate of 061 039985

EPA one-liner: "IBT-supplementary; Dynamac Corp.; Contract # 68-01-6561; accepted by EPA 5/31/83. (Two year feeding; IBT # 622-06769)." This is probably the study in Vol. 061, Record #039985, mentioned under "Chronic, Rat", earlier in this summary.

095 059080 "Interim Report on Atrazine Chronic/Oncogenicity Feeding Study IARC," (National Institute of Hygiene, Budapest, Hungary.) This was a summary statement only: **not a complete study.** Atrazine technical grade (batch No. 0041424; 98.9%) was administered in the diet at 500 and 1000 ppm for 8 weeks to Fischer F344/LATI rats (51-56 rats/group). Later, the levels were lowered to 375 and 750 ppm due to toxic signs. Body weight, food and water consumption were regularly monitored. At death, animals were completely autopsied and histology was performed on tissues. **Adverse effect indicated.** Dose-related increase in combined leukemia/lymphoma incidence was observed in males (not statistically significant) and in females (statistically significant). A significant increase in benign mammary tumors in males at 1000 ppm and a statistically significant increase in infiltrating uterine adenocarcinomas (dose related) in females was observed. M. Silva, 11/20/87. (A more detailed report is found in Record No. 069686: see 1-liner under oncogenicity, rat, below).

220-112 069686 Wetzel, L.T. "Review of a Hungarian Institute of Hygiene atrazine toxicology study", performed at Hungarian Institute of Hygiene in Budapest under auspices of WHO. Wetzel's evaluation was dated 1/13/87. This was the Institute's first long-term oncogenicity study. Atrazine (98.9%) was fed to 55/group to F344 rats at final dose levels of 0, 375, or 750 ppm until natural deaths of the animals. Males had increased incidence of combined mammary tumors (incidence of 1, 1, and 8 in increasing dose groups). The finding was not considered by investigators to represent a treatment effect, because 6 of the 8 high dose tumor-bearing animals died after the last control male had died, hence preferential survival had biased the incidence. (Survival of control males was very poor, with 100% mortality at about week 110). There was no increase in mammary tumors in females. Study does not appear to offer meaningful data, even after finalization of the report. Aldous, 10/18/93.

220-540 170886 Liu, C. Y. and A. K. Thakur, "Statistical report for survival and mammary tumor analyses from the Fischer 344/Lati rat study (Pinter et al., 1990)". This submission, dated Sept. 2, 1999, was prepared in response to a request by a U.S. EPA reviewer for clarification of study details. The most

complete evaluation of this study provided to DPR is Document No. 220-112, Record No. 069686: an evaluation by L. T. Wetzel, entitled "Review of a Hungarian Institute of Hygiene atrazine toxicology study" dated 1/13/87 (above). The present submission provides individual data on mammary tumors for males and females, plus some statistical analyses. Incidence data of concern (mammary tumors in males) are consistent with results previously reported. Results do not lead to change in acceptability or "possible adverse effect" status from previous submissions. Aldous, 10/15/99.

Interpretative Writings by Investigators (generally without substantial new data)

220-541 171843 Breckenridge, C., J. McFarland, and J. Stevens, "Summary of Atrazine's mode of action in the female Sprague-Dawley rat". Addendum to 220-511:160554, 10/29/99. This summary, by Novartis Crop Protection, Inc., reviewed several themes, none of which are unique to this submission, considered to support treatment of atrazine as a threshold toxicant. The first tab provides several figures and tables relating to major studies previously reported by registrants, and several published studies. Additional ongoing studies were identified in this section. The second tab is an analysis by JSC Sielkin (authors: Sielkin, R. L., C. Valdez-Flores, and L. Holden,) entitled "Palpable tumors in Sprague-Dawley rats: time-to-tumor analyses". Investigators evaluated 35 predictor variables using Multistage-Weibull time-to-tumor analyses with respect to mammary adenocarcinoma and fibroadenoma incidence in Record No.160554. Investigators concluded that fibroadenoma had a very strong correlation with the parameter, "mammary secretory" activity. Additional parameters correlated with fibroadenoma were mammary galactocele, pituitary adenoma, and number of abnormal days in diestrus during weeks 1 to 26. The executive summary of this record stated that "high level stimulation of the mammary gland with prolactin has been shown to be linked to the development of fibroadenoma as a result of ductal enlargement, lobulo-alveolar development, the development of secretory activity and the formation of milk cysts". The data in Record No.160554, however, do not show a positive association between mammary secretory activity and fibroadenoma at any of several selected time periods in the study, as shown in the DPR review. The same investigators' analyses of adenocarcinoma correlates reported the strongest association to be with "number of observed abnormal estrous days in weeks 5 to 26", followed by "ppm". The latter is consistent with previous DPR review conclusions, but was not analyzed by DPR in response to this submission. DPR would particularly welcome a response to the

above concern about the relationship between fibroadenoma and mammary secretory activity, as well as reviewable data on the effects of GnRH on the LH surge in rats. Aldous, 1/4/00.

220-365 137858 Stevens, J. T. et al., "Hypothesis for mammary tumorigenesis in Sprague-Dawley rats exposed to certain triazine herbicides", J. Toxicol. Environ. Health 43:139-153 (1994). Atrazine and simazine, and to a lesser extent two other chloro-s-triazines, propazine and terbuthylazine, elicited increases in mammary tumors in female Sprague-Dawley rats in previously completed studies (the atrazine study cited was DPR Record No. 044294). Data from three 2-thiomethyl-s-triazines and two methoxy-s-triazines were presented: these tended to be, at best, weak inducers of mammary tumors. There was no consistent pattern of effect on any class of substituted triazines on pituitary tumors. Limited hormone level data (week 104 observations only) indicated marked changes in hormone profiles in aged females. Investigators concluded that some of these triazines elicited tumors by altering hormonal levels over time, however this report did not give supporting information. Aldous 7/27/95 (no worksheet).

220-366 137859 Eldridge, J. C. et al., "Factors affecting mammary tumor incidence in chlorotriazine-treated female rats: hormonal properties, dosage and animal strain", Environmental Health Perspectives 102:29-36 (1994). Reviewed in draft form as 220-172:113603 by Aldous on 10/14/93. Report presents plots and bar graphs, but no individual data. Major findings were as follows. **(1) Effects on uterine weights:** Ovariectomized rats [strain was not specified: judging from a similar publication by the same group (Tennant, M. K. et al., J. Toxicol. Environ. Health 43:183-196 (1994), DPR Record No. 137864), strain was probably Sprague-Dawley] were dosed with 0, 20, 100, or 300 mg/kg/day atrazine for 2 days. On day 2, half of each group was injected with estradiol. All rats were killed 24 hr later. A dose-related decrease in uterine weights was obtained at the 100 and 300 mg/kg/day dose levels in rats with estradiol treatment: without estradiol treatment, atrazine had no effect on uterine weight. **(2) [3H]-thymidine binding in uterine slices from treated rats:** Intact, immature (23-day old) female rats were gavaged with 0, 1, 10, 50, 100, or 300 mg/kg/day atrazine for 2 days. On day 2, all animals received estradiol injections. After 24 hr, all animals were killed, and uterine slices were prepared for incubation with [3H]-thymidine. Radiolabel incorporation into slices was decreased at 50 to 300 mg/kg/day only. **(3) Binding studies in uterine cytosol:** A competitive binding study was similar to a simazine study (Record No. 128788, summarized toward the end of this Summary of Toxicology

Data). Initial attempts to study competitive binding of atrazine to the estrogen receptor in uterine cytosol were futile: no attainable atrazine concentration was sufficient to measurably displace estradiol when atrazine and estradiol were applied simultaneously. Pre-incubation of 0.1 mM atrazine with cytosol at RT, followed by chilling the preparation prior to addition of 3 nM labeled estradiol, led to appreciable but reversible reduction in estradiol binding. Investigators concluded that atrazine possessed a very weak inhibition of estradiol, possibly with competitive and noncompetitive components. The inhibition seen in vitro was considered to be plausibly relevant to effects seen in high dose levels in chronic studies. **(4) Vaginal cytology studies to evaluate estrus cycles:** Investigators discussed data reviewed under Record Nos. 123799 and 123801: analyses of vaginal cytology over time in chronic studies using Sprague-Dawley and F-344 rats that suggested that the Sprague-Dawley females dosed with high amounts of atrazine tended to have elevated estradiol levels and greater percentage of time in estrus, presumed to enhance mammary tumor yields in affected strains. **(5) Hypothalamic histopathology:** The arcuate nucleus of the rat hypothalamus is known to influence estrous cycles. The di-dealkylated chlorotriazine metabolite, diaminochlorotriazine (DACT) was shown to cause premature formation of inclusion granules of astrocytes in that nucleus (EM evaluation of hypothalamic tissues following dosing of 0 or 1000 ppm DACT for 20 to 48 wk). These inclusions are part of a normal aging process, which has been shown to be blocked by ovariectomy, and enhanced by chronic estrogen administration. Thus, study results suggest a possible central mode of action for atrazine-like molecules. **Investigator conclusions:** differences between humans and rodents relating to the process of reproductive aging and to the physiological nature and hormonal responsiveness of mammary tumors were presented as reasons why triazines may not be as serious a health concern to humans as might be inferred from rodent studies. Aldous, 7/28/95 (only highly reduced data were presented, hence no worksheet).

220-172 113603 Draft of the publication in Record No. 137859, above.

220-367 137860 Stevens, J. T., "Rat mammary tumorigenesis: Relevance of hormonal imbalance to dose selection", (published in The Toxicology Forum, The Given Institute of Pathobiology, Aspen CO, July, 1994). This is principally a risk assessment document, recommending that tumors associated with atrazine be regulated on the basis of a NOEL for chronic effects associated with hormonal imbalance, rather than by extrapolation of tumor incidence curves to the origin. Major points are differences

between strains of rats (i.e., Sprague-Dawley females have protracted estrus associated with elevated estradiol upon aging, compared to F-344 rats, which are characterized by extensive proestrus and elevated progesterone in geriatric life). The author contends that the doses that produce highly significant elevations in tumor incidence (i.e. $p < 0.01$), substantially exceed the MTD, based on body weight gain criteria. Aldous, 8/1/95 (only highly reduced data provided, hence no DPR worksheet).

220-369 137862 Wetzel et al., "Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague-Dawley and Fischer 344 rats", J. Toxicol. Environ. Health., 43:169-182 (1994). This is a review of some of the information submitted in Record No. 123802 and related records. No DPR review is appropriate. Aldous, 7/31/95.

220-456 151471 Breckenridge, C., "Summary of additional comments on the response to the Special Review Position Document 1 for pesticide products containing atrazine and simazine: Supplement II" 10/30/96. This document summarizes main points associated with risk assessment. It contains brief reviews of toxicology studies, atrazine metabolism, human exposure assessment, and human safety and ecological risk evaluations. No new studies; no worksheet. Aldous, 3/18/98.

NOTE: In response to a U.S. EPA Special Review on atrazine, registrants have organized a "Consensus Panel", which has convened on several occasions. The panel has gathered data to highlight new findings about the mechanism by which atrazine elicits mammary tumors in female Sprague-Dawley rats, to discuss physiological differences between test animals and humans, and to advocate "margin of exposure" rather than linear extrapolation techniques to address human risk. Some studies cited here, particularly those by Tyrey *et al.* and Cooper *et al.* have not been submitted to DPR, although they may contribute to the evaluation of mechanisms of reproductive risks. "Consensus Panel" reports are summarized below. Aldous, 3/18/98.

220-460 151479 [Consensus Panel] "Weight of the evidence on the oncogenic potential of atrazine: Consensus Panel report", 3/21/95. This appears to be the first of 3 Consensus Panel reports available to date. This report reviews several major atrazine chronic rodent studies, and summarizes mammary tumor findings associated with several analogs of atrazine. There are no new data requiring DPR review (no worksheet). Aldous, 3/18/98.

220-459 151477 Simkins, J. W., "Evaluation of a hormonal mode of action for mammary carcinogenesis of the chlorotriazine herbicides: second consensus panel report", 10/30/96. This report cites data by Cooper *et al.*, 1995, which have not yet been submitted to DPR. There are no fundamentally new insights on atrazine (no worksheet). Aldous, 3/18/98.

220-491 156970 Simkins, J. W., "Evaluation of a hormonal mode of action for mammary carcinogenesis of the chloro-s-triazine herbicides: third consensus panel report", 6/30/97. This panel analysis cites many studies relating to mechanism of atrazine-elicited Sprague-Dawley female rat mammary tumor responses. Some cited studies are not yet received by DPR, and some information on a chronic supplemental study involving ovariectomized rats (DPR Record No. 151475) is more recent than the interim report presently available. This report does not indicate fundamental changes from previous conclusions based on studies in progress. Page 19 of this report suggests that a study (possibly Tyrey *et al.*, 1996) has found that atrazine does not affect pituitary responsiveness to GnRH. That study, and all other studies relating to the disposition of hormones related to reproduction should be submitted to DPR. Aldous, 3/18/98.

220-374 137869 Simkins, J. W., "Evaluation of a hormonal mechanism for mammary carcinogenesis of the chlorotriazine herbicides: Consensus panel report", 3/14/95. A panel of an independent group of scientists determined that atrazine is neither genotoxic nor intrinsically estrogenic, however atrazine appears to accelerate reproductive senescence (as maintained by other Ciba documents). The panel recommended several new studies, for which the protocols are given in another record (220-394 137917, under "Proposed Protocols", above). No reviewable data. Aldous, 7/30/96.

220-395 137928 [Consensus Panel] "Weight of the evidence on the oncogenic potential of simazine: Consensus panel report", 3/21/95. Conclusions were essentially the same as for atrazine (Record No. 137869, above). No reviewable data. Aldous, 7/30/96.

220-375 137870 Wetzel, L. T. and M. O. Tisdel, "Ciba response to May 3, 1994 letter by Penelope Fenner-Crisp, Director, Health Effects Division, Office of Prevention, Pesticides and Toxic Substances, U.S. EPA", 3/20/95. Primary text is a response to a letter by Dr. Fenner-Crisp, who referred in large part to data presented in a Nov. 1993 meeting with U.S. EPA, much of which data are embodied in an analysis by R.F. McConnell (dated 3/10/95, submitted as DPR Document No. 220-376, Record No.

137871, for which a DPR worksheet has been prepared. The letter stated that the etiology of human mammary carcinogenesis is not sufficiently understood to definitively state that the F-344 rat is a better surrogate than the S-D rat. Several possible flaws in the Ciba data were noted, including large SD's in hormone assay means, difficulty in conducting an experiment that could standardize the segment of proestrus in which rats were sampled (considering the large estradiol drop from early to late proestrus), technical problems with reading and interpreting air-dried vaginal smear samples, lack of plausible consistency in estrous cycle staging over the time period of 9 to 18 months, lack of estrous stage sampling between 3 and 9 months (a critical time period if atrazine is shortening the period before which S-D females attain constant estrus), and certain concerns about Ciba interpretation of hormone level data. Ciba authors defend data integrity and maintain that data are consistent with the hypothesis that atrazine works by modulating the LH surge, which is critical to maintenance of estrous cycling in S-D rats. No worksheet is relevant for the present record, however see review of the cited record (No. 137871). Aldous, 7/31/96.

CHRONIC, DOG

** **099, 129 062676, 087668** O'Connor *et al.*, "Atrazine Technical: 52-week Oral Feeding Study in Dogs," (Ciba-Geigy Corporation, 10/27/87). Atrazine Technical (Batch FL 850612, purity = 97%), was fed in the diet to Beagle dogs at 0 (6 dogs/sex/group), 15 and 150 (4 dogs/sex/group) and 1000 ppm (6/sex/group) for 52 weeks. NOEL = 15 ppm (increased mortality at 150 and 1000 ppm, decreased food consumption and body weight gain at 1000 ppm; decreased RBC, Hb, HCT, total protein and albumin as well as increases in platelet counts, phosphorus, sodium, glucose and liver and ovary relative weights at 1000 ppm). **Possible adverse effect.** At 150 and 1000 ppm, females experienced increased heart weights and in both sexes treatment related electrocardiographic changes in the heart accompanied by gross and histologically detectable pathology were observed. Previously reviewed as having a NOEL of 15 ppm (Silva, 5/20/88), the study has been re-evaluated based upon information submitted to DPR by Ciba-Geigy. The status, however, remains unchanged. M. Silva, 11/29/89.

049 016883 (Woodard Research Corp., 1964) 3/sex/group; summary. See EPA one-liner. Two-page summary noted tremors and stiffness of hindlimbs at 1500 ppm (HDT). Lacrimation at 15-1500 ppm. Findings **not considered as significant adverse effects** requiring risk assessment. J. Schreider 5/09/86.
EPA one liner: "Systemic NOEL = 150 ppm; systemic LEL = 1500 ppm (LDT) [sic: should read HDT] reduced food intake, decreased body weight, reduced Hb and hematocrit values. Levels tested = 0, 15, 150, 1500 ppm. Core grade: Supplementary. (Two-year feeding - dog; Woodard Research Lab, 1964; MRID # 00059213)." Aldous, 8/86.

ONCOGENICITY, MOUSE

** 100 064254 "Oncogenicity Study in Mice," (Ciba-Geigy Corporation, NJ, 10/30/87). Atrazine Technical (Batch FL # 841802; purity = 97.6%) was administered in the diet at 0, 10, 300, 1500 and 3000 ppm to 60 CD-1 mice/sex/group for 91 weeks. NOEL = 10 ppm (increased mortality in females at 3000 ppm; decrease in body

weight gain at 300, 1500 and 3000 ppm, decrease in water and food consumption at 1500 ppm in males and 3000 ppm in both sexes; reductions in RBC, HCT and Hb at 1500 ppm in males and 3000 ppm in both sexes; decrease in absolute brain weight at 3000 ppm in both sexes and decreases in mean absolute kidney weight at 3000 ppm in females; cardiac thrombi incidence was increased in 1500 ppm females and 3000 ppm in both sexes). No adverse effect. No oncogenic effects were observed with atrazine under the conditions of this study. ACCEPTABLE**.** M. Silva, 5/20/88.

220-407 138980 One page of historical control data for the above mouse oncogenicity study. Data were provided on U.S. EPA request. No DPR review.

014 942529 (Bionetics Research Labs, 4/29/69) NCI journal article. Study not upgradeable: insufficient information to make meaningful judgement. No individual necropsy/histology data. Too few animals. Too few doses. No indication of adverse effects noted. J. Christopher, 3/5/85.

061 039986 (1969, NCI) One-sentence summary, apparently referring to 014 942529. J. Schreider, 4/18/86.
053 016904 Exact duplicate of 061 039986.

061 039987 (IBT, 1981) Summary. No adverse effects reported. See EPA one-liner, below. J. Schreider, 4/18/86.
EPA one-liner: (Oncogenic, mice; IBT, #8580-08906; 6/30/81) Study contracted to Dynamac Corporation for review. Classified by Dynamac as "Core Supplementary Data" (hence study not acceptable by EPA to fill data gaps). EPA reviewers accepted the Dynamac judgement, hence replacement study required. 5/31/83.

053 016903 Exact duplicate of 061 039987.

REPRODUCTION, RAT

** 101, 118 063785, 072136 "Atrazine Technical: A Two Generation Toxicity Study in Rats." (Ciba-Geigy, Summit, NJ, Laboratory Study # 852063, 11/17/87 and 12/14/88) Atrazine technical, Batch FL-841802, 97.6% purity, was administered to 30 CR CD, VAF/PLUS rats/sex/group in the diet at 0, 10, 50 or 500 ppm continuously for 2 parental generations of animals and their offspring throughout all phases of the study. Parental NOEL = 50 ppm (decreased body weight, body weight gain and food consumption in both sexes at 500 ppm). Reproductive NOEL > 500 ppm (no effects on reproduction was observed). No adverse effect. Initially reviewed as unacceptable but upgradeable with submission of missing data on culled pups for F1 and F2 and no necropsy data for F1 pups and only 5/sex/group were given for F2 pups; also noted that P2 generation had only 18 litters at 10 ppm. M. Silva, 5/20/88. Record # 072136 in vol. #118 cites the pages on which the requested data are found in part 6 of 6 in volume 101. These data upgrade the study to ACCEPTABLE status. J. Gee, 2/9/89. A re-evaluation of this study was performed in response to an EPA review stating a reproductive effect in F2 pups at $\geq$ 50 ppm based upon weight decrease in males at 21 days. CDFA notes the effect at 21, but not 14 days. Therefore, the weight decrease was probably due to increased consumption of treated diet (rather than milk). On a mg/kg basis, the treated diet would be an overdose for the pups. The weight decrease is not considered to be of toxicological importance. The study status remains unchanged. M. Silva, 6/28/90.

014 942530 (Woodard Research Corp., 1966) NOT ACCEPTABLE nor upgradeable. No adverse effects reported. See EPA one-liner. J. Christopher 3/5/85.
EPA one-liner: "Systemic NOEL > 100 ppm (HDT); reproductive NOEL > 100 ppm (HDT); dietary regime altered. Levels tested = 0, 50, 100 ppm. Core grade: Supplementary. (Three-generation; Woodard Research Labs, 1966; MRID #00024471)."

061 039990 (Woodard Research Corp., 1966) Brief summary of 014 942530. J. Schreider, 4/18/86.
053 016900 Exact duplicate of 061 039990.

220-231 128906 Supplement to 101, 118 063785, 072136. U.S. EPA had argued that the NOEL for reproductive effects was 10 ppm, based on a modest decrement in body weights of 50 ppm F2 male pups at day 21 post-partum. This supplement states that the pup weights were not correctly evaluated, and that the pup weight decrements should not have been tagged as statistically significant in the first place. CDFA (now DPR) had placed the reproductive effects NOEL at > 500 ppm (attributing the pup weight changes to direct consumption of diet by rapidly growing pups after day 14). For this reason, this supplemental submission is of unlikely importance to study evaluation. Aldous, 9/3/96.

TERATOGENICITY, RAT

050 016887 "A Teratology Study of Atrazine Technical in Charles River Rats", Ciba-Geigy, 9/8/84. Atrazine technical (96.7%); administered by gavage on days 6 - 15 of gestation to groups of 27 mated rats at 0, 10, 70, or 700 mg/kg/day. Initially reviewed (Parker, 4/30/86) as a possible adverse effect since the Maternal NOEL = 70 mg/kg/day (mortality) while the developmental NOEL < 10 mg/kg/day (increased visceral and skeletal variants). Additional data supplied, 094 54802, included historical control data. Variants were in the range of historical control values; therefore, no adverse effects are indicated and the developmental NOEL = 70 mg/kg/day. Study remains NOT ACCEPTABLE and not upgradeable (excessive mortality, only five litters at high dose). J. Parker, 7/3/87.

094 054802 Ciba-Geigy rebuttal to 016887 includes historical control values.

220-361 137854 Infurna, R. et al. "Teratological evaluations of atrazine technical, a triazine herbicide, in rats and rabbits", J. Toxicol. Environ. Health. 24:307-319 (1988). Publication corresponding to Record No. 16887, reviewed by CDFA in 1987. No separate worksheet is appropriate.

220-407 138991 Ciba response to U.S. EPA regarding purity of atrazine in Record No. 016887, above. Response also provided historical frequency of runted fetuses. No review is necessary for DPR, since fetal body weight was not a noted concern in the review of the above study, and was specifically noted as not evident at the HDT of 100 mg/kg/day in the accepted rat teratology study (Record No. 073682, below). Aldous, 9/4/96.

014 942531 (Ciba Geigy, 1971) Doses of 0, 100, 500, and 1000 mg/kg/day by gavage. Summary and assessment data, tables included. See EPA one liner, below. NOT ACCEPTABLE, not upgradeable. High dosage apparently too high. No individual data. Test article not defined. J. Christopher, 3/5/85.
EPA one-liner: "Maternal NOEL = 100 mg/kg; Maternal LEL = 500 mg/kg (wt. loss); Fetotoxic NOEL = 100 mg/kg; Fetotoxic LEL = 500 mg/kg (fetal resorptions, wt. loss); developmental NOEL > 1000 mg/kg (HDT). Core grade: Minimum, (Ciba Geigy, Switzerland, 1971; MRID # 00038041)."

050 016886 Exact duplicate of 014 942531.

061 039988 (Ciba Geigy, 1971) Summary of 014 942531. J. Schreider, 4/18/86.

053 017902 Exact duplicate of 061 039988.

118 072136 Document providing data for dosing suspensions for the rabbit teratology study (see below) contains a statement that there is an ongoing rat teratology study with Atrazine. The document is signed and dated December 14, 1988. J. Gee, 2/9/89.

** 120, 73682, "Atrazine Technical: A Teratology Study (Segment II) in Rats", (Ciba-Geigy Corporation, Summit, NJ, Project No. 89006, 2/23/89), Atrazine Batch No. FL 841802, 97.6% purity (Document No. 220-101, page 1341), 0.05, 0.25, 1.00% in 3% aqueous cornstarch containing 0.5% Tween 80 dosed at 5, 25, 100 mg/kg/day, vehicle controls 10 ml/kg/day, day 6-15 gestation, dose adjusted for body weights on days 6, 8, and 12;

26 sperm positive females/dose; no adverse effects; Developmental NOEL = 25 mg/kg/day (delayed ossification without decreased body weight), maternal NOEL = 25 mg/kg/day (decreased feed consumption, body weights, and body weight gains). ACCEPTABLE. (DiBiasio 5/26/89)

220-292 129149 Duplicate of Document No. 220-120, Record No. 73682, above.

220-228 128903 Chemical analysis summary relating to Record No. 073682, above.

TERATOGENICITY, RABBIT

** 050, 118 016885, 072135 "Teratology Study of Atrazine Technical in New Zealand White Rabbits." (Ciba-Geigy Pharmaceuticals, NJ, 9/18/84 and 12/8/88) Atrazine technical, 96.3%, lot FL-821014; given by oral gavage at 0 (3% corn starch plus 0.5% Tween 80), 1, 5 or 75 mg/kg/day, days 7 - 19 of gestation to 19 New Zealand White rabbits/group; maternal NOEL = 1 mg/kg/day (decreased weight gain and food consumption); developmental NOEL = 5 mg/kg/day (increased resorptions, decreased fetal weight and number of live fetuses). Initially reviewed as unacceptable but upgradeable based on the lack of data on dosing suspensions. J. Parker, 4/30/86. Record # 072135 in 118 contains data on homogeneity, stability and adequacy of preparation of suspensions at the same facility and in the same vehicle. Status is upgraded to ACCEPTABLE with no adverse effect noted. J. Gee, 2/9/89.

061 039989 (Ciba-Geigy). Summary of 050 016885, see above. J. Schreider, 4/18/86

053 016901 Exact duplicate of 061 039989.

220-361 137854 Infurna, R. et al. "Teratological evaluations of atrazine technical, a triazine herbicide, in rats and rabbits", J. Toxicol. Environ. Health. 24:307-319 (1988). Publication corresponding to Record No. 16887, reviewed by CDFA in 1987. No separate worksheet is appropriate.

220-407 138981 One-page record states purity of atrazine in the above report (Record Nos. 016885 and 072135) was 96.3%. No DPR worksheet.

TERATOGENICITY, MICE

EPA one-liner: "Use of DMSO and insufficient data on litters; fetuses make evaluation impossible. AKRC3H, decreased # of live fetuses, AKR exhibited reduced fetal wt. Core grade: Supplementary. (Bionetics Research Labs, 1968; MRID # 00023558)." DPR does not have this study.

TERATOGENICITY, (GENERAL OVERVIEW)

220-362 137855 Johnson, E.M., "An evaluation and critique of atrazine developmental toxicology safety evaluations and human epidemiological data: a review of published and unpublished studies for hazard potential and risk estimation". Author concludes that the database supports the conclusion that atrazine does not pose special risks for developmental toxicity. Since the DPR evaluations of teratology studies to date do not disagree, there is no need for critical review of this document. Aldous, 7/25/95.

GENE MUTATION

** 105 064333 "Salmonella/Mammalian-Microsome Mutagenicity Test," (Ciba-Geigy, 12/86). Atrazine technical (G 30027, lot no. 210200, purity = 98.3%) was used in a mutagenicity test with Salmonella typhimurium

strains TA98, TA100, TA1538 and TA1537, with and without activation at levels of 0 (DMSO = vehicle), 20, 78, 313, 1250, and 5000 ug/0.1 ml DMSO. A toxicity test was also run at concentrations of 0 (DMSO), 0.08, 0.31, 1.2, 4.9, 19.5, 78.1, 312.5, 1250 and 5000 ug/0.1 ml DMSO. Plates were incubated for 48 hours at 37ºC. Positive controls were included with and without activation and for the toxicity test. No mutagenicity was observed with atrazine. ACCEPTABLE. M. Silva, 5/3/88.

088 065713 "An Assessment of the Genetic Toxicity of Atrazine: Relevance to Health and Environmental Effects," (Ciba-Geigy Corporation, 12/87). The objective of this discussion was to 1) reconcile the inconsistencies of test results within the data base, 2) interpret the significance of the possible difference in bioactivation of atrazine in plants and animals and 3) draw a conclusion regarding the possible genetic toxicity of atrazine to humans. The discussion concluded that a) it is not scientifically possible or necessary to reconcile all the test-response conflicts for atrazine, however using the weight-of-evidence system developed by ICPEMC, atrazine is considered non-mutagenic, b) extrapolation of genotoxic activity from the plant activation studies to effects in mammals or mammalian cells has not been documented, c) no case has been adequately established to show that atrazine is a genotoxic agent capable of initiating neoplasia or inducing transmissible mutation in mammalian germ cells. This information is supplementary. M. Silva, 5/20/88.

014 942532 (Natl. Institute of Genetics, 1975) Mass survey of 166 pesticides. No adverse effects indicated. No data. Report NOT ACCEPTABLE. J. Christopher, 3/5/85.

061 039991 (SRI) One-paragraph summary. Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100. Concentrations ranged from 50 to 5000 mg/plate with and without rat liver activation. No adverse effects reported. Report NOT ACCEPTABLE in summary form as submitted: insufficient information for assessment. See EPA one-liner, below. J. Schreider, 4/18/86.
EPA one liner: "Not mutagenic up to 5000 micrograms/plate with and without activation in TA 1535, 1537, 1538, 98, 100. Core grade: Acceptable. (SRI, 1977; MRID # 0006064)."

061 039992 (SRI) Summary - Mouse/Host-Mediated Assay. Salmonella strains TA1535 or TA1538 injected i.p. following oral administration of one or five doses. Atrazine apparently not mutagenic in this system. Report NOT ACCEPTABLE. Insufficient information for independent assessment. J. Schreider, 4/18/86.

061 039993 (Ciba-Geigy) One paragraph summary - Ames. Technical atrazine; Salmonella strains TA98, TA100, TA1535 and TA1537; with and without activation at 0, 10, 30, 90, 270 or 810 ug/0.1 ml/ plate. No apparent mutagenic effect reported. Report NOT ACCEPTABLE. Insufficient information for review. J. Schreider, 4/18/86.

061 040005 (Abstract from Mutagen Society, 1981) Ames assay with water-soluble extracts from corn grown in treated and untreated soils. No adverse effect reported. Summary. Report NOT ACCEPTABLE. Insufficient information for assessment. J. Schreider, 4/18/86.

061 040007 (Drug & Chemical Toxicology, 1984) Summary report, not acceptable. Salmonella TA100 was tested with corn extracts in the reversion assay and TM-677 in the forward mutation assay. Insufficient information for assessment. No adverse effect indicated. J. Schreider, 4/18/86.
EPA one-liner: "No metabolic activation. Procedure not delineated. Cytotoxicity limit improperly chosen, interpretation not acceptable. Core grade: Unacceptable. (Entomology Dept., P.S.U., by K. A. Rashid, MRID # 00079923)." DPR does not have this study.

Summary: While a number of studies have been conducted with atrazine, a number are incomplete reports or are publications as on file at DPR. The rebuttal submitted in Document 220-094 discusses and evaluates a series of studies in all genotoxicity test types. The complete reports need to be submitted to DPR. The data requirement is, however, fulfilled by Record # 064333. Silva, 5/88 and Gee, 2/89.

220-049 A 1-paragraph summary of negative studies. No DPR review.

CHROMOSOMAL ABERRATIONS

** 105 064335 "Dominant Lethal Test, Mouse," (Ciba-Geigy Ltd, 9/81). Atrazine technical (G 30027, lot 6663) was administered orally (by gavage) in a single dose at 0 (vehicle = 0.5% sodium carboxymethyl cellulose), 444 and 1332 mg/kg to male Tif.MAGf(SPF) mice (20/group). 2-Aminoanthracene at 78 and 234 mg/kg was used as the positive control. Six hours after treatment the males were mated with 2 untreated females. At the end of 1 week, the females were removed from the cages and replaced by another group of 2 females. This procedure was continued for 6 consecutive weeks. Presence of a vaginal plug = "day 0" of gestation. Females were autopsied on the 14th day of gestation. No effects observed at any dose level. The positive control showed a statistically significant increase in the relative number of embryonic deaths at 234 mg/kg in the fourth mating period. No adverse effect. ACCEPTABLE. M. Silva, 5/17/88.

** 181 121283, "Structural Chromosomal Aberration Test Dominant Lethal Test, Mouse, 8 Weeks", (T. Hertner, Ciba-Geigy Limited, Genetic Toxicology, Switzerland, Laboratory Study No. 911247, 1/7/93). G 30027 technical, purity 97.1%, was given in a single oral dose of 0 (corn oil), 500, 1000, 2000 or 2400 mg/kg to 30 male [Tif: MAGf (SPF)] mice/group. Piloerection and reduced locomotor activity were observed in the three high doses. G 30027 did not induce dominant lethal mutations in male mice; NOAEL = 2400 mg/kg. ACCEPTABLE. (Kishiyama and Gee, 10/15/93)

061 039994 (Ciba-Geigy) Nucleus anomaly test in Chinese hamsters given technical atrazine by oral gavage for 2 days at 0, 282, 564 or 1120 mg/kg/ dose. Bone marrow samples were examined from 3 males and 3 females. No adverse effect noted. Summary. Report NOT ACCEPTABLE. Insufficient information for assessment. J. Schreider, 4/18/86.

061 039995 (Ciba-Geigy) Summary - Chromosomal aberration/Mouse spermatogonia. Male mice were given 0, 444 or 1332 mg/kg for 5 consecutive doses, 8 males per group; 100 metaphases from each mouse were examined. No adverse effect indicated. Report NOT ACCEPTABLE. Insufficient information for assessment. J. Schreider, 4/18/86.

061 039996 (Ciba-Geigy) Summary - Dominant Lethal/Mouse Technical atrazine was given by oral intubation in a single dose at 0, 444 or 1332 mg/kg; number of mice not specified; No adverse effects indicated. Report NOT ACCEPTABLE. Insufficient information for assessment. J. Schreider, 4/18/86.

** 220-408 138993 "Atrazine: Structural chromosomal aberration test: Micronucleus test, mouse" (C. Ceresa, Ciba-Geigy Limited, Switzerland, Lab number 871546, 5/31/88) Atrazine, 98.2% purity, batch 210200, was given by oral gavage to male and female mice, Tif: MAGF, SPF. In the first part, doses were 0 (0.5% carboxymethylcellulose) or 2250 mg/kg, to 24/sex/group. Eight per sex per group were sacrificed at 16, 24 or 48 hours after dosing and slides from five per sex were analyzed for micronuclei in polychromatic erythrocytes. In the second part, 8 per sex per group were treated with 0, 562.5, 1125 or 2250 mg/kg and sacrificed at 24 hours. The positive control in both parts was 64 mg/kg cyclophosphamide with sacrifice at 24 hours. In the first part, a total of 7 females died at the high dose. In the second part, one female died at 1125 mg/kg within the 24-hour period. There was no treatment-related increase in micronuclei formation with any sacrifice time or dose. The positive control was functional. **No adverse effect** under test conditions. **Acceptable.** (Gee, 8/21/96.)

DNA DAMAGE

** 105 064334 "Autoradiographic DNA Repair Test on Rat Hepatocytes," (Ciba-Geigy, 2/9/84). Atrazine technical (purity = 98.2%; batch no. P 210200) was used on rat hepatocytes in vitro at 1.2, 6, 30 and 150 mg/ml medium (4 cultures/group). Cells were exposed for 5 hours. Dimethylnitrosamine (100 mM) served as a positive control. Vehicle (ethanol) and untreated groups served as negative controls. No adverse effect. No effects were

observed at any dose. At 150 mg/ml a precipitate was observed. Positive controls were within their historical range. ACCEPTABLE. M. Silva, 5/16/88.

061 040002 (Mutation Research, 1978) Very brief summary. Saccharomyces and Aspergillus nidulans tested for mitotic gene conversion with mouse liver microsomal activation. No adverse effect indicated. Report NOT ACCEPTABLE. Insufficient information for assessment. J. Schreider, 4/18/86.
053 016893 Exact duplicate of 061 040002.

061 040006 (Environ. Mutagenesis, 1982) Saccharomyces cerevisiae tested for gene conversion with corn plant extracts. Insufficient experimental control to identify compound-related effect. No adverse effect indicated. Report NOT ACCEPTABLE. J. Schreider, 4/18/86.
EPA one-liner: "No activation was made. The spot test is limited in usefulness. Core grade: Unacceptable. (T4 Bacteriophage systems; Battelle Memorial Inst., 1977; MRID 00025376)." DPR does not have this study.

EPA one-liner: "Not a mutagen inducer in both rec- assay and reversion assay utilizing H17 Rec+ and M45 Rec- strains of Bacillus subtilis, Escherichia coli, and Salmonella typhimurium. Core grade: Minimum, (Shirash, et. al., Mutagenicity. Research, 1976)." DPR has a very brief summary.

** 220-238 128913, "Atrazine, Autoradiographic DNA Repair Test on Rat Hepatocytes, Tests for Other Genotoxic Effects", (Th. Hertner, Genetic Toxicology, Ciba-Geigy Limited, Basel, Switzerland, Report # 911246, 14 April 1992). The test article was atrazine technical with 97.1% purity. Primary hepatocytes from male Tif: RAIf(SPF) rats were exposed in quadruplicate to concentrations of 0 (DMSO), 15.5, 46.4, 139.2, 417.5, 835.0, and 1670.0 mg/ml for 16 to 18 hours. Unscheduled DNA synthesis was measured by autoradiography. **No increase in unscheduled DNA synthesis** under test conditions. **Acceptable**. (H. Green and Gee, 8/16/96).

220-014 046274 Shirasu, Y. et al., "Mutagenicity Screening of Pesticides in the Microbial System", Mutation Research 10:19-30 (1976). Atrazine was among 166 pesticides screened for mutagenicity endpoints, and was negative for the rec- assay in B. subtilis. Point mutation assay in the same publication was given record No. 942532 (also negative). No DPR worksheet. Aldous, 9/4/96.

MUTAGENICITY, Reports not acceptable: not guideline studies.

[no document or record number] Taets, C., S. Aref, and A. L. Rayburn, “The clastogenic potential of triazine herbicide combinations found in potable water supplies”, study conducted at Department of Crop Sciences, Univ. of Illinois, Urbana, published April 1998 in **Environmental Health Perspectives 106**, 197-201. Atrazine, simazine, and cyanazine were tested individually and in combination in CHO cells for 48 hr at the M.C. L. and at the highest level found in Illinois water supplies (3 and 18 ppb for atrazine, respectively). Evaluations of nuclei were done by lysing cells, staining with propidium iodide, followed by flow cytometry using fluorescence detection. The parameter of interest was the coefficient of variation (CV) of the G1 peaks. Evaluations of chromosomes involved similar incubations, followed by colcemid treatment prior to staining. Cells were broken and chromosomes separated by forcing through a syringe needle. The measured parameter was the CV of the peak of largest chromosome. Atrazine by itself and in most combinations with the other triazines significantly increased the CV of whole nuclei G1 peaks and of the largest chromosome. These results were taken by investigators to indicate clastogenicity, however the meaning of the results is unclear, since this is not a validated assay design and the measured parameter cannot unambiguously be attributed to clastogenicity. Aldous, 5/28/98.

[no document or record number] Biradar, D. P., and A. L. Rayburn, “Chromosomal damage induced by herbicide contamination at concentrations observed in public water supplies”, study conducted at Department of Crop Sciences, Univ. of Illinois, Urbana, published in **J. Environ. Qual. 24**:1222-1225 (1995). No essential information is provided here above what is presented in the previous entry from Dr. Rayburn’s laboratory, except that investigators show that a known clastogen [cytosine â-D-arabinofuranoside (“Ara-C”] increases the CV of the

frequency distribution of the largest chromosome of CHO cells, as does atrazine, suggesting that by association, atrazine may be a clastogen. In addition, Ara-C and atrazine significantly reduced the percentage of total chromosomes observed in the peak corresponding to the largest chromosome, however this was a less sensitive measure than the change in the CV frequency distribution of the largest peak. No worksheet. Aldous, 5/29/98.

[no document or record number] Biradar, D. P., and A. L. Rayburn, "Flow cytogenetic analysis of whole cell clastogenicity of herbicides found in groundwater", **Arch. Environ. Contam. Toxicol. 28**, 13-17 (1995). This is the earliest of 3 similar publications (see above). Useful additional information is that the positive control, "Ara-C", causes marked "smearing" of the AG1" peak when tested at 0.3 ì**M** concentrations, however at 3.0 ì**M**, the G1 peak disappeared and a new well-defined peak appeared, suggesting that Ara-C had effectively blocked the cell cycle. In addition, when atrazine was incubated for only 15 minutes in this system at concentrations shown to elicit an increase in the CV of the frequency distribution of the G1 peak following the standard 48 hr incubation, the histogram generated by the cytometer appeared normal, suggesting that the mere presence of atrazine in the nucleus at time of assay did not alter the histogram. No worksheet. Aldous, 5/29/98.

061 040000 (Mutation Research, 1971) One-paragraph summary. No adverse effects indicated. Insufficient information to make any conclusions. J. Schreider, 4/18/86.

053 016896 Exact duplicate of 061 040000.

061 040001 (J. of Toxicology & Environ. Health, 1977) Summary of miscellaneous studies with Drosophila. **Possible adverse effect noted**: reductions in egg hatches, losses of x or y chromosomes. J. Schreider, 4/18/86.

053 016894 Exact duplicate of 061 040001.

061 040003 (Mutation Research, 1980) Half-page summary. Miscellaneous assay systems discussed. Insufficient information for meaningful conclusions. No adverse effects noted. J. Schreider, 4/18/86
053 016894 Exact duplicate of 061 040003.

061 040004 (Abstract from Mutagen Society, 1981) Summary of discussion of different protocols for cytogenetic studies. No adverse effects reported. J. Schreider, 4/18/86

053 016892 Exact duplicate of 061 040004.

220-355 137848 Brusick, D. J., "An assessment of the genetic toxicity of atrazine: Relevance to human health and environmental effects", [not a laboratory study, but an evaluation of the overall mutagenicity study database]. Analysis was by a computer-assisted weight of evidence approach, assessing the composite results of reviewed data under various study designs. Conclusion by Dr. Brusick was that "an adequate case for classifying atrazine as a genotoxic hazard (or genotoxic carcinogen) to humans has not been made". No worksheet (not an independent study), C. Aldous, 7/25/95.

METABOLISM

102 062750 "Disposition of Atrazine in the Rat (General Metabolism)," (Ciba-Geigy Corporation, 10/23/87). ^{14}C-Atrazine (>98% purity) and unlabeled atrazine technical (purity not stated) was administered (gastric intubation) to three groups of 5 CR CD rats/sex/dose and 2/sex/dose were untreated control. Group 1 was given a single oral dose at 1.0 mg/kg. Group 2 was given a single oral dose at 100 mg/kg and Group 3 was subchronically treated for 14 days with unlabeled atrazine given by gavage as a single daily dose at 1.0 mg/kg. On day 15 a single oral dose of ^{14}C-atrazine was administered at 1.0 mg/kg. No significant differences were observed between mean % recoveries of dosage groups or between mean % recoveries for male and female animals within dosage

groups. Urine was the primary route of excretion. In tissues, the highest concentration of radioactivity was associated with RBC's. The whole body half-life of elimination was determined as 31.3 ± 2.8 hours and elimination was from two body compartments by first order process. No adverse effects indicated. UNACCEPTABLE**,** (no analysis of atrazine technical, or dosing material; need to state whether high dose caused toxic or pharmacologic signs; intravenous route of dosing was not utilized). Upgradeable. M. Silva, 5/20/88.

102 062287 "Study of ^{14}C-Atrazine Dose/Response Relationship in the Rat (General Metabolism)," (SRI International and Agrisearch Incorporated, 10/23/87). ^{14}C-Atrazine (purity = 97.9%), in combination with cold atrazine (purity = 98.8%) was administered by gastric intubation to Sprague-Dawley CD female rats at 0 (vehicle = corn starch/polysorbate 80), 1, 3, 7, 10, 50 and 100 mg/kg daily for 10 days (2 rats/group). One rat/group was sacrificed 3 hours after the tenth dose and the remaining animals were sacrificed 72 hours after the tenth dose. Recovery averaged 89.2 (3 hour sacrifice after tenth dose), and 94.2% (72 hour sacrifice after tenth dose). The primary route of excretion was urine. A steady-state RBC concentration was estimated to be achieved only after 30 days of daily dosing. Tissue concentrations at 3 hours after the tenth dose were linearly related to plasma concentrations obtained at the same time point. Tissue concentrations at 72 hours after the tenth dose were 27% (significant) lower. A longer residence time for a portion of tissue ^{14}C-radioactivity than plasma radioactivity was indicated. Subchronic administration does not alter the first-order kinetics pattern of excretion observed for a single dose of atrazine. No adverse effect indicated. This study is supplemental to 102 62750. M. Silva, 5/20/88.

102 062647 "A Summary of the Disposition, Kinetics and Metabolism of Atrazine in the Rat," (Ciba-Geigy Corporation, 11/17/87). ^{14}C-Atrazine (purity not stated), was administered in a single oral dose to Sprague Dawley CD rats (5/sex/group) at 1.0 mg/kg, 100 mg/kg and 1.0 mg/kg following a 14 day period of dosing with nonlabeled material at the same rate. Elimination of radiolabel in urine and feces was monitored over a 7-day period after which the animals were sacrificed. A second test was run where female rats (2/group) were dosed daily for 10 days with ^{14}C-atrazine at 0 (vehicle = corn starch/polysorbate-80), 1, 3, 7, 10, 50 and 100 mg/kg, then sacrificed. In a third test, 5 females were dosed with 100 mg/kg to obtain metabolites for isolation and identification. Urine was the preferred form of elimination (first order, two compartment open system with whole body half-life = 31.3 hrs). The major metabolite was 2-chloro-4,6-diamine-S-triazine. No adverse effect indicated. This study is supplementary to 102 62750. M. Silva, 5/20/88.

102 064323 & 064324 "Characterization and Identification of Atrazine Metabolites From Rat Urine (General Metabolism)," (Ciba-Geigy Corporation, 11/17/87). ^{14}C-Atrazine (> 97% pure) was administered (vehicle = 1% carboxymethyl cellulose and Hi Sil 233 silica gel) by gavage in a single dose to 5 female Sprague-Dawley rats at 100 mg/kg. A mean total of 47.37% of the dose was excreted in the urine, 49.26% in the feces, 1.37% in the blood, and the rest in other tissues. Recovery averaged 103.78% with 93.5% of the administered dose eliminated within 72 hours. Urinary metabolites were isolated from the 0-72 hour urine. Metabolites were isolated by several methods, then identified. A pathway for metabolism of atrazine is proposed. Report 102 064324 "Formulation and Analysis of ^{14}C-CGA-169374 Dose Suspensions for Rat," (Ciba-Geigy, Greensboro, NC 2/10/87) contained an analysis of dosing material. No adverse effects indicated. These data are supplementary to report 102 62750**.** M. Silva, 5/20/88.

103 064325 "Dermal Absorption of ^{14}C-Atrazine in the Rat (General Metabolism)," (Ciba-Geigy Corporation, 11/11/87). Atrazine (radioactive purity = 99.5% for low & mid doses, 99% for the high dose) labeled with C and then dermally applied to male CR Sprague-Dawley rats (4/group) at 0.1, 1.0, or 10.0 mg/rat using 4L formulation. Another group used atrazine (10 mg/rat) in an 80W formulation. The application site was covered with a non-occlusive bandage. Rats treated with atrazine formulated with 4L were sacrificed at 2, 4, 10 or 24 hours after treatment. Rats treated with atrazine formulated with 80W were sacrificed at 10 hours. There was no difference in atrazine absorption, excretion or the amount left unabsorbed at 10 hours using 4L versus 80W. No adverse effects indicated. This study is supplementary to 102 62750. M. Silva, 5/20/88.

103 064326 "Metabolism of ^{14}C-Atrazine in Orally Dosed Rats," ^{14}C-Atrazine, (radioactive purity, 97.5%) was administered orally to male Harlan Sprague-Dawley rats for 7 days at 0.4 and 4.0 mg/kg. Three rats/time point were sacrificed at 5, 7, 9, 10, 14 and 18 days after dosing. The peak levels of radioactivity in RBC, liver and

kidney were reached at Day 8 for 4.0 mg/kg treated animals and Day 10 for 0.4 mg/kg treated animals. There were no significant differences observed in excretion patterns between the two dose levels. No adverse effect indicated. This study is supplementary to 102 062750. M. Silva, 5/20/88.

103 064327 "Dermal Absorption of ^{14}C-Atrazine by Rats," (Ciba-Geigy Corporation, 5/16/83). ^{14}C-Atrazine dissolved in ethanol was applied dermally at 0.25 and 2.5 mg/kg to Harlan Sprague-Dawley rats for 2, 4, 8, 24, and 48 hours (4 rats/sex/dose at each time point), then sacrificed. Atrazine had low solubility at 2.5 mg/kg and flaked off the skin, therefore, only data from the low dose was used in determining half-life skin values. Absorption of total radioactivity by the skin did not vary significantly by sex nor did excretion in urine and feces. Tissue levels of radioactivity did not plateau by 72 hours at the low dose. Most radioactivity was excreted in the form of ^{14}C-atrazine at both dose levels. No adverse effect indicated. This study is supplementary to 102 62750. M. Silva, 5/20/88.

103 064328 "Excretion Rate of ^{14}C-Atrazine From Dermally Dosed Rats," (Ciba-Geigy Corporation, 10/20/88). ^{14}C-Atrazine dissolved in tetrahydrofuran with a specific activity of 17.2 mCi/mg was dermally applied at 0.025, 0.25, 2.5 and 5.0 mg/kg to 2 female Harlan Sprague-Dawley rats/group for 144 hours. Urine and feces were collected at 24-hour intervals. An increase in percent of dose excreted was observed as the dosage level increased. The amount of dose remaining on skin residues was observed to decrease as dosage increased. The main route of excretion was in the urine. No adverse effect indicated**.** This study is supplementary to 102 62750. M. Silva, 5/20/88.

104 064329 "Metabolism of 2-Chloro-4,6-bis(isopropylamino)-s-triazine (Propazine) and 2-Methyl-4,6-bis (isopropylamino)-s-triazine (Prometone) in the Rat. Balance Study and Urinary Metabolite Separation," (USDA, ARS, 1967). The excretion of Propazine-^{14}C and Prometone-^{14}C (98-100% pure) labeled in the triazine ring was determined following administration of a single oral dose (41-56 mg/kg) to Sprague-Dawley male rats (5/group). Prometone-^{14}C activity was quantitatively recovered in the urine and feces within 72 hours after treatment. Propazine-^{14}C activity was excreted more slowly with tissue residues remaining 12 days after treatment. No activity could be detected in the expired CO_2 with either ring-labeled compound. Ion exchange chromatography of the urine revealed at least 11 metabolites from Prometone and 18 from Propazine. M. Silva, 5/2/88.

104 064330 "The Transformation of Triazine Herbicides in Animals" (Max von Pettenkofer-Institut, 1967). Male albino rats and male rabbits (number of animals was not specified) were treated by esophageal probe (number of treatments not specified) with simazine, atrazine, propazine, prometon or prometryn at 50-200 mg/animal (rats) or 600-1000 mg/animal (rabbits). Actual number of groups treated was not indicated. The urine was collected during the first 72 hours after feeding. A number of metabolites were isolated and identified. All metabolites retained the triazine ring intact. Dealkylation was a common metabolic pathway. M. Silva, 5/2/88.

104 064331 "In Vitro Metabolism of Atrazine by Rat Liver," (Ciba-Geigy Ltd., 1973). Male RAI rat livers were homogenized to make a 10,800 g fraction, and 100,000 g supernatant and microsomal fractions. The metabolism of atrazine and 6 possible metabolites by rat liver subcellular fractions was studied in vitro. The dealkylation reaction predominated over the conjugation reaction with glutathione; the isopropyl group was more easily dealkylated than the ethyl group. With the compounds investigated, the reactions involved dealkylation in the microsomal fraction and conjugation with glutathione in the soluble fraction. All of the chloro-s-triazines were able to form conjugates with glutathione. No evidence for the dechlorination of the chloro-s-triazines to hydroxy-s-triazines was observed in vitro. M. Silva, 5/2/88.

104 064332 "Metabolism of Mercapturic Acid-pathway Metabolites of 2-Chloro-N-isopropylacetanilide (Propachlor) by Gastrointestinal Bacteria," (Metabolism and Radiation Research Lab, USDA, 1983). The study was published in Xenobiotica, Vol. 13, No. 2, 115-126, 1983. There was no worksheet done on this supplementary information. M. Silva, 5/23/88.

Summary: Study 102 062750 was unacceptable due to a lack of necessary information and flaws in the design. DPR believes, however, that the supplementary metabolism studies submitted in combination with 102 62750 provide sufficient data to determine the metabolism, distribution and excretion patterns of atrazine. Therefore

even though an intravenous route was not used as required by the guidelines, the collective studies form a complete and acceptable metabolism study. Silva, 5/88.

NEUROTOXICITY, HEN

Not required at this time.

GENERAL REVIEW DOCUMENTS ON ATRAZINE:

220-336 137769 "Atrazine/Simazine Response to the United States Environmental Protection Agency's Position Document 1: Initiation of Special Review", PD-1 was dated 11/23/94. The primary Ciba response, entitled "Evaluation of the carcinogenic potential of atrazine and the relevance to human risk assessment" was dated 3/22/95. This document did not contain unique SB-950 data. Principal issues were the same as discussed in 220-222 128816 (Eldridge et al., 1993), found in the Combined, Rat section, above. No SB-950 review is appropriate at this time. Aldous, 7/30/96.

220-337 137772 [A parallel response to Record No. 137769, above, except relating to simazine]. No SB-950 review is appropriate at this time. Aldous, 7/30/96.

PROPOSED PROTOCOLS FOR UPCOMING STUDIES:

220-394 137917 Breckenridge, C. et al., (proposals to undertake several studies in female Sprague-Dawley rats to evaluate luteinizing hormone surge as it relates to estrous cycling). These studies were completed or well underway as of 3/31/98.

220-424 146006 Proposed protocol for the "fourth study" mentioned in the preceding paragraph. The interim report for this study (CHV Study No. 2386-108) has since been reported as Record No. 151475. No DPR worksheet is necessary. Aldous, 9/5/96, updated 3/19/98.

STUDIES OF ATRAZINE METABOLITES, CONTAMINANTS, OR ANALOGS:

Chronic, Oncogenicity, Or Subchronic Rat (Atrazine-Related Compounds):

220-370 137863 Tennant, M. K. et al., "Ultrastructural changes in rat hypothalamic arcuate nucleus following long-term diaminochlorotriazine feeding", Steroid Biochem. 12:21-26 (1993). Female Sprague-Dawley rats were treated for 12 or 28 weeks with 1000 ppm DACT. EM evaluation of arcuate nucleus sections was performed, in comparison to controls. There was an increase ($p < 0.05$) in astrocyte granule density in treated rats after 28 weeks of treatment only (based on 3 treated rats at that time period). No other treatment-related effects were observed. These granules increase during aging and rate of

appearance is influenced by factors including levels of several circulating hormones. Investigators proposed that circumstances associated with prolonged estrus in treated rats may be related to the hypothalamic changes. Aldous, 7/31/95 (only highly reduced data provided, hence no DPR worksheet).

220-332 134724 Chow, E. and Emeigh Hart, S.G., "2-Year dietary chronic toxicity/oncogenicity study with G-34048 in rats", Ciba-Geigy Corporation, Farmington, CT., 1/27/95. Report # F00125. G-34048 Technical (hydroxyatrazine), purity 97.1%, was administered in diet to 70 to 80 CD7 rats per sex per group at 0, 10, 25, 200, or 400 ppm. Sixty/sex/group were designated for a 2-yr study, other rats were sacrificed at 12 months. NOEL = 25 ppm (progressive nephropathy in females; dilatation with crystalline deposits, particularly in collecting ducts and renal pelves, interstitial papillary fibrosis). High dose rats had an accelerated onset of severe progressive nephropathy, with associated widespread mineralization of tissues, secondary parathyroid hyperplasia, and associated changes in bone (fibrous osteodystrophy), heart (progressive cardiomyopathy), and testes (degeneration or atrophy). These findings showed a sharp dose-response relationship, were limited to relatively high dose levels, and did not represent the active ingredient. **Acceptable** as an ancillary study. (H. Green and C. Aldous, 10/3/96).

220-417 Preliminary U.S. EPA DER of 220-332 134724, above. No DPR worksheet of this preliminary review is necessary. Aldous, 9/5/96.

220-333 134857 This is a 12/22/94 "potential adverse effects" disclosure originally sent to U.S. EPA regarding the nephrotoxicity evaluated in Record No. 134724, above. (No DPR "review" of this disclosure). Aldous, 8/30/96.

220-180 120943 1-year interim report for Record No. 134724, above. Briefly reviewed by Aldous, 10/7/93.

220-314 131713 June 1994 interim report for Record No. 134724, above. No review necessary, since final report is available.

220-216 128786 Rudzki, M. W., G. C. McCormick and A. T. Arthur, "Hydroxyatrazine: 90-day oral toxicity study in rats", Ciba-Geigy Corp., Summit, NJ, 10/25/89. Dose range-finding study for the chronic hydroxyatrazine study (Record No.134724, above). Main finding was nephrosis at 300 to 600 ppm, with associated changes in serum chemistry, urine volume, and urine solute concentrations. Apparent NOEL = 100 ppm. No worksheet

(2-year study has been reviewed, with lower NOEL). No adverse effects. No further information required. Aldous, 5/11/98.

220-215 128785 Pettersen, J. C., A. D. Richter, and P. A. Gilles, "Diaminochlorotriazine (G-28273): 90-day oral toxicity study in rats", Ciba-Geigy Corporation, Farmington, CT, 11/5/91, Laboratory Study # F-00006. Test article is termed "DACT" in this review. Crl:CD7(SD)BR rats, 15/sex/group, were dosed with 0, 10, 100, 250, or 500 ppm DACT (98.2%) in diet in a guideline study. In addition, estrous cycling was evaluated by daily vaginal smears (the main evaluation periods involving all rats encompassed days 42-56 and 70-85). Shortly before termination, blood samples were taken for hormonal evaluations, with an attempt to bleed during proestrus at a fixed time of day to optimize sensitivity and standardize diurnal variables (with analysis by an outside specialist: not under GLP oversight). Apparent NOEL was 10 ppm, based on increased numbers of "variable" or "indeterminate" length estrous cycles (< 4 days or > 5 days) at 100 ppm and above during days 42-56 (a finding more common in higher treatment groups at both of the main evaluation periods). Despite considerable variability in estrous status evaluations, incidence of "persistent estrus" was elevated at 250 and 500 ppm compared to other groups. No hormonal effects could be detected in this study, which was complicated because limited numbers of rats in the higher two dosage groups were in proestrus at blood sampling time, hence few usable sampling units. Other findings were reduced body weights at 500 ppm (significant only in males), and a small but statistically significant reduction of serum calcium, of unlikely physiological importance. This is an **acceptable** ancillary study. Aldous, 6/1/98.

220-213 128783 Schneider, M., "G-28279 Technical: 3-month oral toxicity study in rats (administration in food)", Ciba-Geigy Limited, Stein, Switzerland, 5/8/92, Test #: 901261. Ten Tif: RAIf (SPF) rats/sex/group were dosed with 0, 10, 50, or 500 ppm atrazine metabolite AG-28279" [deisopropylatrazine], batch FL-901747, 96.7% purity for 3 months in a standard subchronic study design. NOEL = 10 ppm [increased extent of splenic extramedullary hematopoiesis (EMH) in females]. Common findings at 500 ppm included body weight decrements (significant, $p < 0.01$, in females), marked increase in water consumption (without associated pathology), pituitary cell hypertrophy with associated thyroid follicular epithelium hypertrophy and fatty change in adrenal cortex in males, liver EMH and increased splenic EMH in females. Acceptable ancillary study, with no adverse effects. Aldous, 5/8/98.

220-213 128784 Gerspach, R., "G-30033 Technical: 3-month oral toxicity study in rats (administration in food)", Test No. 901264, 10/22/91. G-30033 (deethylatrazine) elicited minor body weight decrements and slight alterations in hematology and serum chemistry at 500 ppm, so that the NOEL was the next lower dose of 50 ppm. No worksheet. No adverse effects. **No changes** in splenic extramedullary hematopoiesis at any dose level, in contrast to the deisopropylatrazine subchronic report in Record No. 128783, above. Aldous, 5/11/98.

Chronic or Subchronic, Dog (Atrazine-Related Compounds):

097 060319 "Atrazine: Potential Adverse Effect Finding. Dog Chronic Feeding Study (Metabolite)," (Ciba-Geigy Corporation, Greensboro, NC). A pilot study utilizing the atrazine metabolite, 6-chloro-2,4-diamino-s-triazine (CAS No. 3397-62-4) has been initiated to assess its potential for adverse effects. Dogs were treated with doses of 0, 5, 100 and 1500 ppm in the diet. The study in progress demonstrated a **possible adverse effect.** At the end of week 5, four out of twenty dogs treated at 1500 ppm experienced atrial fibrillation. Two of these four dogs also experienced pre-cordial trill (pronounced atrial fibrillation). Currently (13 weeks into the study), the high dose is being lowered to 1000 ppm and two dogs from the 1500 ppm group are being placed on control diet as a recovery group. NOTE: See final report in 220-132 086117. M. Silva, 11/20/87.

**** 132 086117** "Diaminochlorotriazine, 13/52 Week Oral Toxicity Study in Dogs," (Agricultural Division, Ciba-Geigy Corporation, Summit NJ, 1/17/90). Diaminochlorotriazine (a metabolite of atrazine) technical (98.7% pure; FL 871423) was administered in the feed to Beagle dogs at 0 (10/sex), 5 (8/sex) and 100 ppm (8/sex) for 52 weeks (Groups 1 - 3). Ten males in another group (Group 4) received 1500 ppm (weeks 1-6) and 750 (weeks 7, 8 and 14-52) with an intervening period of 0 ppm (weeks 9-13). The 10 females in Group 4 received 1500 ppm (weeks 1-6) and 750 ppm (weeks 7-52). At wk 13, an interim sacrifice was performed and 2 Group 4 Females were placed on 0 ppm for weeks 14-52 (recovery). **Possible adverse effect.** NOEL = 100 ppm (Heart pathology and dysfunction, abnormalities of electrocardiogram; clinical signs (associated with decreased heart function) with accompanying gross and histopathological changes in heart and liver; increased liver, spleen and kidney weight; decreased animal weight gain and food consumption; hematology and clinical chemistry effects; increased mortality). ACCEPTABLE. (M. Silva, 3/21/90).

220-406 138979 Chau, R. Y., G. C. McCormick and A. T. Arthur, "Hydroxyatrazine: 13-week feeding study in dogs", Ciba-Geigy Corporation, Summit NJ, 3/20/90. Lab Study No. 892076. Four beagles/sex were dosed with 0, 15, 150, 1500, or 6000 ppm of hydroxyatrazine (presumably about 97.1% purity, based on other studies) for 13 weeks. Hematology, clinical chemistry, and urinalysis were examined prestudy and at termination. Standard gross and microscopic pathology was undertaken. The primary finding was chronic nephropathy with associated crystalline casts in renal tubules and papillae. All dogs at 1500 to 6000 ppm were affected. Acceptable as an ancillary subchronic study on an environmental contaminant. Aldous, 8/7/96.

220-211 128781 Thompson, S. S., G. Batastini, and A. T. Arthur, "G-28279 Technical: 13-week feeding study in dogs", Ciba-Geigy Corp., Summit, NJ, 4/22/92. Laboratory Study # MIN 912021. Four beagles/sex/group were dosed in diet with 0, 15, 100, 500, or 1000 ppm G-28279 [deisopropylatrazine] for 14 weeks. The study evaluated parameters typical of a dog subchronic study, plus EKG’s measured on days -14, 22, 50, and 87. Apparent NOEL = 100 ppm (reduced body weight and reduced food consumption, particularly in females; reduced organ weights of heart, testis, epididymides, prostate, and uterus; EKG’s showed reduced amplitude of P wave in both sexes). The EKG data were evident from the individual dog data, but the text in the report failed to acknowledge or discuss these changes; offering instead the impression that there no treatment-related EKG changes. Study is **not acceptable**, since the QAU failed to identify this reporting discrepancy. No adverse effects are indicated, considering the comparatively high NOEL. Aldous, 5/20/98.

220-212 128782 Rudzki, M. W., G. Batastini, and A. T. Arthur, "G-30033 Technical: 13-week feeding study in dogs", Ciba-Geigy Corp., Summit, NJ, 4/16/92. Laboratory Study # MIN 902187. Four beagles/sex/group were dosed in diet with 0, 15, 100, or 1000 ppm G-30033 [deethylatrazine] for 13 weeks. The study evaluated parameters typical of a dog subchronic study, plus EKG’s measured on days -21, 27, 50, and 78. Apparent NOEL = 100 ppm [reduced body weight and reduced food consumption; reduced organ weights, particularly of uterus and thymus in high dose females, with atrophic change seen in histopathology in both organs; heart pathology was indicated by angiomatous hyperplasia of the right atrium in one high dose male, paroxysmal atrial fibrillation observed EKG’s in one high dose female, and quantitative EKG effects including reduced P wave amplitude, increased duration of P waves, and remarkable tachycardia in both sexes; and a reduction of hematology parameters (RBC count,

Hb, and HCT) in both sexes]. The quantitative EKG changes were evident from the individual dog data, but the text in the report failed to acknowledge or discuss these changes; offering instead the impression that there no treatment-related EKG changes other than paroxysmal atrial fibrillation in one high dose dog. Study is **not acceptable**, since the QAU failed to identify this reporting discrepancy. No adverse effects are indicated, considering the comparatively high NOEL. Aldous, 6/1/98.

Teratogenicity (Atrazine-Related Compounds):

220-293 129150 Giknis, M. L. A., "Hydroxyatrazine Technical: A teratology (segment II) study in rats", Ciba-Geigy Corporation, Research Department, Pharmaceuticals Division, Summit, NJ, Report # 88099, 2/14/89. Hydroxyatrazine technical, 97.1% purity, was administered by gavage in aqueous suspensions of cornstarch/Tween 80 to pregnant Crl:COBS7 CD(SD)BR rats (26 dams/group at 0, 5, 25, or 125 mg/kg/day on gestation days 6-15). Maternal NOEL = 25 mg/kg/day (2 high-dose females had enlarged, mottled kidneys; also there was slightly reduced food consumption at 125 mg/kg/day during gestation days 8-12). Developmental NOEL = NOAEL = 25 mg/kg/day [slightly delayed ossification at several sites, very slight decrement in fetal weights, and a low incidence of pups with malformations in the abdominal wall (1 gastroschisis and 1 umbilical hernia in separate high dose litters)]. The malformations appear plausibly related to treatment, and constitute a **"possible adverse effect" relating to hydroxyatrazine**. The study is **Acceptable** for characterization of toxicity of hydroxyatrazine. (H. Green and C. Aldous, 10/3/96).

220-223 128818 Historical control malformation and skeletal variation data supporting Record No. 129150. Considered in review of that record.

220-224 128820 Chemical analyses of hydroxyatrazine supporting Record No. 129150. Considered in review of that record.

220-229 128904 Similar analytical report to Record No. 128820, but bearing an earlier date and missing even-numbered pages.

220-225 128821 Marty, J. H. "Developmental Toxicity (Teratogenicity) Study in Rats with G-28279 Technical (Oral Administration)", Ciba-Geigy Limited, Stein, Switzerland, Report # 901262, 1 June 1992. G 28279 technical, 97.4% purity. This is deisopropylatrazine (see cross index at end of Summary of

Toxicology Data). Tif: RAI f (SPF) rats, hybrids of RII/1 x RII/2, 24 mated females per group, received 0, 5, 25, or 100 mg/kg/day by gavage on gestation days 6-15. Developmental NOEL = NOAEL= 5 mg/kg/day [fused sternebrae (#1 and #2)], a "**possible adverse effect**" for this metabolite. Ossification delays were common at 100 mg/kg/day. Maternal NOEL = 5 mg/kg/day (minor decrements in body weight and food consumption). **Acceptable** as an ancillary study. (H. Green and C. Aldous, 10/3/96).

220-226 128822 Marty, J. H., "Developmental toxicity (teratogenicity) study in rats with G-30033 Technical (oral administration)", Ciba-Geigy Limited, Reproduction Toxicology, Stein, Switzerland, Report # 901265, 1 June 1992. G-30033 technical, 97.5% pure, was administered by gavage to Tif: RAI f (SPF) rats, 24 mated females per group, at 0, 5, 25, or 100 mg/kg/day on gestation days 6-15. Maternal NOEL = 5 mg/kg/day (slightly reduced food consumption at 25 mg/kg/day). Maternal effects at 100 mg/kg/day included more pronounced reduction in food consumption, statistically significant b.w. decrement, and hunched posture in 1 dam. Developmental NOEL = 25 mg/kg/day (sternebrae #1 and #2 fused). **Acceptable** as an ancillary study, with a "possible adverse effect" (fused sternebrae). H. Green, and C. Aldous, 10/3/96.

220-227 128823 Hummel, H. et al., "Diaminochlorotriazine, A teratology (Segment II) study in rats", Ciba-Geigy Corporation, Summit, NJ, 8/15/89, Report No. 89043. Diaminochlorotriazine (DACT) with at least 98.1% purity. Crl:COBS CD (SD)BR rats, 26 per group, received 0, 2.5, 25.0, 75.0, or 150.0 mg/kg/day by gavage on gestation days 6 through 15. Maternal NOEL = 25 mg/kg/day (maternal food consumption and body weight gain decrements). Developmental NOEL = 2.5 mg/kg/day (ossification delays in parietal, interparietal, and hyoid bones at $\geq$ 25 mg/kg/day). Changes at 75 to 150 mg/kg/day included dose-related decrements in fetal body weights, and ossification delays in the skull, hindpaw, and ribs. At 150 mg/kg/day there was also an increase in resorptions. **No adverse effects** (considering that developmental effects at all dose levels below 150 mg/kg/day appeared to be delays in development, without evidence of permanent changes). **Acceptable**. (H. Green and C. Aldous, July 6, 1995).

220-230 128905 Hummel, H. et al., [Analytical confirmation of identity of diaminochlorotriazine]. Relates to 220-227:128823, above. Test article is consistent with diaminochlorotriazine by MS, IR, and NMR. Aldous, 7/5/95.

Reproductive Toxicity Mechanisms (Atrazine-Related Compounds):

220-218 128788 Eldridge, J. C., "Interactions of simazine, a chlorotriazine herbicide, with the estrogen receptor system of rat uterus", Bowman Gray School of Medicine of Wake Forest University, 4/26/91. Several in vitro studies, utilizing pooled uterine cytosol, investigated competition between simazine and 3H-estradiol for specific estrogen receptor interactions. Simazine proved to be a weak competitive inhibitor of estrogen. Investigators determined that at simazine loading levels that might occur during chronic studies (e.g. 100 mg/kg b.w.), it is possible that simazine could compete with or delay binding of biologically significant amounts of estrogens with receptors. Aldous, 10/4/96 (no worksheet).

Mutagenicity (Atrazine-Related Compounds):

Test Type 842

** 220-408 139098 "Diaminochlorotriazine: Gene mutations test: Salmonella/mammalian-microsome mutagenicity test" (E. Deparade, Ciba-Geigy Limited, Switzerland, Lab study number 871372, 11/10/87) Diaminochlorotriazine (G28273) technical, 97% purity, was tested with Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 with and without activation with Aroclor 1254-induced rat liver S9. Concentrations were 0 (DMSO), 20, 78, 313, 1250, or 5000 mg/plate, triplicate plates per concentration, two trials. A precipitate formed at 5000 mg/plate. Positive controls with and without activation were functional. No evidence of an increase in reversion frequency with treatment was reported under the test conditions. **No adverse effect. Acceptable.** (Gee, 8/26/96)

** 220-408 139100 "Hydroxyatrazine: Gene mutations test: Salmonella/mammalian-microsome mutagenicity test" (E. Deparade, Ciba-Geigy Limited, Switzerland, test no. 871376, 2/15/88) Hydroxyatrazine [G34048] technical, 99% purity, was tested for mutagenicity with Salmonella strains TA98, TA100, TA1535 and TA1537, with and without rat liver activation with triplicate plates and two trials. Concentrations tested were 0 (DMSO), 20, 78, 313, 1250 and 5000 ug/0.1 ml/plate with 48 hour incubation. No increase in reversion rate was reported under the test conditions. **No adverse effect. Acceptable.** (Gee, 8/22/96)

** 220-233 128908, "G-30033 Technical, Gene Mutations Test, Salmonella and Escherichia/Liver-Microsome Test", (Eckhard Deparade, Ciba-Geigy Limited, Basel, Switzerland, Report #

891236, 18 December 1989). G-30033 technical (deethylatrazine), 99.3% purity. Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and Escherichia coli strain WP2uvrA were exposed for 48 hours in the presence and absence of rat liver microsomal activation to concentrations of 0 (DMSO), 313, 625, 1250, 2500, and 5000 mg/0.1 ml/plate. **No increase in reversion frequency indicated. Acceptable.** (H. Green and Gee, 8/29/96)

** 220-232 128907, "G-28279 Technical, Gene Mutations Test, Salmonella and Escherichia/Liver-Microsome Test", (Eckhard Deparade, Toxicology II, Ciba-Geigy Limited, Basel, Switzerland, Report # 891243, 18 January 1990). The test article was G-28279 technical [deisopropylatrazine], 97.4% purity. Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and Escherichia coli strain WP2uvrA were exposed for 48 hours in the presence and absence of rat liver microsomal activation at 0 (DMSO), 313, 625, 1250, 2500, and 5000 mg/0.1 ml/plate, triplicate plates, two trials. **No increase in reversion frequency was indicated** under test conditions. **Acceptable**. (H. Green and Gee, 8/29/96).

Test Type 843

** 220-408 139108 "Hydroxyatrazine: Structural Chromosomal aberration test; micronucleus test, mouse" (C. Ceresa, Ciba-Geigy Limited, Switzerland, lab. no. 871373, 8/31/88) Hydroxyatrazine [G34048] technical, 99% purity, was tested with Tif: MAGF, SPF mice of both sexes for the induction of micronuclei in polychromatic erythrocytes of the bone marrow. The study was conducted in two parts. Part 1: 24 mice per sex were given a single dose of 0 (0.5% carboxymethylcellulose) or 5000 mg/kg hydroxyatrazine. Eight per sex were sacrificed at 16, 24 or 48 hours after dosing and the bone marrow harvested for micronuclei analysis. Cyclophosphamide (64 mg/kg) was used as the positive control. Part 2: 8/sex were given 0, 1250, 2500 or 5000 mg/kg in a single dose with sacrifice after 24 hours. Slides from 5 per sex per group of eight were evaluated for the number of micronuclei and the ratio of PCE/NCE per animal. There was no increase in the formation of micronuclei with exposure to hydroxyatrazine. **No adverse effect** under the test conditions. **Acceptable.** (Gee, 8/23/96)

** 220-235 128910, "G-30033 Technical, Structural Chromosomal Aberration Test, Micronucleus Test, Mouse", (B. Ogorek, Genetic Toxicology, Ciba-Geigy Limited, Basel, Switzerland, Report # 901309, 25 March 1991). The test article was G-30033 technical

[deethylatrazine] with 99.3% purity. In the first stage, 24 Tif: MAGf (SpF) mice per sex per group received 0 (0.5% carboxymethylcellulose) or 480 mg/kg by gavage. Five (5) per sex per group were sacrificed at 16, 24, or 48 hours after treatment. In a second part, 8 per sex per group received 0, 120, 240, and 480 mg/kg. Bone marrow from 5 per sex per group was sampled 24 hours after treatment. **No increase in micronucleated polychromatic erythrocytes was reported**. **Acceptable**. (H. Green and Gee, 8/29/96).

** 220-234 128909, "G-28279 Technical, Structural Chromosomal Aberration Test, Micronucleus Test, Mouse", (B. Ogorek, Genetic Toxicology, Ciba-Geigy Limited, Basel, Switzerland, Report # 901307, 23 February 1991). The test article was G-28279 technical [deisopropylatrazine], 97.4% purity. In part one, Tif: MAGf (SpF) mice received 0 (0.5% carboxymethylcellulose) or 480 mg/kg by gavage, then marrow samples from 5/sex/treatment were evaluated 16, 24, or 48 hours after treatment. In part two, mice received 0, 120, 240, or 480 mg/kg. Bone marrow samples from 5 per sex per group were harvested 24 hours after treatment. One thousand (1000) PCE's were scored per animal. **No increase in micronucleated polychromatic erythrocytes was found. Acceptable**. (H. Green and Gee, 8/28/96).

Test Type 844

220-408 139099 "Diaminochlorotriazine: Tests for other genotoxic effects: Autoradiographic DNA repair test on human fibroblasts" (A. Meyer, Ciba-Geigy, Switzerland, lab number 871371, 11/20/87). Diaminochlorotriazine technical [G28273], 97% purity, was tested with human fibroblast cell line CRL 1521 (ATCC), passages 8, 13 and 14 without activation only. Concentrations tested were 0 (medium, DMSO), 5.56, 16.67, 50, 150, 300 or 600 ug/ml, five hours, triplicate coverslips, two trials. The positive control was 4-nitroquinoline-N-oxide. Concentrations were based on a preliminary cytotoxicity test with concentrations to 1000 ug/ml with evaluation of percent viable by dye exclusion and adhesion/morphological evaluation. Unscheduled DNA synthesis was determined by autoradiography following incorporation of ^{3}H-thymidine into nuclear DNA. Cell-free areas of the slides were used for background. Fifty cells from each of three slides per concentration were scored. **No adverse effect of unscheduled DNA synthesis induction** under the test conditions reported. **Unacceptable, not upgradeable** (no activation included, inadequate details regarding minimization of S-phase DNA synthesis, no cytoplasmic counts). (Gee, 8/26/96)

220-408 139103 "Hydroxyatrazine: Tests for other genotoxic effects; Autoradiographic DNA repair test on human fibroblasts" (A. Meyer, Ciba-Geigy Limited, Switzerland, lab no. 871375, 1/11/88) Hydroxyatrazine [G34048] technical, purity of 96-99%, was tested with the human fibroblast cell line CRL 1521 for the induction of unscheduled DNA synthesis by autoradiography. Cells were exposed for five hours without activation only to concentrations of 0 (DMSO), 13.89, 41.67, 125, 375, 750 or 1500 ug/ml in multiplates containing coverslips. Four replicates per concentration were exposed per trial (two trials) with fifty cells from three coverslips scored for each replicate. Cell-free areas were used as background. No measures were taken to minimize S-phase DNA synthesis. No evidence for the induction of unscheduled DNA synthesis under the test conditions was reported. **No adverse effect. Unacceptable** (no activation included). Not upgradeable. (Gee, 8/22/96)

** 220-408 139105 "Hydroxyatrazine: Test for other genotoxic effects: autoradiographic DNA-repair test on rat hepatocytes" (Th. Hertner, Ciba-Geigy Limited, Switzerland, lab. no. 871374, 1/22/88) Hydroxyatrazine [G34048] technical, 96-99% purity, was tested with male rat hepatocytes for the induction of unscheduled DNA synthesis. Primary hepatocytes were exposed on coverslips for 16-18 hours in two trials. First trial: 0 (DMSO, medium), 13.89, 41.67, 125, 375, 750 or 1500 ug/ml. Second trial: 0, 3.125, 6.25, 12.5, 25, 50, 100, 200, 300, 500 or 1500 ug/ml. Precipitates were visible at concentrations of 12.5 ug/ml and higher. Concentration selection was based on a preliminary cytotoxicity test. Unscheduled DNA synthesis was assayed by autoradiography following incubation with ^{3}H-thymidine. Fifty nuclei/slide for each of three slides were scored per concentration. No consistent evidence was reported for the induction of unscheduled DNA synthesis with hydroxyatrazine under the test conditions. **No adverse effect. Acceptable**. (Gee, 8/26/96)

** 220-236 128911, "G-28279 Technical, Tests for Other Genotoxic Effects, Autoradiographic DNA Repair Test on Rat Hepatocytes", (D. Geleick, Genetic Toxicology, Ciba-Geigy Limited, Basel, Switzerland, Report # 901308, 12 April 1991). The test article was G-28279 technical, 97.4% purity. Hepatocytes from adult male Tif.RAIf (SPF) rats were treated in quadruplicate at 0 (DMSO), 7.4, 22.2, 66.6, 200, 400, and 800 mg/ml for 16 to 18 hours. Unscheduled DNA synthesis was determined by incorporation of ^{3}H-thymidine and quantitated by autoradiography. **No induction of unscheduled DNA synthesis was indicated** under test conditions. **Acceptable**. (H. Green and Gee, 8/28/96).

** 220-237 128912, "G-30033 Technical, Tests for Other Genotoxic Effects, Autoradiographic DNA Repair Test on Rat Hepatocytes", (D. Geleick, Genetic Toxicology, Ciba-Geigy Limited, Basel, Switzerland, Report # 901310, 26 April 1991). The test article was G-30033 technical [deethylatrazine] with 99.3% purity. Primary hepatocytes from male Tif: RAIf(SPF) rats were exposed in quadruplicate to test material concentrations of 0 (DMSO), 9.25, 27.7, 83.3, 250, 500, and 1000 mg/ml for 16 to 18 hours. Unscheduled DNA synthesis analyzed by autoradiography. **No increase in unscheduled DNA synthesis** under test conditions. **Acceptable**. (H. Green and Gee, 8/29/96).

Studies of Atrazine Together with Other Active Ingredients and/or Fertilizers in Ground Water Contamination Studies:

220-364 137857 Johnson, E. M., "The effects of representative groundwater pesticides on reproduction and in utero development of experimental animals", (not a study, but a brief critique of 2 developmental toxicity and two reproduction studies employing such mixtures), 6/22/93. Components that commonly contaminate Iowa or California groundwaters, respectively, were tested at up to 100 times the median observed levels in respective areas in "CR" rats (developmental studies) and in CD-1 mice (continuous breeding reproduction studies). The only noteworthy finding was an 11% reduction in seminal vesicle weights in the mouse reproduction study employing the 100-fold California groundwater mixture (without any associated histopathology). No DPR worksheet is relevant for this brief critique. Aldous, 7/25/95.

A number of new studies, mainly on metabolites of atrazine, have been received. Metabolite studies may be evaluated in the future if warranted by ubiquity in the environment, or evidence of toxicity concern. Abstracts of studies have been examined in any case. J. Kishiyama and C. Aldous, 4/20/94.

CROSS INDEX OF CHEMICALS (IF CHARACTERIZED) AND CODE NUMBERS
[from 220-437:146019, pp. 14-15 (Ciba Study #174-91)]

CODE	NAME OR DESCRIPTION OF TEST ARTICLE	WHERE IDENTIFIED
G-28273	Diaminochlorotriazine i.e. 2,4-diamino-6-chloro-s-triazine	
G-34048	Hydroxyatrazine i.e. 2-ethylamino-4-hydroxy-6-isopropylamino-s-triazine	
G-28279	deisopropylatrazine i.e. 2-amino-4-chloro-6-ethylamino-s-triazine	
G-30033	deethylatrazine i.e. 2-amino-4-chloro-6-isopropylamino-s-triazine	
GS-17794	deethylhydroxyatrazine i.e. 2-amino-4-hydroxy-6-isopropylamino-s-triazine	
GS-17792	deisopropylhydroxyatrazine i.e. 2-amino-4-hydroxy-6-ethylamino-s-triazine	
GS-17791	diaminohydroxyatrazine i.e. 2,4-diamino-6-hydroxy-s-triazine	

- end of Summary of Toxicology Data 1-liners -

Record Code Name Findings Reported
Vol. I.D. Number of chemical in Abstract of Report

Study Type: 833- TERATOGENICITY
Test Species: RAT
225 SBR-145382-E 128821 (G28279) NEGATIVE
226 SBR-145382-E 128822 (G30033) NEGATIVE
227 SBR-145382-E 128823 (G28279) NEGATIVE for teratogenicity: LEL for delayed ossification = 25 mg/kg/day, NOEL for same = 2.5 mg/kg/day (lower than maternal NOEL). Probably does not constitute a "possible adverse effect" due to basis of LEL.
224 SBR-145382-E 128820 Revised Supplement to rat teratology study, EPA MRID #41065202 (by M. L. A. Giknis, original report dated 2/14/89). This supplement replaces MRID #42165501. This is a chemical purity and characterization for hydroxyatrazine.

223 SBR-145382-E 128818 Supplement to ??120 073682??
(historical data: Supplement to teratology study in rats with hydroxyatrazine, original study by Giknis, dated 2/14/89)

Record Code Name Findings Reported
Vol. I.D. Number of chemical in Abstract of Report

Study Type: 832- ONCOGENICITY
Test Species: RAT

MUTAGENICITY
Study Type: 842 - Gene Mutation (bacterial point mutation)
232 SBR-145382-E 128907 (G-28279) NEGATIVE
233 SBR-145382-E 128908 (G30033) NEGATIVE

Study Type: 843 - Structural Chromosomal Aberration.
(Micronucleus)
234 SBR-145382-E 128909 (G28279) NEGATIVE
235 SBR-145382-E 128910 (G30033) NEGATIVE

Study Type: 844 - DNA
236 SBR-145382-E 128911 (G28279) NEGATIVE
237 SBR-145382-E 128912 (G30033) NEGATIVE
238 SBR-145382-E 128913 (Atrazine) NEGATIVE

Study Type: 834- REPRODUCTIVE EFFECTS
Test Species: RAT
231 SBR-145382-E 128906 (see text of this toxicology summary)

220 SBR-145382-E 128813 Hormone level study, later superseded by 220-186:123799 (hence no DPR review of this earlier draft is needed).
221 SBR-145382-E 128815 Hormone level study, later superseded by 220-187:123801 (hence no DPR review of this earlier draft is needed).

Study Type: 821- SUBCHRONIC TOXICITY
Test Species: DOG

Study Type: 821- SUB-CHRONIC TOXICITY
Test Species: RAT

Study Type: 811- ACUTE ORAL TOXICITY
Test Species: RAT

209 SBR-145382-E 128779 (G28279) overall LD50 = 1240 mg/kg (tolerated better in males than in females)
210 SBR-145382-E 128780 (G30033) overall LD50 = 1110 mg/kg (tolerated better in males than in females)

Study Type: 833- TERATOGENICITY
Test Species: RAT
292 SBRA-145759-E 129149 (1 Part)(Atrazine) DUPLICATE of Document No. 220-120, Record No. 73682
293 SBRA-145759-E 129150 (1 Part)(Hydroxyatrazine) Giknis, M. L. A., "Hydroxyatrazine technical: A teratology (Segment II) study in rats", 2/14/89. No adverse effects. Both maternal and fetal development NOEL’s were reported to have been at least 25 mg/kg/day.

APPENDIX VI .

Dietary Exposure Assessment

ATRAZINE (Aatrex®)

DIETARY EXPOSURE AND ACUTE

TOLERANCE ASSESSMENTS

Wesley C. Carr, Jr.

HEALTH ASSESSMENT SECTION

MEDICAL TOXICOLOGY BRANCH

CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

August 28, 2000

I. Summary

Acute and chronic dietary exposures along with an acute tolerance assessment were performed for the herbicide atrazine. A NOEL of 5.0 mg/kg/day was used for the acute analysis. The value of 0.5 mg/kg/day was used as the NOEL for the chronic analysis. Seven primary and nine secondary raw agricultural commodities (RAC) and their residues were assessed. The residue data were either registrant supplied field trial data, U.S. EPA tolerances, or DPR data (California well water). The majority of the residue values were derived from the registrant supplied data. The most important RACs with detectable residues were corn and wheat. The sweet corn residue values were: acute, 0.06 ppm and for chronic, 0.022 ppm. The grain and pop corn residues were for acute; 0.04 ppm the limit of detection (LOD) and 0.02 ppm ½ the LOD for chronic. The wheat residue values were all non-detects: acute; 0.02 ppm, flour and 0.04 ppm other forms and chronic forms were all 0.02 ppm. Cane sugar atrazine residues were all non-detectable when measured as refined cane sugar. A separate residue value for cane sugar - molasses in addition to the refined cane sugar was used to reflect the potential dietary concentration in molasses. The chronic dietary exposure analysis used crop adjustment factors to reflect national percentage of crop treated (PCT) for 6 commodities based on registrant supplied national sales and marketing information. The commodities adjusted for reported U.S. usage were corn (sweet, pop and grain), wheat, sugar cane, sorghum, macadamia nuts and guava. There are one acute and two chronic dietary exposure scenarios evaluated by DPR for their impact on the population subgroups. The first chronic scenario which included DPR California well water data together with commodity residues and no percent of the crop treated adjustment was done only for baseline comparative purposes. The second chronic scenario used the same residue values but were then adjusted for percent of the crop treated reductions and represented the definitive DPR chronic dietary exposure scenario. The DPR well water data reported a total atrazine equivalents acute value of 2.73 ppb and a chronic residue value of 0.631 ppb. The acute value used was the single highest measured total atrazine equivalents residue detected in a non- municipal well known to contain atrazine residues. The chronic residue value consisted of the mean of all 16 total atrazine equivalents contaminated wells. The margins of safety at the 95th percentile of exposure were equal to or greater than 5,831 (non-nursing infant, <1 year) for the acute dietary exposure scenario. The margins of safety for the chronic non-oncogenic PCT adjusted dietary exposure were equal to or greater than 2,462 (non-nursing infants < 1year). The Q1 (MLE) oncogenic risk value for the lifetime oncogenic dietary exposure was 4.46E-06 (U.S. population, all seasons) for the PCT adjusted scenario. An acute tolerance assessment at the maximum residue contribution (MRC) was performed on 10 individual RAC tolerances. The margins of safety at the MRC 95th percentile of exposure were greater than 2,023 for each of the individual commodities and population subgroups evaluated.

II. Introduction

Atrazine (40 CFR #180.220) acute tolerance, acute and chronic (both non-oncogenic and oncogenic) dietary exposure assessments were revised and completed in 2000 (TAS, 1996a,b, USDA, 1989-91). All available atrazine raw agricultural commodity (RAC) residue data were evaluated (Table 1). The USEPA and Ciba-Geigy are revising the atrazine 40 CFR 180.220 tolerance from being characterized as atrazine to one of atrazine parent material and the toxicologically significant atrazine chlorometabolites (CFR, 1999). This will require all future residue collection programs to sample and check for atrazine and its toxicologically significant chlorometabolites. The Ciba-Geigy compound names in the submitted field residue studies are: **G-30027** (atrazine) CAS# 1912-24-9, **G-30033** (2-amino-4-chloro-6-isopropylamino-s-triazine) CAS# 6190-65-4, **G-28279** (2-amino-4-chloro-6-ethylamino-s-triazine) CAS# 1007-28-9 and **G-28273** (2,4 diamino-6-chloro-s-triazine) CAS# 3397-62 -4. The dietary exposure from the hydroxyatrazine metabolite of atrazine was also evaluated by Ciba-Geigy and determined to be of little toxicological concern (Ballatine, 1989). The impact of this change has been to render all previous FDA, USDA and DPR atrazine monitoring data essentially useless since they do not detect the significant chlorometabolites. The FDA limit of detection (LOD) for atrazine parent material is 0.01 ppm (FDA, 1992-95). The USDA Food Safety Inspection Service (FSIS) atrazine parent monitoring LOD is 0.005 ppm (USDA, 1994). The DPR atrazine parent material LOD was 0.1 ppm prior to 1988 and 0.05 ppm from 1988 to present (DPR, 1993-1995). The USDA Food Safety Inspection Service (FSIS) meat monitoring and DPR RAC residue monitoring programs do not currently characterize any of the atrazine chlorometabolites. The FDA does not routinely monitor for atrazine chlorometabolites and they are not recoverable using the current FDA methods in fatty foods such as milk, eggs, and cheese (FDA, 1991). Therefore, all the RAC and secondary commodity residue data for atrazine and its chlorometabolites used in the DPR dietary exposure and tolerance assessments were obtained from Ciba-Geigy registrant supplied field residue data or the U.S. EPA tolerance was used. The only exception to this were the water values which used the DPR's special California well water monitoring data obtained from wells known to have been previously contaminated with atrazine (DPR, 1994). Table 2 contains a summary of relevant margin of safety data from conducting acute and chronic dietary exposure assessment.

Currently there are 4 active registrations of atrazine approved for use in California. The registrations are only for agricultural uses . These herbicide ag end use products are for general pre-plant, pre-emergence and post-emergence weed control on various monocot annuals and guava fruit and macadamia nut commodities. The percent active ingredient formulations range from 43% to 90% for the agricultural sprays using atrazine active ingredient. The crop pre-harvest intervals (PHI) for commercial agricultural is 0 days for all of the actively registered crops. The labels have a 45 days PHI for pineapple, however this use has been voluntarily withdrawn by the registrant.

The total pounds (lbs.) of atrazine used in 1995 on California commodities was 38,140 lbs., with 60,080 lbs. for 1996 and during 1997 it was 49,070 lbs. (DPR, 1996, 1999a,b). The 3 year average (1995 - 1997) is 49,097 lbs. per year. The three California crops receiving the greatest application of atrazine over the 1995-97 period are corn (all forms) an average of 13,306 pounds per year, sudangrass an average of 11,786 lbs./yr. and bermudagrass with an average of 6,583 lbs./yr. These three crops accounted, on average, for approximately 65% of the total average atrazine use per year in California.

III. Residue Database

A. Corn (fresh, grain and pop)

The U.S. EPA raw food tolerance for corn (human consumption) is 0.25 ppm. The current corn maximum atrazine use rates call for either 3.0 pounds (lbs.) active ingredient (a.i.) per acre for pre-emergence (grain and sweet corn) or 4.0 lbs. a.i. per acre for post-emergence (sweet corn only). The lowest application rate in the registrant submitted field studies was 3.0 lbs. a.i./acre. The residue data for both grain and sweet corn were generated from this application rate. The residue data for grain corn was employed as the surrogate residue data for pop corn.

The registrant submitted several data volumes containing field trial residue data (Bade, 1987a, Bade and Ballantine, 1986 and Szorik, 1990a). These trials could be divided into two groups; pre-1985 and 1985-later studies. The pre-1985 field trials were not used because atrazine and its three significant chlorometabolites were not characterized at as low of a combined LOD level as the 1985 and later submitted studies. The pre-1985 studies combined LOD was 0.25 ppm versus 0.20 ppm or lower obtained for the 1985 and later studies. In addition, the 1985 and later field studies had better documention regarding plot replication. There were 25 field studies each with 2 replicates to represent the corn grain residue data generated prior to 1990. These 1985 through 1990 studies, while very usable, were superceeded by two studies submitted in 1994 and 1995 which had lower LODs and application rates which conform to the current labels (Selman, 1994 and Gold, 1995). All of the corn grain and sweet corn residue values were derived from the Selman and Gold field trial studies.

The registrant submitted a corn grain commodity residue study in 1994 which measured for atrazine and its 3 chlorometabolites from 1 field study located in Indiana (Selman, 1994). The application rate in the registrant submitted field studies included the 3.0 lbs. a.i./acre label rate and two exaggerated rates of 9.0 lbs. and 15.0 lbs. a.i./acre. The residue values were generated from the whole corn kernels in the analysis. The whole corn grain residue data were all non-detect at 0.01 ppm per analyte (combined LOD non-detect for atrazine and 3 chlorometabolites is 0.04 ppm). Since the three application rates had no detectable residues, they were all included in the sample count to generate a total of 6 samples. The combined LOD non-detect value will represent both grain and pop corn for residue expression in the DPR assessment. The whole kernel grain corn residue value used in the DPR acute analysis was 0.04 ppm (N = 6, all non-detect @ 0.04 ppm total atrazine). All the grain and pop corn residue values were set to ½ the combined LOD (0.02 ppm) in the chronic DPR dietary exposure analysis.

The registrant submitted another corn (sweet corn ears) residue study in 1995 which measured for atrazine and its 3 chlorometabolites from 6 field study locations in 5 states (Florida, Minnesota, North Carolina, Oregon and South Carolina) (Gold, 1995). The application rate in the registrant submitted field studies included both the 3.0 lbs. active ingredient per acre for pre-emergence (grain and sweet corn) and the 4.0 lbs. a.i./acre post-emergence (sweet corn only) rates. The whole ear sweet corn residue data were not all non-detectable at 0.01 ppm per analyte (combined LOD non-detect for atrazine and 3 chlorometabolites is 0.04 ppm) and 3 replicates were reported to have 0.04 ppm, 0.05 pppm and 0.06 ppm combined total atrazine residues. No parent atrazine was detected however, the three detectable residues found in the study were the chlorometabolites G-28279 (2 detections) and G-30033 (1 detection) (Gold, 1995). The sweet corn residue value used in the acute analysis was 0.06 ppm, the highest detected combined total atrazine residue. The value of 0.022 ppm (average sweet corn residue) was used in the chronic DPR dietary exposure analysis. This registrant data submission, Gold, 1995, was the basis for the

residues selected to represent the acute and chronic sweet corn residues in the DPR dietary exposure assessment.

Table 1. Summary of Atrazine Residues (August, 2000)

RAC	Source [1] (reference)	Tolerance (ppm)	Residue (ppm) Acute	Chronic	N [2]	Additional Information
Beef, fat/lean	Reg (Bray, 1997)	0.02 (N)[3]	0.001	0.001	-	no hydroxyatrazine
Beef, kidney	Reg (Bray, 1997)	0.02 (N)	0.004	0.004	-	no hydroxyatrazine
Beef, liver/mbyp	Reg (Bray, 1997)	0.02 (N)	0.02	0.02	-	no hydroxyatrazine
Corn, sweet	Reg (Gold, 1995)	0.25	0.06	0.022	24	combined residues
Corn, grain/pop/oil	Reg (Selman, 1994)	0.25	0.04	0.02	6	combined LODs
Egg	Reg (Bray, 1997)	0.02 (N)	0.005	0.005	-	no hydroxyatrazine
Goat, fat/lean	Reg (Bray, 1997)	0.02 (N)	0.001	0.001	-	no hydroxyatrazine
Goat, kidney	Reg (Bray, 1997)	0.02 (N)	0.004	0.004	-	no hydroxyatrazine
Goat, liver/mbyp	Reg (Bray, 1997)	0.02 (N)	0.02	0.02	-	no hydroxyatrazine
Guava	40 CFR 180	0.05	0.05	0.025	-	EPA toler & ½ toler
Horse	Reg (Bray, 1997)	0.02 (N)	0.02	0.02	-	no hydroxyatrazine
Macadamia nut	Reg (Bade, 1987b)	0.25	0.2	0.1	6	combined LODs
Milk	Reg (Bade, Cheung, 1987b)	0.02(N)	0.005	0.005	-	extrapolated res.
Pork, fat/lean	Reg (Bray, 1997)	0.02 (N)	0.001	0.001	-	no hydroxyatrazine
Pork, kidney	Reg (Bray, 1997)	0.02 (N)	0.004	0.004	-	no hydroxyatrazine
Pork, liver/mbyp	Reg (Bray, 1997)	0.02 (N)	0.02	0.02	-	no hydroxyatrazine
Poultry, fat/lean	Reg (Bray, 1997)	0.02 (N)	0.0003	0.0003	-	no hydroxyatrazine
Poultry, giblets/mbyp	Reg (Bray, 1997)	0.02 (N)	0.006	0.006	-	no hydroxyatrazine
Sheep, fat/lean	Reg (Bray, 1997)	0.02 (N)	0.001	0.001	-	no hydroxyatrazine
Sheep, kidney	Reg (Bray, 1997)	0.02 (N)	0.004	0.004	-	no hydroxyatrazine
Sheep, liver/mbyp	Reg (Bray, 1997)	0.02 (N)	0.02	0.02	-	no hydroxyatrazine
Sorghum grain	Reg (Ross, 1994b)	0.25	0.12	0.105	2	combined residues
Sugar-cane	Reg (Ross, 1994a)	0.25	0.002	0.002	8	refined sugr ½LODs
Water (all)	DPR (1994)	0.003 MCL[4]	0.00273	0.000631	16	DPR CA well water
Wheat	Reg (Ross, 1994c)	0.25	0.04	0.02	16	combined residues

1/ **REG** = Registrant supplied residue study or dietary exposure assessment derived data. **CFR 40** = U.S. EPA 40 CFR 180.220 tolerances. **DPR** = California Department of Pesticide Regulation.
2/ **N** = The number of RAC composite samples analyzed from the selected submitted studies.
3/ **(N)** = U.S. EPA determined that residue is expected to be a negligible residue as defined.
4/ **MCL** = U.S. EPA formulated atrazine maximum contaminant level for drinking water

B. Wheat

The U.S. EPA food tolerance for wheat (human consumption) is 0.25 ppm. The current maximum annual atrazine use rate calls for 1.0 pounds (lbs.) a.i./acre pre plant for wheat. The

application rate in the registrant submitted field studies was 1.0 lbs a.i./acre. The reviewed residue data included in the DPR dietary exposure assessment used this rate of atrazine application.

The registrant submitted one set of data volumes containing the field trial residue data. There were 6 field studies from 1985 each with 2 replicates to generate the wheat residue data (Bade, 1987e). All the residue values from these studies ranged between 0.13 ppm - 0.04 ppm for the combined LODs of atrazine and its three significant chlorometabolite degradation products. The values were; 0.04, 0.04, 0.04, 0.04, 0.04, 0.04, 0.04, 0.04, 0.05, 0.05, 0.09, and 0.13 ppm (N=12). The combined atrazine and metabolite analysis method acute non-detect value would be 0.04 ppm (atrazine and the 3 metabolites each at the LOD level of 0.01 ppm). A chronic combined total atrazine non-detect would be 0.02 ppm which is ½ the LOD.

The registrant submitted another wheat commodity residue study in 1994 which measured for atrazine and its 3 chlorometabolites from 8 field study locations in 5 states (Colorado, Kansas, Montana, Nebraska and South Dakota) (Ross, 1994d). The application rate in the registrant submitted field studies included the 1.0 lbs a.i./acre label rate. In addition to the whole grain residue data, low grade milled wheat flour residue data collected from 1 location was included in the analysis. The whole grain residue data were all non-detect at 0.01 ppm per analyte (combined LOD non-detect for atrazine and 3 chlorometabolites is 0.04 ppm) and wheat flour both are substrates selected for residue expression in the DPR assessment. The wheat whole grain residue value used in the acute analysis was 0.04 ppm (N = 16, all non-detect @ 0.04 ppm total atrazine) to represent bran, germ and rough wheat. The low grade milled wheat flour residues were also all non-detect however, since milled flour is a mixture, the ½ combined LOD value of 0.02 ppm was used to represent acute wheat flour residues. All the wheat residue values were set to ½ the combined LOD (0.02 ppm) in the chronic DPR dietary exposure assessment. This registrant data submission was the basis for the residues selected to represent wheat residues in the DPR dietary exposure assessment.

C. Milk

The U.S. EPA food residue tolerance for milk is 0.02 ppm which is considered a negligible residue level (CFR, 1999). The primary source of milk with atrazine residues would likely occur due to the consumption of feeds (labeled crop forage) with residues in the diets of dairy cows (Bade and Cheung, 1987b). The and registrant presented residue data indicate that the registrant extrapolated feeding study residue data is an accurate representation of potential atrazine residues in milk. The current maximum annual atrazine use rate on a crop with an established forage/fodder tolerance that can be fed to dairy cattle is 4.0 lbs a.i./acre for sugarcane. Based on the U.S. EPA Subdivision O residue chemistry guidelines (USEPA, 1982), no more than 55% of the dairy cows diet would consist of products from sugarcane (forage, bagasse, and molasses). The actual sugarcane contribution would likely be much less on a national basis because the majority of the animal fodder would come from corn, sorghum, or wheat sources. These RAC fodder sources are restricted to the lower maximum application rate of 2.5 lbs a.i./acre for these crops. The percentages of crop feed residue contribution would vary depending on dairy cattle feed sources.

The registrant submitted a data volume containing a feeding study of beef and dairy cattle fed diets fortified with atrazine and its chlorometabolite degradation products (Bade and

Cheung, 1987b). The lowest dietary feeding concentration was 3.75 ppm. The animal diet was grain fodder with the atrazine dosage levels administered daily separately in a capsule. The dairy cow atrazine extrapolated feeding level of 2 ppm most closely matches field rates of 2.5 lbs a.i./acre and resulted in an anticipated milk residue value of 0.013 ppm.

There are also two open literature atrazine detection studies that sampled commercial milk supplies and found residues that were near the registrant anticipated milk levels of 0.005 ppm and 0.013 ppm (Bushway et al., 1992 and Pylypiw and Hankin, 1991).

Finally, the registrant submitted a revised dietary assessment for atrazine which included a calculated value for milk (Bray, 1997). The milk value of 0.005 ppm total triazine residue (TTR) minus the hydroxyatrazine residues is higher than the USEPA anticipated residue of 0.002 ppm but lower than the earlier feeding study (Bade and Cheung, 1987a). The 0.005 ppm value is used in the DPR dietary exposure assessment because the current field application rates, animal forage/feed transfer factors and pre-harvest intervals are more reflective of anticipated milk residues than the 1987 feeding study. The extrapolated residue value of 0.005 ppm for milk is used in both the DPR acute and chronic analyses.

D. Water

There is not a currently established U.S. EPA tolerance for drinking water. However, the U.S. EPA has established a maximum contaminant level (MCL) value for atrazine of 3 ppb in drinking water (USEPA, 1991). Atrazine and its degradation products have been detected in the surface and ground water of many eastern and mid-western states (Ciba-Geigy, 1992). The DPR has conducted surveys of California's ground water to look for and measure atrazine and its chlorometabolites (DPR, 1994). Based on the concerns of potential atrazine contaminated water being consumed and used in the preparation of foods (commercial and homeowner reconstitution of frozen juices, etc), DPR has included in its dietary exposure assessment the potential exposures of the addition of atrazine contaminated drinking water.

The results of a detailed DPR California well water atrazine residue survey reported an acute value of 2.73 ppb and a chronic residue value of 0.631 ppb expressed as atrazine equivalent concentration units. The equivalent concentration units express total detected atrazine, deethylatrazine (DEA), and deisopropylatrazine (DIPA) amounts adjusted to reflect atrazine in total molecular weight equivalents (DPR, 1994). There were thirty wells retested throughout the state that previously had shown detectable levels of atrazine. Upon retesting, only 16 of the thirty wells still had detectable levels of atrazine. Atrazine degradates, DEA and DIPA, were also found in 14 of the 16 of these atrazine positive wells. The acute (2.73 ppb) and chronic (0.631 ppb) atrazine equivalent concentration residue values used in the DPR dietary scenarios is understood to likely be an overestimation of the amount of atrazine found in the California water supply. California drinking water comes from many sources; surface sources (rivers and lakes), agricultural and non-agricultural use wells (DWR, 1998). About 30% of California’s drinking water originates from ground water and 70% is obtained from surface water sources (DWR, 1998). Many California municipalities blend water from multiple sources, ground and surface water, and also same source (wells) assets. Given these uncertainties and likely overestimation of atrazine levels in California state drinking water, DPR did use these atrazine well water residues to provide a starting point for potential dietary estimation of exposure from atrazine contaminated water.

E. Other Agricultural Commodities

A summary of the residue data for the other primary and secondary RACs with U.S. EPA atrazine tolerances; cane sugar, guava, macadamia nut, sorghum, beef, all tissues, eggs, goat, horse, pork, poultry and sheep are presented in Table 1. These RACs are not explained in detail because of a combination of various factors such as established as a negligible tolerance or low commodity consumption rates made these RACs of lesser concern and the residue values do not require the explanation that the corn and wheat residue data received. Guava is an exception in that no field trial data were available to DPR and the registrant requested that the U.S. EPA evaluate guava based on its earlier pre-reregistration field residue data that established the tolerance of 0.05 ppm in the original U.S. EPA registration standard (Ciba-Geigy, 1993).

IV. Residue Adjustments

A. Percent of the Crop Treated

The current DPR chronic dietary exposure analysis default assumption is that 100% of any crop is treated with the pesticide under consideration. When quality data are available that indicate that less than 100% of a commodity is treated with a specific pesticide, then on an individual commodity by pesticide combination basis, exceptions to the default assumptions can be made.

The assumption that people under normal eating conditions would be continuously exposed to the averaged residue level of a pesticide for every labeled commodity for either for 1 year (chronic) or 70 years (lifetime) is unrealistic based on available substantial dietary information. This assumption does not take into account the fact that a significant amount of a commodity is often untreated with the pesticide under consideration. This is not reflective of actual practices and is borne out by the lower residue levels encountered in various market basket surveys versus the registrant field studies. The actual percentage of the crop treated with a specific pesticide varies from year to year depending upon biotic and abiotic factors. Using the existing percent crop treated data, it is reasonable to revise the 100% treated assumption downward using more realistic pesticide treatment rates and use patterns. Commodities that used residues obtained from registrant field trial or state and federal monitoring data in the chronic dietary exposure assessment were considered for percent crop treated adjustments.

The percent of the crop treated (PCT) adjustment method has been employed as the definitive comparison using 6 commodities (corn, wheat, sugar cane, sorghum, macadamia nuts and guava) that have atrazine tolerances to a chronic dietary exposure assessment without PCT adjustment. Only the commodities corn and wheat have reported atrazine use at the federal and state levels based on DPR Pesticide Use Reports and USDA Ag Field Crops Summary annuals (DPR, 1996, 1999a,b and USDA, 1997, 1998, 1999). The registrant also used percent of the crop treated adjustments in their submitted chronic dietary exposure assessment which were based on market share data (Bray, 1997). The registrant used marketing data from the research companies Maritz and Doane based on active ingredient sales and market share to estimate percent of the crop treated adjustments for not only corn and wheat but also for 4 other commodities (Bray, 1997). The PCT values submitted by the registrant for 6 commodities were used by DPR in the chronic dietary exposure analysis instead of the USDA and DPR derived PUR data.

B. Commercial Processing

The were no open literature processing studies reviewed that contained information regarding the combinations of the active ingredient atrazine and specific commodities. There was not any specific registrant supplied processing information supplied. Several registrant submitted studies contained residue data based on more specific food forms rather than the whole raw forms. These more refined RACs residues were used when available. Wheat flour values were used instead of using whole wheat grain to represent wheat flour (Ross, 1994c). The sugar cane residues in earlier studies were based on raw sugar cane, later studies used refined sugar from cane which is the form encountered by the majority of consumers (Ross, 1994a). Therefore, no specific processing reduction factors (appearing under adjustment factor #1 in the residue file) for any commodity food form measurements were used to modify the default adjustment factors used in the DPR acute and chronic dietary exposure assessment.

V. Dietary Exposure (Summary)

A. Acute Dietary Exposure

The acute dietary exposure values resulting from the use of the atrazine no-observed-effect-level (NOEL) of 5.0 mg/kg/day (rabbit oral developmental toxicity study; fetal effects) and anticipated residues were examined and the results are presented in Table 2 (TAS, 1996a, USDA, 1989-91). The 95th percentile acute dietary exposures using the rabbit NOEL ranged from 0.000857 mg/kg/day, non-nursing infants <1 year (MOE: 5,831) to 0.000234 mg/kg/day, seniors 55^{+} years (MOE: 21,333). The 97.5th percentile acute dietary MOEs using the same NOEL ranged from 5,334, non-nursing infants to seniors 55^{+} years at 18,084. There were no population subgroups, at either the 95th or 97.5th percentiles, that had margins of exposure values of less the 1000. The complete acute dietary exposure analysis includes all current U.S. EPA label approved atrazine uses.

B. Seasonal Dietary Exposure for California Workers

Atrazine, because of its pervasive year around utilization on California crops, does not present a clearly defined sub-chronic use season for workers applying the pesticide. The Worker Health and Safety branch therefore has not calculated a seasonal California worker occupational exposure. The Health Assessment Section (HAS) of the Medical Toxicology branch has determined that no seasonal worker exposure exists which would result in no sub-chronic worker dietary exposure. Therefore, none was calculated.

C. Chronic Dietary Exposure

The chronic non-oncogenic dietary exposure values obtained by using a NOEL of 0.5 mg/kg/day, derived from a 1 year dog study (cardio-toxicity effects) were examined (TAS, 1996b, USDA, 1989-91) (Table 3). There were two chronic exposure scenarios. The first scenario consisted of dietary exposure data with the use of percent of the crop treated adjustments (PCT) to 6 commodities and represents the primary DPR chronic dietary exposure. The second did not employ any PCT adjustments to the chronic dietary exposure data.

The chronic dietary exposures for commodities modified by the use of percent of the crop treated (PCT) adjustments ranged from 0.000203 mg/kg/day, non-nursing infants <1 year

(MOE: 2,491) to 0.000038 mg/kg/day, nursing infants (MOE: 13,082). The chronic dietary exposures for unmodified label approved commodity contributions ranged from 0.000286 mg/kg/day, children 1-6 years (MOE: 1,749) to 0.000046 mg/kg/day, nursing infants (MOE: 10,935). Under either scenario, there were no population subgroups that had margins of exposure values of less than 1000.

D. Lifetime (Oncogenic) Dietary Exposure

The chronic oncogenic dietary exposure values for the U.S. Population (all seasons), to represent potential lifetime dietary exposure, are presented in the following text. The cancer risk from chronic dietary exposure to atrazine was determined and the Q1 (MLE: maximum likelihood estimate) cancer potency value of 0.064 $(mg/kg/day)^{-1}$ and the $Q1^{*}$ (UB: upper bound) cancer potency value of 0.12 $(mg/kg/day)^{-1}$ were determined and used. The lifetime dietary exposure risk, using only the modified with marketing data (as PCT) and DPR well water residue data was 4.46E-06 for the MLE and 8.36E-06 for the UB using the U.S. population (all seasons) subgroup.

Table 2. Dietary Margins of Exposure [a] from Anticipated Atrazine Residues on Raw Agricultural Commodities When Using the Acute Rabbit NOEL.

Population Subgroups	Acute Dietary Exposure [b] 95 th percentile (Margins of Exposure) [c]	97.5 th percentile (Margins of Exposure) [c]
US Pop. all seasons	11,410 (0.000438 mg/kg/day)	9,190 (0.000544 mg/kg/day)
Western Region	11,220 (0.000446)	9,310 (0.000537)
Pacific Region	11,550 (0.000433)	9,470 (0.000528)
Hispanics	10,170 (0.000492)	8,210 (0.000609)
Non-Hispanic Whites	11,720 (0.000427)	9,450 (0.000529)
Non-Hispanic Blacks	10,510 (0.000476)	8,800 (0.000568)
Non-Hispanic Other	11,020 (0.000454)	8,790 (0.000569)
All Infants	6,020 (0.000831)	5,600 (0.000892)
Infants (nursing, < 1 year)	12,290 (0.000407)	11,170 (0.000448)
Infants (non-nursing, < 1 year)	5,830 (0.000857)	5,330 (0.000937)
Children (1-6 years)	6,820 (0.000733)	6,010 (0.000832)
Children (7-12 years)	10,520 (0.000475)	9,170 (0.000545)
Females (13-19 years) (not pregnant, not nursing)	17,720 (0.000282)	14,800 (0.000338)
Females (20+ years) (not pregnant, not nursing)	20,470 (0.000244)	17,240 (0.000290)
Females (13-50 years)	19,200 (0.000260)	16,020 (0.000312)
Females (13+ years) (pregnant, not nursing)	17,340 (0.000288)	16,200 (0.000309)
Females (13+ years) (nursing)	14,140 (0.000354)	12,960 (0.000386)
Males (13-19 years)	14,800 (0.000338)	12,360 (0.000405)
Males (20+ years)	20,000 (0.000250)	16,880 (0.000296)
Seniors (55+ years)	21,330 (0.000234)	18,080 (0.000276)
U.S. Population (16^+ Years) [d]	19,920 (0.000251)	16,630 (0.000301)

a/ MOEs based on all label approved commodities. Exposure levels have been rounded off to 4 significant figures and are based on the 1989-1992 Continuing Survey of Food Intakes of Individuals (CSFII).
b/ Acute residue files used non-Monte Carlo point estimate anticipated residue values for the commodities.
c/ MOE = NOEL ÷ Exposure. A MOE of at least 100 is generally considered to be protective of human health when the NOEL (non-oncogenic) is based on animal data. The acute NOEL value of 5.0 mg/kg/day was used (rabbit; developmental toxicity, fetal effects).
d/ A workers surrogate custom population subgroup not available in the chronic dietary exposure program.

Table 3. Chronic Dietary Margins of Exposure [a] from Anticipated Atrazine Residues on Raw Agricultural Commodities When Using the Chronic Dog NOEL.

	Chronic Exposure [b] Annualized Average (Margins of Exposure) [c]	
Population Subgroups	**No Percent Crop Treated [d]**	**With PCT Adjustments [d]**
US Pop. all seasons	4,685 (0.000107 mg/kg/day)	7,177 (0.000070 mg/kg/day)
Western Region	4,760 (0.000105)	7,190 (0.000070)
Pacific Region	4,850 (0.000103)	7,278 (0.000069)
Hispanics	4,612 (0.000108)	6,581 (0.000076)
Non-Hispanic Whites	4,683 (0.000107)	7,244 (0.000069)
Non-Hispanic Blacks	4,804 (0.000104)	7,282 (0.000069)
Non-Hispanic Other	4,459 (0.000112)	6,872 (0.000073)
All Infants	2,820 (0.000177)	3,206 (0.000156)
Infants (nursing, < 1 year)	10,935 (0.000046)	13,082 (0.000038)
Infants (non-nursing, < 1 yr)	2,172 (0.000230)	2,462 (0.000203)
Children (1-6 years)	1,749 (0.000286)	2,491 (0.000201)
Children (7-12 years)	2,856 (0.000175)	4,361 (0.000115)
Females (13-19 years) (not pregnant, not nursing)	5,445 (0.000092)	8,714 (0.000057)
Females (20+ years) (not pregnant, not nursing)	7,062 (0.000071)	11,190 (0.000045)
Females (13-50 years)	6,571 (0.000076)	10,562 (0.000047)
Females (13+ years) (pregnant, not nursing)	5,416 (0.000092)	8,149 (0.000061)
Females (13+ years) (nursing)	5,151 (0.000097)	8,212 (0.000061)
Males (13-19 years)	4,390 (0.000114)	6,995 (0.000071)
Males (20+ years)	6,716 (0.000074)	11,100 (0.000045)
Seniors (55+ years)	7,256 (0.000069)	11,284 (0.000044)

a/ MOEs based on all label approved commodities. Exposure levels have been rounded off to 3 significant figures and are based on the 1989-1992 Continuing Survey of Food Intakes of Individuals (CSFII).
b/ The chronic residue files used anticipated residue values for the commodities.
c/ MOE = NOEL ÷ Exposure. A MOE of at least 100 is generally considered to be protective of human health when the NOEL (non-oncogenic) is based on animal data. The chronic NOEL value of 0.5 mg/kg/day was used (dog; 1 year; cardio-toxicity).
d/ PCT = percent of the crop treated adjustment. The modification is made to adjustment factor 2 in the chronic residue file.

VI. Acute Tolerance Assessment

An acute tolerance assessment was performed for atrazine using the current U.S. EPA tolerances (CFR, 1999). The atrazine acute NOEL of 5.0 mg/kg-body wt/day was used to calculate margins of exposure based on a rabbit developmental toxicity study (fetal effects). There are currently 27 human consumption commodities (RACs) that have atrazine tolerances (CFR, 1999). A total of 10 individual commodities were analyzed. The 10 human consumption RACs with use tolerances listed in the 1999 Code of Federal Regulations were included in the acute tolerance assessment.

All 10 commodities analyzed had MOE values much greater than 100 for each population subgroup when the tolerance value was used. The highest acute tolerance residue contribution exposure (lowest MOE) was 0.002472 mg/kg-bw (MOE: 2,023) which occurred in the non-nursing infants <1 year population subgroup from potential sweet corn consumption. The lowest exposure (highest MOE) was obtained from the guava tolerance assessment of the population subgroup males 13-19 years; with a value of 0.000003 mg/kg-bw (MOE: >1,000,000). The 10 commodities analyzed were: beef; fat, beef; lean, cane sugar, corn; grain, corn; sweet, guava, milk, poultry; lean, poultry; fat and wheat.

The RAC sweet corn tolerance MOE range is non-nursing infants; 2,023 (0.002472 mg/kg-bw) - female 13^+ years (pregnant/non-nursing); 7,868 (0.000635 mg/kg-bw). The MOE range for the grain corn tolerance is all infants (nursing and non-nursing); 2,023 (0.002085 mg/kg-bw) - seniors 55^+ years; 15,170 (0.000330 mg/kg-bw). The RAC wheat tolerance MOE range is children 1-6 years; 2,753 (0.001816 mg/kg-bw) - seniors 55^+ years; 8,777 (0.000570 mg/kg-bw). The MOE range for the milk tolerance is non-nursing infants; 2,417 (0.002069 mg/kg-bw) - seniors 55^+ years; 23,698 (0.000211 mg/kg-bw). The MOE range for the cane sugar tolerance is children 1-6 years; 11,421 (0.000438 mg/kg-bw) - seniors 55^+ years; 43,276 (0.000116 mg/kg-bw). The MOE range for lean beef is non-nursing infants; 36,379 (0.000137 mg/kg-bw) - female 13^+ years (nursing); 105,886 (0.000047 mg/kg-bw). The RAC lean poultry tolerance MOE range is non-nursing infants; 31,526 (0.000159 mg/kg-bw) - female 13^+ years (pregnant/non-nursing); 88,758 (0.000056 mg/kg-bw). The MOE range for guava is western U.S.; 98,318 (0.000051 mg/kg-bw) - males 13-19 years; >1,000,000 (0.000003 mg/kg-bw), also there was no recorded infant guava consumption. The MOE range for beef fat is all infants; 150,206 (0.000033 mg/kg-bw) - female 13^+ years (nursing); 598,542 (0.000008 mg/kg-bw). Finally, the MOE range for poultry fat is non-nursing infants; 229,431 (0.000022 mg/kg-bw) - female 13^+ years (nursing); 871,120 (0.000006 mg/kg-bw).

Atrazine Dietary Exposure References (August, 2000)

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Chronic Exposure (EX1) Analysis for Atrazine Section 3 Registration
RESIDUE FILE NAME: ATRA1CH ANALYSIS DATE: 07-20-2000
NFCS Combined 89-92 DATA ADJUSTMENT FACTOR #2 NOT USED
EPA (RfD) = 0.005 mg/kg body-wt/day, DPR NOEL (Chronic) = 0.5 mg/kg bd-wt/day
COMMENT 1: REG studies, USEPA tolerance, DPR well H20, no %PCT.

RESIDUE FILE LISTING

TAS CODE	CROP GRP	FOOD NAME	RESIDUE (PPM)	ADJ. FCTRS #1	#2	SOURCE CODE
46	R	MACADAMIA NUTS (BUSH NUTS)	0.100000	1.00	1.00	REG-f
79	A	GUAVA	0.025000	1.00	1.00	EPA1/2
237	O	CORN/POP	0.020000	1.00	1.00	REG-fp
238	O	CORN/SWEET	0.022000	1.00	1.00	REG-f
266	O	CORN/GRAIN-ENDOSPERM	0.020000	1.00	1.00	REG-fp
267	O	CORN/GRAIN-BRAN	0.020000	1.00	1.00	REG-fp
268	O	CORN SUGAR	0.020000	1.00	1.00	REG-fp
275	O	SORGHUM (INCLUDING MILO)	0.105000	1.00	1.00	REG-f
276	O	WHEAT-ROUGH	0.020000	1.00	1.00	REG-fp
277	O	WHEAT-GERM	0.020000	1.00	1.00	REG-fp
278	O	WHEAT-BRAN	0.020000	1.00	1.00	REG-fp
279	O	WHEAT-FLOUR	0.020000	1.00	1.00	REG-fp
283	A	CANE SUGAR	0.002000	1.00	1.00	REG-fp
284	A	SUGAR-MOLASSES	0.005500	6.00	1.00	REG-fp
289	O	CORN GRAIN-OIL	0.020000	1.00	1.00	REG-fp
318	X	MILK-NONFAT SOLIDS	0.005000	1.00	1.00	REG-fp
319	X	MILK-FAT SOLIDS	0.005000	1.00	1.00	REG-fp
320	X	MILK SUGAR (LACTOSE)	0.005000	1.00	1.00	REG-fp
321	U	BEEF-MEAT BYPRODUCTS	0.020000	1.00	1.00	REG-hy
322	U	BEEF(ORGAN MEATS)-OTHER	0.020000	1.00	1.00	REG-hy
323	U	BEEF-DRIED	0.001000	1.00	1.00	REG-hy
324	U	BEEF(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
325	U	BEEF(ORGAN MEATS)-KIDNEY	0.004000	1.00	1.00	REG-hy
326	U	BEEF(ORGAN MEATS)-LIVER	0.020000	1.00	1.00	REG-hy
327	U	BEEF(BONELESS)-LEAN (FAT/FREE)	0.001000	1.00	1.00	REG-hy
328	U	GOAT-MEAT BYPRODUCTS	0.020000	1.00	1.00	REG-hy
329	U	GOAT(ORGAN MEATS)-OTHER	0.020000	1.00	1.00	REG-hy
330	U	GOAT(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
331	U	GOAT(ORGAN MEATS)-KIDNEY	0.004000	1.00	1.00	REG-hy
332	U	GOAT(ORGAN MEATS)-LIVER	0.020000	1.00	1.00	REG-hy
333	U	GOAT(BONELESS)-LEAN (FAT/FREE)	0.001000	1.00	1.00	REG-hy
334	U	HORSE	0.020000	1.00	1.00	REG-hy
336	U	SHEEP-MEAT BYPRODUCTS	0.020000	1.00	1.00	REG-hy

337	U	SHEEP(ORGAN MEATS)-OTHER	0.020000	1.00	1.00	REG-hy

338	U	SHEEP(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
339	U	SHEEP(ORGAN MEATS)-KIDNEY	0.004000	1.00	1.00	REG-hy
340	U	SHEEP(ORGAN MEATS)-LIVER	0.020000	1.00	1.00	REG-hy
341	U	SHEEP(BONELESS)-LEAN (FAT FREE)	0.001000	1.00	1.00	REG-hy
342	U	PORK-MEAT BYPRODUCTS	0.020000	1.00	1.00	REG-hy
343	U	PORK(ORGAN MEATS)-OTHER	0.020000	1.00	1.00	REG-hy
344	U	PORK(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
345	U	PORK(ORGAN MEATS)-KIDNEY	0.004000	1.00	1.00	REG-hy
346	U	PORK(ORGAN MEATS)-LIVER	0.020000	1.00	1.00	REG-hy
347	U	PORK(BONELESS)-LEAN (FAT FREE)	0.001000	1.00	1.00	REG-hy
355	V	TURKEY-BYPRODUCTS	0.006000	1.00	1.00	REG-hy
356	V	TURKEY-GIBLETS (LIVER)	0.006000	1.00	1.00	REG-hy
357	V	TURKEY-(BONELESS)-FAT	0.000300	1.00	1.00	REG-hy
358	V	TURKEY-(BONELESS)LEAN/FAT FREE	0.000300	1.00	1.00	REG-hy
359	V	TURKEY-UNSPECIFIED	0.006000	1.00	1.00	REG-hy
360	V	POULTRY-OTHER-LEAN (FAT FREE)	0.000300	1.00	1.00	REG-hy
361	V	POULTRY-OTHER-GIBLETS(LIVER)	0.006000	1.00	1.00	REG-hy
362	V	POULTRY-OTHER-FAT	0.000300	1.00	1.00	REG-hy
363	X	EGGS-WHOLE	0.005000	1.00	1.00	REG-hy
364	X	EGGS-WHITE ONLY	0.005000	1.00	1.00	REG-hy
365	X	EGGS-YOLK ONLY	0.005000	1.00	1.00	REG-hy
366	V	CHICKEN-BYPRODUCTS	0.006000	1.00	1.00	REG-hy
367	V	CHICKEN-GIBLETS(LIVER)	0.006000	1.00	1.00	REG-hy
368	V	CHICKEN (BONELESS)-FAT	0.000300	1.00	1.00	REG-hy
369	V	CHICKEN(BONELESS)LEAN/FAT FREE	0.000300	1.00	1.00	REG-hy
385	V	CHICKEN-GIBLETS (EXCL. LIVER)	0.006000	1.00	1.00	REG-hy
388	O	CORN SUGAR-MOLASSES	0.020000	1.00	1.00	REG-fp
393	A	GUAVA-NECTAR	0.025000	1.00	1.00	EPA1/2
398	X	MILK-BASED WATER	0.005000	1.00	1.00	REG-fp
424	U	VEAL-(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
425	U	VEAL-(BONELESS)-LEAN (FAT FREE	0.001000	1.00	1.00	REG-hy
426	U	VEAL-(ORGAN MEATS)-KIDNEY	0.004000	1.00	1.00	REG-hy
427	U	VEAL-(ORGAN MEATS)-LIVER	0.020000	1.00	1.00	REG-hy
428	U	VEAL-(ORGAN MEATS)-OTHER	0.020000	1.00	1.00	REG-hy
429	U	VEAL-DRIED	0.001000	1.92	1.00	REG-hy
430	U	VEAL-MEAT BYPRODUCTS	0.020000	1.00	1.00	REG-hy
432	A	WATER-BOTTLED	0.000631	1.00	1.00	DPR-X
433	A	WATER-TAP	0.000631	1.00	1.00	DPR-X
434	A	WATER-COMMERCIAL PROCESSING	0.000050	1.00	1.00	1/2LOD
435	A	WATER-NON-FOOD BASED	0.000631	1.00	1.00	DPR-X
437	O	WHEAT-GERM OIL	0.020000	1.00	1.00	REG-fp
449	V	TURKEY-(ORGAN MEATS)-OTHER	0.006000	1.00	1.00	REG-hy

911 A MOLASSES-NFS 0.020000 1.00 1.00 REG-f

Chronic Exposure (EX1) Analysis for Atrazine Section 3 Registration
RESIDUE FILE NAME: ATRA1CH ANALYSIS DATE: 07-20-2000
NFCS Combined 89-92 DATA ADJUSTMENT FACTOR #2 NOT USED
EPA Reference dose (RfD, chronic) = 0.005000 mg/kg body-wt/day
DPR NOEL (Chronic) = 0.500000 mg/kg body-wt/day
COMMENT 1: REG studies, USEPA tolerance, DPR well H20, no %PCT.
COMMENT 2: Most current DPR chronic assessment with no %CT adjustment.

TOTAL EXPOSURE BY POPULATION SUBGROUP

POPULATION SUBGROUP	mg/kg body-wt/day	Margin of Exposure 1	Percent of RfD
U.S. POP - 48 STATES - ALL SEASONS	0.000107	4,685	2.1%
NORTHEAST REGION	0.000105	4,780	2.1%
MIDWEST REGION	0.000114	4,400	2.3%
SOUTHERN REGION	0.000104	4,820	2.1%
WESTERN REGION	0.000105	4,760	2.1%
PACIFIC REGION	0.000103	4,850	2.1%
HISPANICS	0.000108	4,612	2.2%
NON-HISPANIC WHITES	0.000107	4,683	2.1%
NON-HISPANIC BLACKS	0.000104	4,804	2.1%
NON-HISPANIC OTHER THAN BLACK OR WHITE	0.000112	4,459	2.2%
ALL INFANTS	0.000177	2,820	3.5%
NURSING INFANTS (<1 YEAR OLD)	0.000046	10,935	0.9%
NON-NUR. INFANTS (<1 YEAR OLD)	0.000230	2,172	4.6%
CHILDREN (1-6 YEARS)	0.000286	1,749	5.7%
CHILDREN (7-12 YEARS)	0.000175	2,856	3.5%
FEMALES (13-19 YRS/NOT PREG/NUR)	0.000092	5,445	1.8%
FEMALES (20+ YRS/NOT PREG/NUR)	0.000071	7,062	1.4%
FEMALES (13-50 YEARS)	0.000076	6,571	1.5%
FEMALES (13+/PREG/NOT NURSING)	0.000092	5,416	1.8%
FEMALES (13+/NURSING)	0.000097	5,151	1.9%
MALES (13-19 YEARS)	0.000114	4,390	2.3%
MALES (20+ YEARS)	0.000074	6,716	1.5%
SENIORS (55+)	0.000069	7,256	1.4%

1. Margin of Exposure = DPR NOEL / Dietary Exposure

Chronic Exposure (EX1) Analysis for Atrazine Section 3 Registration
RESIDUE FILE NAME: ATRA1CH ANALYSIS DATE: 07-20-2000
NFCS Combined 89-92 DATA ADJUSTMENT FACTOR #2 NOT USED
Q1* = 0.120000
COMMENT 1: REG studies, USEPA tolerance, DPR well H20, no %PCT.
COMMENT 2: Most current DPR chronic assessment with no %CT.

TOTAL EXPOSURE BY POPULATION SUBGROUP

POPULATION SUBGROUP	TOTAL EXPOSURE mg/kg body-wt/day	Life-Time Risk (Q1*=0.120000) UB
U.S. POP - 48 STATES - ALL SEASONS	0.000107	1.28E-05
NORTHEAST REGION	0.000105	1.26E-05
MIDWEST REGION	0.000114	1.36E-05
SOUTHERN REGION	0.000104	1.24E-05
WESTERN REGION	0.000105	1.26E-05
PACIFIC REGION	0.000103	1.24E-05
HISPANICS	0.000108	1.30E-05
NON-HISPANIC WHITES	0.000107	1.28E-05
NON-HISPANIC BLACKS	0.000104	1.25E-05
NON-HISPANIC OTHER - THAN BLACK OR WHITE	0.000112	1.35E-05
ALL INFANTS	0.000177	2.13E-05
NURSING INFANTS (<1 YEAR OLD)	0.000046	5.49E-06
NON-NURSING INFANTS (<1 YEAR OLD)	0.000230	2.76E-05
CHILDREN (1-6 YEARS)	0.000286	3.43E-05
CHILDREN (7-12 YEARS)	0.000175	2.10E-05
FEMALES (13-19 YRS/NOT PREG or NUR)	0.000092	1.10E-05
FEMALES (20+ Yrs/NOT PREG OR NUR)	0.000071	8.50E-06
FEMALES (13-50 YEARS)	0.000076	9.13E-06
FEMALES (13+/PREGNANT/NOT NUR)	0.000092	1.11E-05
FEMALES (13+/NURSING)	0.000097	1.16E-05
MALES (13-19 YEARS)	0.000114	1.37E-05
MALES (20+ YEARS)	0.000074	8.93E-06
SENIORS (55+)	0.000069	8.27E-06

Chronic Exposure (EX1) Analysis for Atrazine Section 3 Registration
RESIDUE FILE NAME: ATRA1CH ANALYSIS DATE: 07-20-2000
NFCS Combined 89-92 DATA ADJUSTMENT FACTOR #2 NOT USED
Q1 = 0.064000
COMMENT 1: REG studies, USEPA tolerance, DPR well H20, no %PCT.
COMMENT 2: Most current DPR chronic assessment with no %CT.

TOTAL EXPOSURE BY POPULATION SUBGROUP

TOTAL EXPOSURE

POPULATION SUBGROUP	mg/kg body-wt/day	Life-Time Risk (Q1=0.064, MLE)
U.S. POP - 48 STATES - ALL SEASONS	0.000107	6.83E-06
NORTHEAST REGION	0.000105	6.69E-06
MIDWEST REGION	0.000114	7.27E-06
SOUTHERN REGION	0.000104	6.64E-06
WESTERN REGION	0.000105	6.72E-06
PACIFIC REGION	0.000103	6.60E-06
HISPANICS	0.000108	6.94E-06
NON-HISPANIC WHITES	0.000107	6.83E-06
NON-HISPANIC BLACKS	0.000104	6.66E-06
NON-HISPANIC OTHER THAN BLACK OR WHITE	0.000112	7.18E-06
ALL INFANTS	0.000177	1.13E-05
NURSING INFANTS (<1 YEAR OLD)	0.000046	2.93E-06
NON-NURSING INFANTS (<1 YEAR OLD)	0.000230	1.47E-05
CHILDREN (1-6 YEARS)	0.000286	1.83E-05
CHILDREN (7-12 YEARS)	0.000175	1.12E-05
FEMALES (13-19 YRS/NOT PREG. OR NUR)	0.000092	5.88E-06
FEMALES (20+ YEARS/NOT PREG/NUR)	0.000071	4.53E-06
FEMALES (13-50 YEARS)	0.000076	4.87E-06
FEMALES (13+/PREGNANT/NOT NURSING)	0.000092	5.91E-06
FEMALES (13+/NURSING)	0.000097	6.21E-06
MALES (13-19 YEARS)	0.000114	7.29E-06
MALES (20+ YEARS)	0.000074	4.76E-06
SENIORS (55+)	0.000069	4.41E-06

Chronic Exposure (EX1) Analysis for Atrazine Section 3 Registration
RESIDUE FILE NAME: ATRA1CH ANALYSIS DATE: 07-20-2000
NFCS Combined 89-92 DATA ADJUSTMENT FACTOR #2 NOT USED
EPA (RfD) = 0.005 mg/kg body-wt/day, DPR NOEL (Chronic) = 0.5 mg/kg bd-wt/day
COMMENT 1: REG studies, USEPA tolerance, DPR well H20, with %PCT adjustment.

RESIDUE FILE LISTING

TAS CODE	CROP GRP	FOOD NAME	RESIDUE (PPM)	ADJ. FCTRS #1	#2	SOURCE CODE
46	R	MACADAMIA NUTS (BUSH NUTS)	0.100000	1.00	0.70	REG-f
79	A	GUAVA	0.025000	1.00	0.10	EPA1/2
237	O	CORN/POP	0.020000	1.00	0.65	REG-fp
238	O	CORN/SWEET	0.022000	1.00	0.50	REG-f
266	O	CORN/GRAIN-ENDOSPERM	0.020000	1.00	0.65	REG-fp
267	O	CORN/GRAIN-BRAN	0.020000	1.00	0.65	REG-fp
268	O	CORN SUGAR	0.020000	1.00	0.65	REG-fp
275	O	SORGHUM (INCLUDING MILO)	0.105000	1.00	0.60	REG-f
276	O	WHEAT-ROUGH	0.020000	1.00	0.01	REG-fp
277	O	WHEAT-GERM	0.020000	1.00	0.01	REG-fp
278	O	WHEAT-BRAN	0.020000	1.00	0.01	REG-fp
279	O	WHEAT-FLOUR	0.020000	1.00	0.01	REG-fp
283	A	CANE SUGAR	0.002000	1.00	0.90	REG-fp
284	A	SUGAR-MOLASSES	0.005500	6.00	0.90	REG-fp
289	O	CORN GRAIN-OIL	0.020000	1.00	0.65	REG-fp
318	X	MILK-NONFAT SOLIDS	0.005000	1.00	1.00	REG-fp
319	X	MILK-FAT SOLIDS	0.005000	1.00	1.00	REG-fp
320	X	MILK SUGAR (LACTOSE)	0.005000	1.00	1.00	REG-fp
321	U	BEEF-MEAT BYPRODUCTS	0.020000	1.00	1.00	REG-hy
322	U	BEEF(ORGAN MEATS)-OTHER	0.020000	1.00	1.00	REG-hy
323	U	BEEF-DRIED	0.001000	1.00	1.00	REG-hy
324	U	BEEF(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
325	U	BEEF(ORGAN MEATS)-KIDNEY	0.004000	1.00	1.00	REG-hy
326	U	BEEF(ORGAN MEATS)-LIVER	0.020000	1.00	1.00	REG-hy
327	U	BEEF(BONELESS)-LEAN (FAT/FREE)	0.001000	1.00	1.00	REG-hy
328	U	GOAT-MEAT BYPRODUCTS	0.020000	1.00	1.00	REG-hy
329	U	GOAT(ORGAN MEATS)-OTHER	0.020000	1.00	1.00	REG-hy
330	U	GOAT(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
331	U	GOAT(ORGAN MEATS)-KIDNEY	0.004000	1.00	1.00	REG-hy
332	U	GOAT(ORGAN MEATS)-LIVER	0.020000	1.00	1.00	REG-hy
333	U	GOAT(BONELESS)-LEAN (FAT/FREE)	0.001000	1.00	1.00	REG-hy
334	U	HORSE	0.020000	1.00	1.00	REG-hy
336	U	SHEEP-MEAT BYPRODUCTS	0.020000	1.00	1.00	REG-hy
337	U	SHEEP(ORGAN MEATS)-OTHER	0.020000	1.00	1.00	REG-hy

338	U	SHEEP(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
339	U	SHEEP(ORGAN MEATS)-KIDNEY	0.004000	1.00	1.00	REG-hy
340	U	SHEEP(ORGAN MEATS)-LIVER	0.020000	1.00	1.00	REG-hy
341	U	SHEEP(BONELESS)-LEAN (FAT FREE)	0.001000	1.00	1.00	REG-hy
342	U	PORK-MEAT BYPRODUCTS	0.020000	1.00	1.00	REG-hy
343	U	PORK(ORGAN MEATS)-OTHER	0.020000	1.00	1.00	REG-hy
344	U	PORK(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
345	U	PORK(ORGAN MEATS)-KIDNEY	0.004000	1.00	1.00	REG-hy
346	U	PORK(ORGAN MEATS)-LIVER	0.020000	1.00	1.00	REG-hy
347	U	PORK(BONELESS)-LEAN (FAT FREE)	0.001000	1.00	1.00	REG-hy
355	V	TURKEY-BYPRODUCTS	0.006000	1.00	1.00	REG-hy
356	V	TURKEY-GIBLETS (LIVER)	0.006000	1.00	1.00	REG-hy
357	V	TURKEY-(BONELESS)-FAT	0.000300	1.00	1.00	REG-hy
358	V	TURKEY-(BONELESS)LEAN/FAT FREE	0.000300	1.00	1.00	REG-hy
359	V	TURKEY-UNSPECIFIED	0.006000	1.00	1.00	REG-hy
360	V	POULTRY-OTHER-LEAN (FAT FREE)	0.000300	1.00	1.00	REG-hy
361	V	POULTRY-OTHER-GIBLETS(LIVER)	0.006000	1.00	1.00	REG-hy
362	V	POULTRY-OTHER-FAT	0.000300	1.00	1.00	REG-hy
363	X	EGGS-WHOLE	0.005000	1.00	1.00	REG-hy
364	X	EGGS-WHITE ONLY	0.005000	1.00	1.00	REG-hy
365	X	EGGS-YOLK ONLY	0.005000	1.00	1.00	REG-hy
366	V	CHICKEN-BYPRODUCTS	0.006000	1.00	1.00	REG-hy
367	V	CHICKEN-GIBLETS(LIVER)	0.006000	1.00	1.00	REG-hy
368	V	CHICKEN (BONELESS)-FAT	0.000300	1.00	1.00	REG-hy
369	V	CHICKEN(BONELESS)LEAN/FAT FREE	0.000300	1.00	1.00	REG-hy
385	V	CHICKEN-GIBLETS (EXCL. LIVER)	0.006000	1.00	1.00	REG-hy
388	O	CORN SUGAR-MOLASSES	0.020000	1.00	0.65	REG-fp
393	A	GUAVA-NECTAR	0.025000	1.00	0.10	EPA1/2
398	X	MILK-BASED WATER	0.005000	1.00	1.00	REG-fp
424	U	VEAL-(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
425	U	VEAL-(BONELESS)-LEAN (FAT FREE	0.001000	1.00	1.00	REG-hy
426	U	VEAL-(ORGAN MEATS)-KIDNEY	0.004000	1.00	1.00	REG-hy
427	U	VEAL-(ORGAN MEATS)-LIVER	0.020000	1.00	1.00	REG-hy
428	U	VEAL-(ORGAN MEATS)-OTHER	0.020000	1.00	1.00	REG-hy
429	U	VEAL-DRIED	0.001000	1.92	1.00	REG-hy
430	U	VEAL-MEAT BYPRODUCTS	0.020000	1.00	1.00	REG-hy
432	A	WATER-BOTTLED	0.000631	1.00	1.00	DPR-X
433	A	WATER-TAP	0.000631	1.00	1.00	DPR-X
434	A	WATER-COMMERCIAL PROCESSING	0.000050	1.00	1.00	1/2LOD
435	A	WATER-NON-FOOD BASED	0.000631	1.00	1.00	DPR-X
437	O	WHEAT-GERM OIL	0.020000	1.00	0.01	REG-fp
449	V	TURKEY-(ORGAN MEATS)-OTHER	0.006000	1.00	1.00	REG-hy
911	A	MOLASSES-NFS	0.020000	1.00	0.65	REG-f

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
COMMENT 1: REG studies, USEPA tolerance & DPR well water data.
COMMENT 2: Most current DPR Acute Dietary Exposure Assessment.

RESIDUE FILE LISTING

TAS CODE	CROP GRP	FOOD NAME	RESIDUE (PPM)	ADJ. FCTRS #1	#2	SOURCE CODE
46	R	MACADAMIA NUTS (BUSH NUTS)	0.200000	1.00	1.00	REG-f
79	A	GUAVA	0.050000	1.00	1.00	EPA
237	O	CORN/POP	0.040000	1.00	1.00	REG-fp
238	O	CORN/SWEET	0.060000	1.00	1.00	REG-f
266	O	CORN/GRAIN-ENDOSPERM	0.040000	1.00	1.00	REG-fp
267	O	CORN/GRAIN-BRAN	0.040000	1.00	1.00	REG-fp
268	O	CORN SUGAR	0.020000	1.00	1.00	REGmix
275	O	SORGHUM (INCLUDING MILO)	no consumption in survey			
276	O	WHEAT-ROUGH	0.040000	1.00	1.00	REG-fp
277	O	WHEAT-GERM	0.040000	1.00	1.00	REG-fp
278	O	WHEAT-BRAN	0.040000	1.00	1.00	REG-fp
279	O	WHEAT-FLOUR	0.020000	1.00	1.00	REGmix
283	A	CANE SUGAR	0.002000	1.00	1.00	REGmix
284	A	SUGAR-MOLASSES	0.005500	6.00	1.00	REGmix
289	O	CORN GRAIN-OIL	0.020000	1.00	1.00	REGmix
318	X	MILK-NONFAT SOLIDS	0.005000	1.00	1.00	REG-fp
319	X	MILK-FAT SOLIDS	0.005000	1.00	1.00	REG-fp
320	X	MILK SUGAR (LACTOSE)	0.005000	1.00	1.00	REG-fp
321	U	BEEF-MEAT BYPRODUCTS	0.020000	1.00	1.00	REG-hy
322	U	BEEF(ORGAN MEATS)-OTHER	0.020000	1.00	1.00	REG-hy
323	U	BEEF-DRIED	0.001000	1.92	1.00	REG-hy
324	U	BEEF(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
325	U	BEEF(ORGAN MEATS)-KIDNEY	0.004000	1.00	1.00	REG-hy
326	U	BEEF(ORGAN MEATS)-LIVER	0.020000	1.00	1.00	REG-hy
327	U	BEEF(BONELESS)-LEAN (FAT/FREE)	0.001000	1.00	1.00	REG-hy
328	U	GOAT-MEAT BYPRODUCTS	no consumption in survey			
329	U	GOAT(ORGAN MEATS)-OTHER	0.020000	1.00	1.00	REG-hy
330	U	GOAT(BONELESS)-FAT	no consumption in survey			
331	U	GOAT(ORGAN MEATS)-KIDNEY	no consumption in survey			
332	U	GOAT(ORGAN MEATS)-LIVER	no consumption in survey			
333	U	GOAT(BONELESS)-LEAN (FAT/FREE)	no consumption in survey			
334	U	HORSE	no consumption in survey			

TAS CODE	CROP GRP	FOOD NAME	RESIDUE (PPM)	ADJ. FCTRS #1	#2	SOURCE CODE
336	U	SHEEP-MEAT BYPRODUCTS	no consumption in survey			
337	U	SHEEP(ORGAN MEATS)-OTHER	no consumption in survey			
338	U	SHEEP(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
339	U	SHEEP(ORGAN MEATS)-KIDNEY	no consumption in survey			
340	U	SHEEP(ORGAN MEATS)-LIVER	no consumption in survey			
341	U	SHEEP(BONELESS)-LEAN (FAT FREE	0.001000	1.00	1.00	REG-hy
342	U	PORK-MEAT BYPRODUCTS	0.020000	1.00	1.00	REG-hy
343	U	PORK(ORGAN MEATS)-OTHER	no consumption in survey			
344	U	PORK(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
345	U	PORK(ORGAN MEATS)-KIDNEY	no consumption in survey			
346	U	PORK(ORGAN MEATS)-LIVER	0.020000	1.00	1.00	REG-hy
347	U	PORK(BONELESS)-LEAN (FAT FREE)	0.001000	1.00	1.00	REG-hy
355	V	TURKEY-BYPRODUCTS	0.006000	1.00	1.00	REG-hy
356	V	TURKEY-GIBLETS (LIVER)	0.006000	1.00	1.00	REG-hy
357	V	TURKEY-(BONELESS)-FAT	0.000300	1.00	1.00	REG-hy
358	V	TURKEY-(BONELESS)LEAN/FAT FREE	0.000300	1.00	1.00	REG-hy
359	V	TURKEY-UNSPECIFIED	no consumption in survey			
360	V	POULTRY-OTHER-LEAN (FAT FREE)	0.000300	1.00	1.00	REG-hy
361	V	POULTRY-OTHER-GIBLETS(LIVER)	no consumption in survey			
362	V	POULTRY-OTHER-FAT	0.000300	1.00	1.00	REG-hy
363	X	EGGS-WHOLE	0.005000	1.00	1.00	REG-hy
364	X	EGGS-WHITE ONLY	0.005000	1.00	1.00	REG-hy
365	X	EGGS-YOLK ONLY	0.005000	1.00	1.00	REG-hy
366	V	CHICKEN-BYPRODUCTS	no consumption in survey			
367	V	CHICKEN-GIBLETS(LIVER)	0.006000	1.00	1.00	REG-hy
368	V	CHICKEN (BONELESS)-FAT	0.000300	1.00	1.00	REG-hy
369	V	CHICKEN(BONELESS)LEAN/FAT FREE	0.000300	1.00	1.00	REG-hy
385	V	CHICKEN-GIBLETS (EXCL. LIVER)	0.006000	1.00	1.00	REG-hy
388	O	CORN SUGAR-MOLASSES	0.020000	1.00	1.00	REGmix
393	A	GUAVA-NECTAR	no consumption in survey			
398	X	MILK-BASED WATER	0.005000	1.00	1.00	REG-fp
424	U	VEAL-(BONELESS)-FAT	0.001000	1.00	1.00	REG-hy
425	U	VEAL-(BONELESS)-LEAN (FAT FREE	0.001000	1.00	1.00	REG-hy
426	U	VEAL-(ORGAN MEATS)-KIDNEY	no consumption in survey			
427	U	VEAL-(ORGAN MEATS)-LIVER	no consumption in survey			
428	U	VEAL-(ORGAN MEATS)-OTHER	no consumption in survey			
429	U	VEAL-DRIED	no consumption in survey			
430	U	VEAL-MEAT BYPRODUCTS	no consumption in survey			
432	A	WATER-BOTTLED	no consumption in survey			
433	A	WATER-TAP	0.002730	1.00	1.00	DPR-hi

TAS CODE	CROP GRP	FOOD NAME	RESIDUE (PPM)	ADJ. FCTRS #1	#2	SOURCE CODE
434	A	WATER-COMMERCIAL PROCESSING	0.000100	1.00	1.00	DPRLOD
435	A	WATER-NON-FOOD BASED	0.002730	1.00	1.00	DPR-hi
437	O	WHEAT-GERM OIL	no consumption in survey			
449	V	TURKEY-(ORGAN MEATS)-OTHER	0.006000	1.00	1.00	REG-hy
911	A	MOLASSES-NFS	0.020000	1.00	1.00	REGmix

Chronic Exposure (EX1) Analysis for Atrazine Section 3 Registration
RESIDUE FILE NAME: ATRA2CH ANALYSIS DATE: 07-20-2000
NFCS Combined 89-92 DATA
EPA Reference dose (RfD, chronic) = 0.005000 mg/kg body-wt/day
DPR NOEL (Chronic) = 0.500000 mg/kg body-wt/day
COMMENT 1: REG studies, USEPA tolerance, DPR well water data, %PCT.
COMMENT 2: Most current DPR assessment with %PCT.

TOTAL EXPOSURE BY POPULATION SUBGROUP

POPULATION SUBGROUP	TOTAL EXPOSURE mg/kg body-wt/day	Margin of Exposure1	Percent of RfD
U.S. POP - 48 STATES - ALL SEASONS	0.000070	7,177	1.4%
NORTHEAST REGION	0.000067	7,469	1.3%
MIDWEST REGION	0.000075	6,646	1.5%
SOUTHERN REGION	0.000067	7,458	1.3%
WESTERN REGION	0.000070	7,190	1.4%
PACIFIC REGION	0.000069	7,278	1.4%
HISPANICS	0.000076	6,581	1.5%
NON-HISPANIC WHITES	0.000069	7,244	1.4%
NON-HISPANIC BLACKS	0.000069	7,282	1.4%
NON-HISPANIC OTHER THAN BLACK OR WHITE	0.000073	6,872	1.5%
ALL INFANTS	0.000156	3,206	3.1%
NURSING INFANTS (<1 YEAR OLD)	0.000038	13,082	0.8%
NON-NURSING INFANTS (<1 YEAR OLD)	0.000203	2,462	4.1%
CHILDREN (1-6 YEARS)	0.000201	2,491	4.0%
CHILDREN (7-12 YEARS)	0.000115	4,361	2.3%
FEMALES (13-19 YRS/NOT PREG. OR NUR)	0.000057	8,714	1.1%
FEMALES (20+ YEARS/NOT PREG. OR NUR)	0.000045	11,190	0.9%
FEMALES (13-50 YEARS)	0.000047	10,562	0.9%
FEMALES (13+/PREGNANT/NOT NURSING)	0.000061	8,149	1.2%
FEMALES (13+/NURSING)	0.000061	8,212	1.2%
MALES (13-19 YEARS)	0.000071	6,995	1.4%
MALES (20+ YEARS)	0.000045	11,100	0.9%
SENIORS (55+)	0.000044	11,284	0.9%

1. Margin of Exposure = DPR NOEL / Dietary Exposure

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
Initial estimate of user-days as % of person-days in survey = 100.00%
COMMENT 1: REG studies, USEPA tolerance & DPR well water data.
COMMENT 2: Most current DPR Acute Dietary Exposure Assessment.
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U.S. POP - ALL SEASONS Daily Exposure Analysis 1/
---------- (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000171	0.000171
Standard Deviation	0.000129	0.000129
Standard Error	0.000001	0.000001

Percent of Person-Days that are User-Days = 99.85%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE. in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000065	77,417	90.00	0.000329	15,192
20.00	0.000083	60,179	95.00	0.000438	11,413
30.00	0.000099	50,386	97.50	0.000544	9,194
40.00	0.000116	43,254	99.00	0.000674	7,422
50.00	0.000133	37,537	99.50	0.000770	6,490
60.00	0.000154	32,442	99.75	0.000876	5,705
70.00	0.000182	27,412	99.90	0.001032	4,845
80.00	0.000229	21,814			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE. in mg/kg body-wt/day and corresponding Margin of Exposure)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000064	78,491	90.00	0.000329	15,199
20.00	0.000083	60,342	95.00	0.000438	11,417
30.00	0.000099	50,473	97.50	0.000544	9,197
40.00	0.000115	43,309	99.00	0.000674	7,423
50.00	0.000133	37,574	99.50	0.000770	6,492
60.00	0.000154	32,469	99.75	0.000876	5,706
70.00	0.000182	27,432	99.90	0.001032	4,846
80.00	0.000229	21,828			

1/ Analysis based on all participant-days in NFCS 1989-92 survey.
2/ Margin of Exposure = NOEL / Dietary Exposure.

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
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WESTERN REGION

Daily Exposure Analysis
(mg/kg body-weight/day)

	per Capita	per User
Mean	0.000171	0.000172
Standard Deviation	0.000130	0.000130
Standard Error	0.000002	0.000002

Percent of Person-Days that are User-Days = 99.71%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000064	78,131	90.00	0.000342	14,624
20.00	0.000082	60,617	95.00	0.000446	11,223
30.00	0.000099	50,698	97.50	0.000537	9,314
40.00	0.000115	43,438	99.00	0.000667	7,497
50.00	0.000133	37,582	99.50	0.000756	6,615
60.00	0.000155	32,335	99.75	0.000857	5,834
70.00	0.000183	27,288	99.90	0.000984	5,081
80.00	0.000232	21,522			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000062	80,203	90.00	0.000342	14,638
20.00	0.000082	60,931	95.00	0.000445	11,230
30.00	0.000098	50,866	97.50	0.000537	9,318
40.00	0.000115	43,545	99.00	0.000667	7,500
50.00	0.000133	37,655	99.50	0.000756	6,618
60.00	0.000154	32,386	99.75	0.000857	5,836
70.00	0.000183	27,325	99.90	0.000984	5,082
80.00	0.000232	21,548			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
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HISPANICS

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.000178	0.000179
Standard Deviation	0.000142	0.000141
Standard Error	0.000002	0.000002

Percent of Person-Days that are User-Days = 99.70%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000064	78,689	90.00	0.000362	13,824
20.00	0.000082	61,114	95.00	0.000492	10,171
30.00	0.000098	51,210	97.50	0.000609	8,206
40.00	0.000115	43,548	99.00	0.000694	7,201
50.00	0.000132	38,004	99.50	0.000801	6,242
60.00	0.000156	32,107	99.75	0.000902	5,546
70.00	0.000191	26,203	99.90	0.001019	4,908
80.00	0.000246	20,323			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000062	80,875	90.00	0.000361	13,838
20.00	0.000081	61,443	95.00	0.000491	10,179
30.00	0.000097	51,385	97.50	0.000609	8,211
40.00	0.000115	43,666	99.00	0.000694	7,203
50.00	0.000131	38,077	99.50	0.000801	6,244
60.00	0.000155	32,167	99.75	0.000901	5,548
70.00	0.000191	26,246	99.90	0.001019	4,909
80.00	0.000246	20,351			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
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NON-HISPANIC WHITES

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.000169	0.000169
Standard Deviation	0.000125	0.000125
Standard Error	0.000001	0.000001

Percent of Person-Days that are User-Days = 99.90%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000067	75,155	90.00	0.000321	15,568
20.00	0.000085	59,065	95.00	0.000427	11,721
30.00	0.000100	49,761	97.50	0.000529	9,454
40.00	0.000117	42,862	99.00	0.000667	7,497
50.00	0.000134	37,383	99.50	0.000757	6,603
60.00	0.000154	32,436	99.75	0.000863	5,793
70.00	0.000181	27,626	99.90	0.001023	4,886
80.00	0.000225	22,254			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000066	75,815	90.00	0.000321	15,572
20.00	0.000085	59,163	95.00	0.000426	11,724
30.00	0.000100	49,814	97.50	0.000529	9,456
40.00	0.000117	42,897	99.00	0.000667	7,498
50.00	0.000134	37,406	99.50	0.000757	6,604
60.00	0.000154	32,452	99.75	0.000863	5,793
70.00	0.000181	27,638	99.90	0.001023	4,886
80.00	0.000225	22,262			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day

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NON-HISPANIC BLACKS

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.000171	0.000172
Standard Deviation	0.000143	0.000143
Standard Error	0.000002	0.000002

Percent of Person-Days that are User-Days = 99.74%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000055	90,393	90.00	0.000356	14,039
20.00	0.000074	67,966	95.00	0.000476	10,507
30.00	0.000090	55,524	97.50	0.000568	8,796
40.00	0.000106	46,966	99.00	0.000728	6,867
50.00	0.000125	40,111	99.50	0.000838	5,963
60.00	0.000150	33,315	99.75	0.000902	5,544
70.00	0.000185	27,040	99.90	0.001094	4,570
80.00	0.000244	20,511			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000054	92,532	90.00	0.000356	14,051
20.00	0.000073	68,314	95.00	0.000476	10,514
30.00	0.000090	55,707	97.50	0.000568	8,800
40.00	0.000106	47,078	99.00	0.000728	6,869
50.00	0.000124	40,186	99.50	0.000838	5,965
60.00	0.000150	33,373	99.75	0.000902	5,545
70.00	0.000185	27,080	99.90	0.001094	4,571
80.00	0.000243	20,536			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

NON-HISPANIC OTHER

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.000185	0.000186
Standard Deviation	0.000140	0.000139
Standard Error	0.000004	0.000004

Percent of Person-Days that are User-Days = 99.14%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000062	80,955	90.00	0.000379	13,203
20.00	0.000084	59,719	95.00	0.000454	11,015
30.00	0.000106	47,040	97.50	0.000569	8,789
40.00	0.000127	39,448	99.00	0.000692	7,224
50.00	0.000146	34,192	99.50	0.000767	6,517
60.00	0.000169	29,571	99.75	0.000819	6,108
70.00	0.000198	25,304	99.90	0.001025	4,879
80.00	0.000272	18,393			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000057	87,839	90.00	0.000378	13,236
20.00	0.000082	60,831	95.00	0.000453	11,031
30.00	0.000105	47,657	97.50	0.000568	8,804
40.00	0.000126	39,783	99.00	0.000691	7,232
50.00	0.000145	34,391	99.50	0.000767	6,522
60.00	0.000168	29,711	99.75	0.000818	6,111
70.00	0.000197	25,400	99.90	0.001024	4,884
80.00	0.000271	18,481			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

NURSING INFANTS (<1 YEAR) Daily Exposure Analysis
---------- (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000090	0.000142
Standard Deviation	0.000112	0.000112
Standard Error	0.000009	0.000012

Percent of Person-Days that are User-Days = 63.15%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000026	191,113	90.00	0.000292	17,126
20.00	0.000066	75,968	95.00	0.000407	12,293
30.00	0.000073	68,458	97.50	0.000448	11,167
40.00	0.000080	62,298	99.00	0.000472	10,601
50.00	0.000088	56,902	99.50	0.000480	10,425
60.00	0.000100	50,167	99.75	0.000522	9,584
70.00	0.000210	23,796	99.90	0.000595	8,408
80.00	0.000232	21,556			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000000	>1,000,000	90.00	0.000257	19,460
20.00	0.000000	>1,000,000	95.00	0.000340	14,717
30.00	0.000000	>1,000,000	97.50	0.000424	11,798
40.00	0.000013	383,282	99.00	0.000462	10,814
50.00	0.000066	75,290	99.50	0.000475	10,527
60.00	0.000078	64,230	99.75	0.000497	10,057
70.00	0.000091	55,059	99.90	0.000566	8,829
80.00	0.000192	26,088			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

NON-NURSING INFANTS (<1)

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.000494	0.000494
Standard Deviation	0.000200	0.000200
Standard Error	0.000010	0.000010

Percent of Person-Days that are User-Days =100.00%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000270	18,530	90.00	0.000756	6,612
20.00	0.000327	15,285	95.00	0.000857	5,831
30.00	0.000371	13,467	97.50	0.000937	5,334
40.00	0.000428	11,671	99.00	0.001087	4,601
50.00	0.000484	10,339	99.50	0.001354	3,693
60.00	0.000522	9,577	99.75	0.001425	3,510
70.00	0.000572	8,742	99.90	0.001467	3,408
80.00	0.000645	7,755			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000270	18,530	90.00	0.000756	6,612
20.00	0.000327	15,285	95.00	0.000857	5,831
30.00	0.000371	13,467	97.50	0.000937	5,334
40.00	0.000428	11,671	99.00	0.001087	4,601
50.00	0.000484	10,339	99.50	0.001354	3,693
60.00	0.000522	9,577	99.75	0.001425	3,510
70.00	0.000572	8,742	99.90	0.001467	3,408
80.00	0.000645	7,755			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

FEMALES (13+/PREG/NOT NSG)

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.000144	0.000144
Standard Deviation	0.000065	0.000065
Standard Error	0.000003	0.000003

Percent of Person-Days that are User-Days =100.00%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000069	72,377	90.00	0.000236	21,190
20.00	0.000087	57,451	95.00	0.000288	17,337
30.00	0.000106	47,177	97.50	0.000309	16,203
40.00	0.000117	42,722	99.00	0.000321	15,591
50.00	0.000134	37,213	99.50	0.000325	15,398
60.00	0.000153	32,670	99.75	0.000327	15,303
70.00	0.000173	28,945	99.90	0.000344	14,515
80.00	0.000198	25,208			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000069	72,377	90.00	0.000236	21,190
20.00	0.000087	57,451	95.00	0.000288	17,337
30.00	0.000106	47,177	97.50	0.000309	16,203
40.00	0.000117	42,722	99.00	0.000321	15,591
50.00	0.000134	37,213	99.50	0.000325	15,398
60.00	0.000153	32,670	99.75	0.000327	15,303
70.00	0.000173	28,945	99.90	0.000344	14,515
80.00	0.000198	25,208			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

FEMALES (13+/NURSING) Daily Exposure Analysis
---------- (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000169	0.000169
Standard Deviation	0.000087	0.000087
Standard Error	0.000006	0.000006

Percent of Person-Days that are User-Days =100.00%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000066	75,808	90.00	0.000305	16,407
20.00	0.000092	54,251	95.00	0.000354	14,140
30.00	0.000120	41,705	97.50	0.000386	12,963
40.00	0.000136	36,816	99.00	0.000417	11,977
50.00	0.000152	32,917	99.50	0.000428	11,681
60.00	0.000175	28,623	99.75	0.000441	11,342
70.00	0.000200	25,042	99.90	0.000478	10,467
80.00	0.000231	21,673			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000066	75,808	90.00	0.000305	16,407
20.00	0.000092	54,251	95.00	0.000354	14,140
30.00	0.000120	41,705	97.50	0.000386	12,963
40.00	0.000136	36,816	99.00	0.000417	11,977
50.00	0.000152	32,917	99.50	0.000428	11,681
60.00	0.000175	28,623	99.75	0.000441	11,342
70.00	0.000200	25,042	99.90	0.000478	10,467
80.00	0.000231	21,673			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day

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CHILDREN (1-6 YEARS)

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.000400	0.000400
Standard Deviation	0.000179	0.000179
Standard Error	0.000003	0.000003

Percent of Person-Days that are User-Days =100.00%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000210	23,823	90.00	0.000634	7,887
20.00	0.000255	19,592	95.00	0.000733	6,824
30.00	0.000293	17,050	97.50	0.000832	6,012
40.00	0.000331	15,113	99.00	0.000998	5,008
50.00	0.000366	13,668	99.50	0.001161	4,305
60.00	0.000409	12,214	99.75	0.001290	3,877
70.00	0.000464	10,776	99.90	0.001403	3,564
80.00	0.000532	9,390			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000210	23,823	90.00	0.000634	7,887
20.00	0.000255	19,592	95.00	0.000733	6,824
30.00	0.000293	17,050	97.50	0.000832	6,012
40.00	0.000331	15,113	99.00	0.000998	5,008
50.00	0.000366	13,668	99.50	0.001161	4,305
60.00	0.000409	12,214	99.75	0.001290	3,877
70.00	0.000464	10,776	99.90	0.001403	3,564
80.00	0.000532	9,390			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

CHILDREN (7-12 YEARS) Daily Exposure Analysis
---------- (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000249	0.000249
Standard Deviation	0.000119	0.000119
Standard Error	0.000002	0.000002

Percent of Person-Days that are User-Days =100.00%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000125	39,985	90.00	0.000411	12,168
20.00	0.000155	32,251	95.00	0.000475	10,517
30.00	0.000178	28,065	97.50	0.000545	9,168
40.00	0.000199	25,101	99.00	0.000635	7,869
50.00	0.000225	22,220	99.50	0.000716	6,988
60.00	0.000254	19,708	99.75	0.000796	6,284
70.00	0.000287	17,396	99.90	0.000900	5,554
80.00	0.000335	14,940			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000125	39,985	90.00	0.000411	12,168
20.00	0.000155	32,251	95.00	0.000475	10,517
30.00	0.000178	28,065	97.50	0.000545	9,168
40.00	0.000199	25,101	99.00	0.000635	7,869
50.00	0.000225	22,220	99.50	0.000716	6,988
60.00	0.000254	19,708	99.75	0.000796	6,284
70.00	0.000287	17,396	99.90	0.000900	5,554
80.00	0.000335	14,940			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

MALES (13-19 YEARS) Daily Exposure Analysis
---------- (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000163	0.000163
Standard Deviation	0.000095	0.000095
Standard Error	0.000002	0.000002

Percent of Person-Days that are User-Days =100.00%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000069	72,265	90.00	0.000273	18,308
20.00	0.000090	55,270	95.00	0.000338	14,803
30.00	0.000110	45,604	97.50	0.000405	12,357
40.00	0.000127	39,275	99.00	0.000471	10,626
50.00	0.000146	34,224	99.50	0.000546	9,163
60.00	0.000165	30,322	99.75	0.000870	5,745
70.00	0.000185	27,020	99.90	0.000897	5,572
80.00	0.000220	22,682			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000069	72,265	90.00	0.000273	18,308
20.00	0.000090	55,270	95.00	0.000338	14,803
30.00	0.000110	45,604	97.50	0.000405	12,357
40.00	0.000127	39,275	99.00	0.000471	10,626
50.00	0.000146	34,224	99.50	0.000546	9,163
60.00	0.000165	30,322	99.75	0.000870	5,745
70.00	0.000185	27,020	99.90	0.000897	5,572
80.00	0.000220	22,682			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

FEMALES (13-19 YRS/NP/NN) Daily Exposure Analysis
---------- (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000137	0.000137
Standard Deviation	0.000073	0.000073
Standard Error	0.000002	0.000002

Percent of Person-Days that are User-Days =100.00%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000062	81,220	90.00	0.000226	22,109
20.00	0.000077	64,572	95.00	0.000282	17,715
30.00	0.000094	53,118	97.50	0.000338	14,803
40.00	0.000109	45,830	99.00	0.000395	12,650
50.00	0.000124	40,456	99.50	0.000426	11,733
60.00	0.000139	35,915	99.75	0.000458	10,913
70.00	0.000157	31,815	99.90	0.000518	9,656
80.00	0.000181	27,568			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000062	81,220	90.00	0.000226	22,109
20.00	0.000077	64,572	95.00	0.000282	17,715
30.00	0.000094	53,118	97.50	0.000338	14,803
40.00	0.000109	45,830	99.00	0.000395	12,650
50.00	0.000124	40,456	99.50	0.000426	11,733
60.00	0.000139	35,915	99.75	0.000458	10,913
70.00	0.000157	31,815	99.90	0.000518	9,656
80.00	0.000181	27,568			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

MALES (20+ YEARS)

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.000127	0.000127
Standard Deviation	0.000067	0.000067
Standard Error	0.000001	0.000001

Percent of Person-Days that are User-Days = 99.99%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000059	84,151	90.00	0.000209	23,902
20.00	0.000076	66,077	95.00	0.000250	19,995
30.00	0.000088	56,519	97.50	0.000296	16,881
40.00	0.000101	49,414	99.00	0.000368	13,597
50.00	0.000114	43,760	99.50	0.000422	11,857
60.00	0.000129	38,859	99.75	0.000472	10,603
70.00	0.000146	34,180	99.90	0.000530	9,439
80.00	0.000171	29,249			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000059	84,196	90.00	0.000209	23,902
20.00	0.000076	66,084	95.00	0.000250	19,995
30.00	0.000088	56,522	97.50	0.000296	16,881
40.00	0.000101	49,417	99.00	0.000368	13,597
50.00	0.000114	43,762	99.50	0.000422	11,857
60.00	0.000129	38,860	99.75	0.000472	10,603
70.00	0.000146	34,181	99.90	0.000530	9,439
80.00	0.000171	29,249			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

FEMALES (20+ YEARS/NP/NN) Daily Exposure Analysis
---------- (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000127	0.000127
Standard Deviation	0.000066	0.000066
Standard Error	0.000001	0.000001

Percent of Person-Days that are User-Days =100.00%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000060	82,717	90.00	0.000207	24,199
20.00	0.000076	65,669	95.00	0.000244	20,465
30.00	0.000090	55,670	97.50	0.000290	17,242
40.00	0.000102	48,838	99.00	0.000372	13,433
50.00	0.000116	43,245	99.50	0.000438	11,413
60.00	0.000130	38,506	99.75	0.000492	10,170
70.00	0.000146	34,241	99.90	0.000557	8,973
80.00	0.000168	29,799			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000060	82,752	90.00	0.000207	24,199
20.00	0.000076	65,674	95.00	0.000244	20,465
30.00	0.000090	55,673	97.50	0.000290	17,242
40.00	0.000102	48,839	99.00	0.000372	13,433
50.00	0.000116	43,246	99.50	0.000438	11,413
60.00	0.000130	38,506	99.75	0.000492	10,170
70.00	0.000146	34,242	99.90	0.000557	8,973
80.00	0.000168	29,799			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

SENIORS (55+) Daily Exposure Analysis
---------- (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000128	0.000128
Standard Deviation	0.000061	0.000061
Standard Error	0.000001	0.000001

Percent of Person-Days that are User-Days = 99.99%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000067	74,319	90.00	0.000201	24,911
20.00	0.000082	61,111	95.00	0.000234	21,333
30.00	0.000094	53,032	97.50	0.000276	18,084
40.00	0.000105	47,605	99.00	0.000360	13,873
50.00	0.000117	42,827	99.50	0.000435	11,506
60.00	0.000130	38,530	99.75	0.000502	9,960
70.00	0.000145	34,564	99.90	0.000553	9,036
80.00	0.000163	30,630			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000067	74,375	90.00	0.000201	24,911
20.00	0.000082	61,119	95.00	0.000234	21,333
30.00	0.000094	53,036	97.50	0.000276	18,084
40.00	0.000105	47,607	99.00	0.000360	13,873
50.00	0.000117	42,829	99.50	0.000435	11,506
60.00	0.000130	38,532	99.75	0.000502	9,960
70.00	0.000145	34,565	99.90	0.000553	9,036
80.00	0.000163	30,631			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

PACIFIC REGION

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.000166	0.000166
Standard Deviation	0.000124	0.000124
Standard Error	0.000002	0.000002

Percent of Person-Days that are User-Days =100.00%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000063	79,649	90.00	0.000326	15,331
20.00	0.000080	62,410	95.00	0.000433	11,550
30.00	0.000095	52,419	97.50	0.000528	9,468
40.00	0.000111	45,014	99.00	0.000643	7,780
50.00	0.000128	38,943	99.50	0.000729	6,859
60.00	0.000150	33,384	99.75	0.000830	6,026
70.00	0.000177	28,218	99.90	0.000947	5,280
80.00	0.000222	22,532			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000063	79,649	90.00	0.000326	15,331
20.00	0.000080	62,410	95.00	0.000433	11,550
30.00	0.000095	52,419	97.50	0.000528	9,468
40.00	0.000111	45,014	99.00	0.000643	7,780
50.00	0.000128	38,943	99.50	0.000729	6,859
60.00	0.000150	33,384	99.75	0.000830	6,026
70.00	0.000177	28,218	99.90	0.000947	5,280
80.00	0.000222	22,532			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

ALL INFANTS

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.000380	0.000424
Standard Deviation	0.000255	0.000233
Standard Error	0.000011	0.000010

Percent of Person-Days that are User-Days = 89.61%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000090	55,704	90.00	0.000744	6,724
20.00	0.000216	23,125	95.00	0.000831	6,018
30.00	0.000310	16,132	97.50	0.000892	5,603
40.00	0.000357	14,018	99.00	0.000989	5,054
50.00	0.000416	12,029	99.50	0.001371	3,646
60.00	0.000466	10,724	99.75	0.001463	3,417
70.00	0.000525	9,530	99.90	0.001518	3,293
80.00	0.000619	8,083			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000000	>1,000,000	90.00	0.000729	6,858
20.00	0.000099	50,552	95.00	0.000821	6,092
30.00	0.000234	21,380	97.50	0.000885	5,648
40.00	0.000324	15,424	99.00	0.000982	5,092
50.00	0.000381	13,107	99.50	0.001327	3,768
60.00	0.000443	11,292	99.75	0.001452	3,442
70.00	0.000504	9,914	99.90	0.001514	3,303
80.00	0.000597	8,378			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========

FEMALES (13-50 YEARS)

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.000130	0.000130
Standard Deviation	0.000070	0.000070
Standard Error	0.000001	0.000001

Percent of Person-Days that are User-Days =100.00%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000058	86,749	90.00	0.000217	23,081
20.00	0.000075	66,959	95.00	0.000260	19,197
30.00	0.000089	55,984	97.50	0.000312	16,022
40.00	0.000103	48,410	99.00	0.000382	13,082
50.00	0.000118	42,491	99.50	0.000427	11,702
60.00	0.000133	37,647	99.75	0.000467	10,707
70.00	0.000150	33,247	99.90	0.000566	8,839
80.00	0.000175	28,539			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000058	86,749	90.00	0.000217	23,081
20.00	0.000075	66,959	95.00	0.000260	19,197
30.00	0.000089	55,984	97.50	0.000312	16,022
40.00	0.000103	48,410	99.00	0.000382	13,082
50.00	0.000118	42,491	99.50	0.000427	11,702
60.00	0.000133	37,647	99.75	0.000467	10,707
70.00	0.000150	33,247	99.90	0.000566	8,839
80.00	0.000175	28,539			

ACUTE EXPOSURE (EX4) ANALYSIS FOR ATRAZINE Section 3 Registration
Residue file name: ATRA2AC Analysis date: 07-20-2000
1989-92 DATA Adjustment factor #2 NOT used
DPR NOEL (Acute) = 5.000000 mg/kg body-wt/day
==========
CUSTOM DEMOGRAPHICS 1: U.S. Population 16+ Years
All Seasons, All Regions, Sex: M/F-all/, All Races, Age-Low: 16 yrs High: 110 yrs
---

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.000128	0.000128
Standard Deviation	0.000067	0.000067
Standard Error	0.000000	0.000000

Percent of Person-Days that are User-Days =100.00%

ESTIMATED PERCENTILE OF USER-DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000060	83,311	90.00	0.000211	23,738
20.00	0.000076	65,638	95.00	0.000251	19,919
30.00	0.000090	55,770	97.50	0.000301	16,632
40.00	0.000102	48,814	99.00	0.000374	13,376
50.00	0.000116	43,149	99.50	0.000428	11,670
60.00	0.000131	38,308	99.75	0.000480	10,407
70.00	0.000148	33,849	99.90	0.000546	9,161
80.00	0.000171	29,172			

ESTIMATED PERCENTILE OF PER-CAPITA DAYS LESS THAN/EQUAL TO CALCULATED EXPOSURE
in mg/kg body-wt/day and corresponding Margin of Exposure (MOE)

PERCENTILE	EXPOSURE	MOE 2/	PERCENTILE	EXPOSURE	MOE
10.00	0.000060	83,347	90.00	0.000211	23,739
20.00	0.000076	65,643	95.00	0.000251	19,920
30.00	0.000090	55,773	97.50	0.000301	16,632
40.00	0.000102	48,816	99.00	0.000374	13,376
50.00	0.000116	43,150	99.50	0.000428	11,670
60.00	0.000131	38,309	99.75	0.000480	10,407
70.00	0.000148	33,849	99.90	0.000546	9,161
80.00	0.000171	29,172			

Chronic Exposure (EX1) Analysis for Atrazine Section 3 Registration
RESIDUE FILE NAME: ATRA2CH ANALYSIS DATE: 07-20-2000
NFCS Combined 89-92 DATA
Q1* (UB) = 0.120000
COMMENT 1: REG studies, USEPA tolerance, DPR well water data, %PCT.
COMMENT 2: Most current DPR assessment with %PCT adjustment.

TOTAL EXPOSURE BY POPULATION SUBGROUP

POPULATION SUBGROUP	TOTAL EXPOSURE mg/kg body-wt/day	TOTAL EXPOSURE Life-Time Risk (Q1*=0.12, UB)
U.S. POP - 48 STATES - ALL SEASONS	0.000070	8.36E-06
NORTHEAST REGION	0.000067	8.03E-06
MIDWEST REGION	0.000075	9.03E-06
SOUTHERN REGION	0.000067	8.04E-06
WESTERN REGION	0.000070	8.34E-06
PACIFIC REGION	0.000069	8.24E-06
HISPANICS	0.000076	9.12E-06
NON-HISPANIC WHITES	0.000069	8.28E-06
NON-HISPANIC BLACKS	0.000069	8.24E-06
NON-HISPANIC OTHER THAN BLACK OR WHITE	0.000073	8.73E-06
ALL INFANTS	0.000156	1.87E-05
NURSING INFANTS (<1 YEAR OLD)	0.000038	4.59E-06
NON-NURSING INFANTS (<1 YEAR OLD)	0.000203	2.44E-05
CHILDREN (1-6 YEARS)	0.000201	2.41E-05
CHILDREN (7-12 YEARS)	0.000115	1.38E-05
FEMALES (13-19 YRS/NOT PREG OR NUR)	0.000057	6.89E-06
FEMALES (20+ YEARS/NOT PREG OR NUR)	0.000045	5.36E-06
FEMALES (13-50 YEARS)	0.000047	5.68E-06
FEMALES (13+/PREGNANT/NOT NURSING)	0.000061	7.36E-06
FEMALES (13+/NURSING)	0.000061	7.31E-06
MALES (13-19 YEARS)	0.000071	8.58E-06
MALES (20+ YEARS)	0.000045	5.41E-06
SENIORS (55+)	0.000044	5.32E-06

Chronic Exposure (EX1) Analysis for Atrazine Section 3 Registration
RESIDUE FILE NAME: ATRA2CH ANALYSIS DATE: 07-20-2000
NFCS Combined 89-92 DATA
Q* (MLE) = 0.064000
COMMENT 1: REG studies, USEPA tolerance, DPR well water data, %PCT.
COMMENT 2: Most current DPR assessment with %PCT adjustment.

TOTAL EXPOSURE BY POPULATION SUBGROUP

POPULATION SUBGROUP	TOTAL EXPOSURE mg/kg body-wt/day	Life-Time Risk (Q*=0.064, MLE)
U.S. POP - 48 STATES - ALL SEASONS	0.000070	4.46E-06
NORTHEAST REGION	0.000067	4.28E-06
MIDWEST REGION	0.000075	4.82E-06
SOUTHERN REGION	0.000067	4.29E-06
WESTERN REGION	0.000070	4.45E-06
PACIFIC REGION	0.000069	4.40E-06
HISPANICS	0.000076	4.86E-06
NON-HISPANIC WHITES	0.000069	4.42E-06
NON-HISPANIC BLACKS	0.000069	4.39E-06
NON-HISPANIC OTHER THAN BLACK OR WHITE	0.000073	4.66E-06
ALL INFANTS	0.000156	9.98E-06
NURSING INFANTS (<1 YEAR OLD)	0.000038	2.45E-06
NON-NURSING INFANTS (<1 YEAR OLD)	0.000203	1.30E-05
CHILDREN (1-6 YEARS)	0.000201	1.28E-05
CHILDREN (7-12 YEARS)	0.000115	7.34E-06
FEMALES (13-19 YRS/NOT PREG OR NUR)	0.000057	3.67E-06
FEMALES (20+ YEARS/NOT PREG OR NUR)	0.000045	2.86E-06
FEMALES (13-50 YEARS)	0.000047	3.03E-06
FEMALES (13+/PREGNANT/NOT NURSING)	0.000061	3.93E-06
FEMALES (13+/NURSING)	0.000061	3.90E-06
MALES (13-19 YEARS)	0.000071	4.57E-06
MALES (20+ YEARS)	0.000045	2.88E-06
SENIORS (55+)	0.000044	2.84E-06

APPENDIX VII.

Reviews of Draft Final RCD and Correspondence

10 December 2000

FROM: Karl P. Baetcke, PhD, Senior Scientist
Vicki Dellarco, PhD, Senior Scientist
Catherine Eiden, Chemist
Health Effects Division, Office of Pesticide Programs (7509C)
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue NW
Washington, DC 20460

TO: Dr. Gary Patterson, Chief
Medical Toxicology Branch
California Department of Pesticide Regulation
1020 N Street, Rm 100
Sacramento, California 95814-5624

As requested, we have reviewed the hazard assessment document on atrazine that was prepared by the California Department of Pesticide Regulation. We found your review comprehensive and data on the effects of atrazine well presented. It is an excellent hazard assessment document. Per our discussions, our comments are focused on areas of the California review that appear to differ from our findings and conclusions.

There are only a few areas where there appear to be substantive differences between our individual hazard assessments of atrazine. Specifically, our interpretation of data on atrazine led us to different conclusions regarding the events leading to mammary tumor formation in the SD female rat and the mutagenic potential of atrazine and its metabolites. As discussed in our review, we recognize that some mutagenicity/genotoxicty testing yielded positive results but feel that when a weight of the evidence approach is applied, there is not much support for a role of mutagenicity as a component of the effects of atrazine. The potential mutagenicity of plant metabolites as well as atrazines mammalian metabolites is also not considered an issue. We considered the high doses required to produce the possible mutagenic effects, the nature of the test, and whether the results were reproducible. Regarding the mode of action for the carcinogenicity of atrazine, we relied heavily on the concordance of doses that lead to a suppression of the LH surge, extended estrus cycles, histomorphologic changes in the mammary tissue, pituitary histology, and tumor responses; the absence of a tumor response in ovariectomized rats also provided evidence supporting a linkage of sustained estrogen levels with tumor responses in intact female rats. We recognize, and long struggled with, the high variability in LH measurements but felt that with more recent studies on LH and estrus cycle effects, the issue was resolved based on consistency in the data from all such studies. We recognize that data are not robust regarding estrogen and prolactin levels (better data are needed) but do not think that this deficiency outweighs the evidence supporting perturbations of LH and estrus cycles as key events leading to the mammary tumor response.

Other differences we noted between your assessment and HEDs are as follows.

The study/endpoint the Hazard Identification Assessment Review Committee (HIARC) recently selected for chronic dietary assessment is the suppression of the LH surge and estrus cycle alterations reported in a 6 month study conducted by Morseth (NOAEL 1.8 mg/kg/day) (Report attached).

The HIARC selected 10 mg/kg/day as the NOAEL for acute dietary risk assessment. This decision was based on considertion of the large difference between the NOAEL and LOAEL in the rabbit developmental toxicity study and the NOAELs and LOAELs reported for effects in two rat developmental studies. The committee also felt that more emphasis should be placed on the rat studies because the effects observed in the fetuses of the rabbit developmental study occurred at maternally toxic doses. The committee also thought that the pubertal assays conducted by Ralph Cooper supported the use of a NOAEL of 10 mg/kg/day for an acute dietary risk assessment. The internet address for studies published NHEERL is:

http://toxsci.oupjournals.org/cgi/search?fulltext=atrazine&sendit=Enter&volume=58&issue=1

After consulting with a number of pathologists, we concluded that the mammary fibroadenoma and carcinoma incidences should not be combined because they are of a different cell type origin. The Scientific Advisory Panel agreed the two tumor types should not be combined for a quantitative risk assessment.

We have forwarded separately the report of the Scientific Advisory Panel (SAP). Note that the SAP disagreed with our provisional conclusion that Atrazine should be classified as a “likely” human carcinogen. The SAP agreed that the mode of action for the mammary carcinogenic potential of atrazine in female SD rats has been established but, contrary to our assessment, felt the mode of action for tumor formation in the SD rat was unlikely to be relevant for humans. The SAP provided information showing that the human conditions discussed in HEDs review that might indicate sensitivity of humans to the endocrine disrupting properties of atrazine that could lead to tumor formation were not appropriate models. The SAP did feel that the reproductive/developmental potential of atrazine raises a concern for humans as the effects of atrazine at the level of the hypothalamus- pituitary-gonadal axis would be expected to be expressed in humans. The panel was uncertain whether such effects would be clinically important. The SAP report provides more detailed discussions of the potential toxicity of atrazine and implications for human cancer and reproductive/developmental effects in humans.

Overall, it seems the differences in our hazard assessments of atrazine can be attributed to how convincing are the data on atrazines mode of action. Given that we until very recently had concerns about the robustness of some of the key data, it is not surprising that we have up to now arrived at different conclusions. We benefited from our discussions with the SAP and felt that their comments strongly supported our belief that the mode of action of atrazine has been demonstrated, recognizing that there is still some potential that other modes (such as an involvement of aromatase) may be contribute to the tumor response. This possibility is being investigated by our NHEERL research colleagues.

There are also some differences in our individual approaches to dietary exposure assessments. Cal DPR used estimates of residues based on limits of detection from field trials for the major consumption items like corn and sorghum whereas HED used estimates of atrazine residues from TRR in metabolism studies for corn and sorghum, monitoring data from PDP for wheat, LODs for sugar, macadamia nuts, and the tolerance level for guavas only. Regardless of these differences, Cal PDRs acute and chronic dietary exposures were below levels of concern. This may be because Cal PDR used an uncertainty factor of 100 and did not apply an additional FQPA UF as HED did. Both organizations included residues in drinking water in their assessments. However, Cal PDR used data from rural wells in California only, using the maximum value from the wells in the acute assessment and an average across wells in the chronic assessment. HED used data from community water supplies across the US, as well as rural well data. Our practice is not to average residues across wells in a chronic assessment. Cal PDRs risk estimates for non-occupational exposures for all population subgroups resulted in MOEs well above 100. Cal PDRs cancer risk estimates are $4X10^{-5}$, which is about the value HED would estimate if a quantitative cancer risk assessment was conducted. Finally, Cal PDR combined occupational exposures with dietary (food + water) exposures, which is not the practice of HED.

Please contact us if you wish to have further discussions regarding the toxicological profile of atrazine. Atrazine is an interesting chemical!

Attachment
HED DOC. NO. 014308

DATE: 8/28/00

MEMORANDUM

SUBJECT: ***Atrazine*** - 2nd Report of the Hazard Identification Assessment Review Committee.

FROM: Roger Hawks, Toxicologist.
Reregistration Branch III
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Cathy Eiden, Risk Assessor
Reregistration Branch III
Health Effects Division (7509C)

PC Code: 080803

On May 4, 2000, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for atrazine with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. This chemical was evaluated by HIARC in August of 1998. The current reevaluation of atrazine is based on the receipt of additional data pertaining to atrazine's potential effects on infants and children generated by the Agency's National Health Effects Environmental Research Laboratories and by the availability of a draft cancer assesment document - neither of which was available at the August 1998 HIARC meeting. The potential for increased susceptibility of infants and children from exposure to atrazine was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996. The conclusions drawn at this meeting are presented in this report.

Committee Members in Attendance

Members present were:
Elizabeth Mendez, David Nixon, Yung Yang, Beth Doyle, Jonathon Chen, Jess Rowland, Brenda Tarplee, Bill Burnam, Vicki Dellarco, Pam Hurly

Member(s) in absentia: Tina Levine

Data evaluation prepared by: Roger Hawks, Reregistration Branch III

Also in attendance were: Karl Baetcke, HED Richard Hill, OPPTS

Data Evaluation / Report Presentation _____

Roger Hawks

Toxicologist

1.

INTRODUCTION

On May 4, 2000, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for atrazine with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. This chemical was evaluated by HIARC in August of 1998. The current reevaluation of atrazine is based on the receipt of additional data pertaining to atrazine's potential effects on infants and children generated by the Agency's National Health Effects Environmental Research Laboratories and by the availability of a draft cancer assesment document - neither of which was available at the August 1998 HIARC meeting. The potential for increased susceptibility of infants and children from exposure to atrazine was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996. The conclusions drawn at this meeting are presented in this report.

1. HAZARD IDENTIFICATION

.1 Acute Reference Dose (RfD)

- Female 13-50 Subpopulation

Study Selected: A weight of the evidence consideration using evidence provided by four studies: two developmental toxicity studies in rats; a rabbit developmental toxicity study and a study examining the effects of maternal atrazine exposure during lactation on prostate effects in male offspring.
The actual NOAEL and endpoint upon which the reference dose is calculated is derived from one of the above-mentioned developmental studies in the rat - MRID 40566302.

Study #1 - MRID No.: 40566302

Executive Summary: In a developmental toxicity study (MRID 40566302) atrazine (96.7%) was administered to 104 Charles River CD rats 27/dose by gastric intubation at dose levels of 0, 10, 70, or 700 mg/kg/day from days 6 through 15 of gestation.

Mortality was very high for the 700 mg/kg/day animals in this study. All but 6 of the 27 females in this group died during the gestation period. Other statistically significant findings in this group included: salivation; oral and nasal discharge; ptosis; swollen abdomens; blood on the vulva; enlarged stomachs and adrenal; and discolored lungs. Body weight gains were statistically significantly reduced throughout most of the gestation period. Significant reductions in food consumption were observed. Although mortality was quite high in the high dose group, pregnancy rates were comparable to controls (85.2% for controls *vs* 96.3% high dose) and the numbers of live fetuses at c-section was comparable to controls (mean of 12.7 per litter for controls *vs* 13.4 for the high dose).

There were few findings in either the low or mid dose animals. Alopecia was observed in mid dose animals, but was not considered to be biologically significant. Body weight gain in the mid dose group was significantly reduced for the first 5 days of compound exposure (gestation

days 6-10). Food consumption in the intermediate group was reduced, but only at day 17 of gestation. Low dose body weight gain was increased on days 6-10 of gestation and food consumption was increased on gestation day 9.

The maternal LOAEL is 70 mg/kg/day, based on reduced body weight gain. The maternal NOAEL is 10 mg/kg/day.

Fetal weights were statistically significantly reduced in the high dose group. Skeletal examinations were not conducted in the high dose group due to the extremely low fetal weights. Visceral and external examinations were conducted, though, and no group, including the high dose group, displayed any findings significantly different from control values. Skeletal anomalies, however, were observed in the mid dose group. The original reviewers of this study concluded that none of these skeletal anomalies indicated a direct compound related toxic effect to the embryo/fetus (TXR 006131). An evaluation by the Ad Hoc Committee Regarding the Use of Developmental Toxicity Studies (Memorandum- From: Marion Copley. March 25, 1993) focused on findings of delayed ossification at several sites to conclude that atrazine *did* induce delayed ossification.

The developmental LOAEL was found to be 70 mg/kg/day, based on delayed or no ossification at several sites. The developmental NOAEL is 10 mg/kg/day.

The developmental toxicity study in the rat is classified **Acceptable-Guideline** and does satisfy the guideline requirement for a developmental toxicity study §83-3a in rats.

Study #2 - MRID No.: 41065201

Executive Summary: In a developmental toxicity study (MRID 41065201) atrazine (97.6%) was administered by gavage to 104 mated female Sprague-Dawley rats, 26/dose, at dose levels of 0, 5 (LDT), 25 (MDT), 100 (HDT) mg/kg/day from days 6 through 15 of gestation. Maternal toxicity findings were almost exclusively confined to the high dose group. Compared to controls high dose dams displayed: reduced food consumption (decreased 13%, $p \leq 0.5$); reduced total body weight gain (reduced 18% during dosing period, $p \leq 0.5$); reduced corrected (minus uterine weight) weight gain (reduced 20% for entire gestation, $p \leq 0.5$); and increased alopecia 1/26 controls vs 5/26 high dose). One high dose animal died on gestation day 20 and salivation was noted as an observation in 18/26 high dose animals. The only observations seen outside the high dose group were: an abortion from one of the mid-dose animals on gestation day 19; a fluid-filled hollow right kidney in a mid-dose animal; and hollow discolored kidneys in a low dose animal.

The maternal LOAEL is 100 mg/kg/day based on reduced body weight gain and food consumption. The maternal NOAEL is 25 mg/kg/day.

The few malformations seen upon external examination of the fetuses were seen only in the control groups and clearly could not be compound related. Likewise, there was no increased incidence of visceral malformation in dosed groups *vs* control groups. There were no skeletal malformations observed but there was an increased incidence of incomplete ossification of various bones in the HDT. Hyoids (control fetal incidence of 11% *vs* 21.7% HDT), occipitals (7.7% *vs* 21.1%) and parietals (2.2% *vs* 8.4%) showed incomplete ossification. There was also

an increased incidence ($p \leq 0.05$) of incomplete ossification of the interparietals in all dose groups compared to controls.

Fetal body weight, number of resorptions and implantations, and live fetuses/litter were not significantly affected by atrazine treatment.

Exposure of gravid Sprague-Dawley rats to atrazine under the conditions described in this study seemed to have few embryo/fetotoxic effects.

The developmental LOAEL is 100 mg/kg/day, based on increased incidence of delayed ossification of skull bones. The developmental NOAEL is 25 mg/kg/day.

The developmental toxicity study (MRID 41065201) in the rat is classified **Acceptable-Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3a) in the rat.

Study #3 - MRID Nos.: 00143006, 40566301

Executive Summary: In a developmental toxicity study (Acc. No. 254979; MRIDs 00143006, 40566301) atrazine (96.3%) was administered by gavage to 76 mated female New Zealand White rabbits, 19/dose, at dose levels of 0, 1, 5, or 75 mg/kg/day, from days 7 through 19 of gestation.

Clinical signs seen in 75 mg/kg/day (HDT) animals that were considered to be related to compound treatment were stool changes (none, little or soft stool; 9/19 controls *vs* 19/19 HDT), and the appearance of blood in the cage or on the vulva (0/19 controls *vs* 4/19 HDT). Body weight gain was reduced in high dose dams and, at many time points, body weight was below day zero values. At gestation days 14, 19, 21 and 25, mean maternal body weights were 12%, 19%, 18%, and 10% below control values($p \leq 0.01$ for all four of these time points).

High dose animals displayed significantly reduced food consumption during treatment. During gestation days 12 to 17 the HDT average feed consumption was only 1-6 grams of feed per animal per day compared to 175-182 grams for the controls.

The mid and low dose groups had no alterations that could be attributed to atrazine exposure.

The maternal toxicity LOAEL is 75 mg/kg/day based on decreased body weight, food consumption and increased incidence of clinical signs. The maternal toxicity NOAEL is 5 mg/kg/day.

Increased resorptions - mean of 1.3/dam in controls *vs* 4.8/dam in HDT - ($p \leq 0.01$), reduced live fetuses per litter - mean of 8.8/dam in controls *vs* 5.9/dam in HDT - ($p \leq 0.05$), and increased delayed ossification of appendicular elements were observed in the high dose group.

The low and intermediate groups had no fetal findings that could be attributed to compound exposure.

The findings in the high dose group were determined to be secondary to maternal toxicity and thus the LOEL and NOEL for embryo/fetotoxicity match the maternal LOEL and NOEL.

The developmental toxicity LOAEL is 75 mg/kg/day based on reduced litter size, increased resorptions and delayed ossification. The developmental toxicity NOAEL is 5 mg/kg/day

The study is considered **Acceptable-Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3b) in rabbit.

Study #4 - MRID No.: 45166902

Executive Summary: Hyperprolactinemia prior to puberty in male rats has been shown to lead to lateral prostate inflammation in young adult rats. One possible cause of hyperprolactinemia in immature male rats is a deficiency in milk-derived prolactin. Milk-derived prolactin plays a critical role in the development of the tuberoinfundibular dopaminergic neurons (TIDA) of the hypothalamus of a developing rat. The TIDA neurons function to inhibit prolactin secretion from the anterior pituitary. Organization and development of these neurons occurs mainly during the first postnatal week in the rat (Ojeda and McCann, 1974).

Thus, if developing rats do not receive a sufficient amount of prolactin from their mothers milk during the first week after birth, the TIDA neurons will not develop properly and may not be able to sufficiently provide an inhibitory check to prolactin secretion in the adult animal. The resultant hyperprolactinema is associated with development of prostatitis in the adult.

Atrazine has been shown to depress the secretion of prolactin. The role of milk-derived prolactin in development of the TIDA neurons in the neonatal rat hypothalamus, and the resulting hyperprolactinmia followed by lateral prostatitis that is the consequence of incomplete development of these neurons is described above. To summarize these points: without early lactational exposure to PRL, TIDA neuronal growth is impaired and elevated PRL levels are present in the prepubertal male. Hyperprolactinemia in the adult male rat has been implicated in the development of prostatitis.

Thus, early lactational exposure of dams to agents that suppress suckling-induced PRL release (possibly atrazine) could lead to a disruption in TIDA development in the suckling male offspring, followed by altered PRL regulation and subsequent hyperprolactinemia and prostatitis in these male offspring.

To test the hypothesis that atrazine exposure of dams during lactation could initiate the above-described sequence of events, suckling-induced PRL release was measured in Wistar dams treated with atrazine (by gavage, twice daily on PND 1-4 at 0, 6.25, 12.5, 25, and 50 mg/kg) or the dopamine receptor agonist bromocriptine (BROM, sc, twice daily at 0.052, 0.104, 0.208 and 0.417 mg/kg). BROM is known to suppress PRL release. Serum PRL was measured on PND 3 using a serial sampling technique and indwelling cardiac catheters.

A significant rise in serum PRL release was noted in all control females within 10 minutes of the initiation of suckling. Fifty mg/kg ATR inhibited suckling-induced PRL release in all females, whereas 25 and 12.5 mg/kg ATR inhibited this measure in some dams and had no discernible effect in others. The 6.25 mg/kg dose of ATR was without effect. BROM also inhibited suckling-induced PRL release at the two highest doses.

To examine the effect of postnatal ATR and BROM on the incidence and severity of inflammation (INF) of the lateral prostate of the offspring, adult males were examined at 90 and 120 days. While no effect was noted at 90 days of age, at 120 days, both the incidence and severity of prostate inflammation was increased in those offspring of ATR-treated dams (25 and 50 mg/kg). The 12.5 mg/kg ATR and the two highest doses of BROM increased the incidence, but not severity, of prostatitis. Combined treatment of ovine prolactin (oPRL) and 25 or 50 mg/kg ATR on PN1-4 reduced the incidence of inflammation observed at 120 days, indicating that this increase in INF seen after ATR alone resulted from the suppression of PRL in the dam. Testing to determine whether or not there is a critical period for these effects revealed that the critical period for this effect is PND1-9.

Acute RfD = $\frac{10 \text{ mg/kg}}{100}$ = **0.1 mg/kg**	Summary/conclusion

These data demonstrate that ATR suppresses suckling-induced PRL release and that this suppression results in an increase in lateral prostate inflammation in the offspring and that the critical period for this effect is PND1-9.

Dose and Endpoint for Establishing RfD: 10 mg/kg/day based on delayed or lack of ossification of several sites at 70 mg/kg/day (LOAEL).

Uncertainty Factor (UF): 100

Comments about Study/Endpoint/Uncertainty Factor: Any of the four studies described above may be appropriate for selection of an endpoint for acute risk assessment. The developmental effects seen in the two rat and one rabbit developmental study are assumed to have the potential to occur after a single dosing. The effects seen in the open literature prostatitis paper occurs after only 4 days of dosing.

The lowest NOAEL seen in the above studies was 5 mg/kg/day, which is the developmental NOAEL from the rabbit developmental toxicity study (MRID 41065201). Though the NOAEL from this study would be acceptable for use as an acute RfD, HIARC notes that there was a large dose spread in this study. The mid-dose tested (and the NOAEL) in this study was 5 mg/kg/day while the next highest dose tested (the highest dose tested and the LOAEL) was 75 mg/kg/day. This dose is a full 15 times higher than the mid-dose tested. The large spread between 5 and 75 mg/kg/day raises the possibility that had intermediate doses between 5 and 75 been used then the NOAEL would have been higher.

Examination of the rat developmental toxicity studies indicates that intermediate doses in the rabbit study between 5 and 75 may not have shown any adverse effects. The NOAEL in both the rat studies are greater than 5 mg/kg/day (10 mg/kg/day for 40566302 and 25 mg/kg/day for 41065201). The effects seen in the rabbit and two rat developmental toxicity studies are similar with all three studies seeing delayed or no ossification in certain cranial bones at their respective LOAELS of 75 (rabbit), 70 (40566302) and 100 mg/kg/day (41065201). Other effects on which the developmental NOAEL were based in the rabbit study - reduced litter size and increased resorptions - were not seen either of the rat studies and are not considered to be frank malformations, or even variations. In this respect it should be noted that maternal effects were more severe at the LOAEL in the rabbit study than at the LOAELs in either of the two rat studies. The maternal LOAELs in the two rat studies were based on decreased food consumption and body, while the maternal LOAEL in the rabbit study was based on clinical signs such as none, little or soft stool, blood on the vulva, in addition to decreased food consumption and body weight.

HIARC also notes that an acute RfD based on a NOAEL of 10 mg/kg/day would be protective of the prostatitis effects seen in the open literature study at a NOAEL of 12.5 mg/kg/day.

Dose/endpoint for General Population including infants and children:

An appropriate end point for the general population attributable to a single exposure was not available from the oral toxicity studies including the developmental toxicity studies in rats and rabbits.

.1 **Chronic Reference Dose (RfD)**

Study Selected: Six-month LH surge study Special study

MRID No.: 44152102

Executive Summary: In a study to evaluate the effect of long-term atrazine exposure on the proestrus afternoon luteinizing hormone (LH) surge (MRID 44152102) atrazine, 97.1% a.i., was administered to 360 female Sprague Dawley rats in the diet. Dose levels were 0 (negative control), 25, 50, and 400 ppm (0, 1.80, 3.65, 29.44 mg/kg/day) for 26 weeks (approximately six months).

Body weight, body weight gain and food consumption were significantly (p 0.05) decreased in HDT animals compared to controls (body weight decreased 8.5% at the end of the study and food consumption decreased 3.75% for the entire study). The percentage of days in estrus were significantly increased (p 0.01) during the 21-22 and 25-26 week time periods at the HDT. Percent days in estrus were also increased during the 21-22 and 25-26 week time periods at the MDT, but the increase was only significant (p 0.05) for the 21-22 week time period. The proestrus afternoon LH surge was severely attenuated at the HDT (LH levels at most sampling time points were actually decreased compared to baseline) and less so at the MDT (maximum increase over baseline was 157% compared to maximum increase over baseline in controls of 273%). Pituitary weight were increase at the HDT (absolute weight increased 22% and weight relative to body weight was increased 28%). Pituitary weights at the other two doses were not affected. There was a slight increase at the HDT of animals displaying enlarged pituitaries (0% in controls compared to 3.4% at 29.44 mg/kg/day) and thickened mammary glands (0% in controls compared to 6.7% at 29.44 mg/kg/day). There were no other gross necropsy findings in the HDT that could be attributed to compound exposure and there were no compound-related gross pathology findings at the MDT or LDT. Selected tissues were saved for histopathology but those results have yet to be reported.
There were no compound related effects in mortality or clinical signs. The proestrus afternoon prolactin surge was not affected by compound exposure at any dose. The LDT had no effects on the estrous cycle, LH or prolactin surges.

The LOAEL is 3.65 mg/kg/day, based on estrous cycle alterations and LH surge attenuation. The NOAEL is 1.8 mg/kg/day.

Chronic RfD = $\frac{1.8 \text{ mg/kg/day}}{100}$ = **0.018 mg/kg/day**

This special study in the rat is **Acceptable-nonguideline**. this study does not satisfy any guideline requirements and was not submitted with the intention of satisfying a guideline requirement.

Dose and Endpoint for Establishing RfD: 1.8 mg/kg/day based on estrous cycle alterations and LH surge attenuation at 3.65 mg/kg/day (LOAEL).

Uncertainty Factor(s): 100

Comments about Study/Endpoint/Uncertainty Factor: The attenuation of the LH surge is deemed to be a critical event in the mode of action of atrazine-associated carcinogenesis. This six-month study is considered adequate for use in selecting a chronic endpoint without an additional safety factor being added to account for study duration of less than 12 months. An LH surge study of longer duration may be of limited value given that the attenuation of LH surge occurs in normally aging Sprague-Dawley rats.

Though this endpoint (LH surge attenuation and estrous cycle disruption) is applicable only to females 13-50, HIARC notes that this dose is the lowest NOAEL available in the toxicology database and therefore would be protective of other adverse effects, including those occurring in males, infants and children. Therefore, a separate endpoint is not needed for this population (i.e., males, infants and children).

This dose and endpoint replaces the previous dose and endpoint of 3.5 mg/kg/day based on decreased body weight gain and food consumption in a two-year rat bioassay selected by HIARC in 1998. The dose of 1.8 mg/kg/day for use in risk assessment would be protective of effects that occur at the higher dose of 3.5 mg/kg/day as well as protective of effects such as LH surge attenuation and estrous cycle alterations, and any effects that may be associated with alteration of these parameters.

.1 **Occupational/Residential Exposure**

.1 **Short-Term (1-7 days) Incidental Oral Exposure**

Study Selected: Developmental toxicity in rats § 83-3a
MRID No.: 40566302

Executive Summary: See Study #1 above under "Acute RfD"

Dose and Endpoint for Risk Assessment: 10 mg/kg/day based on statistically significant decrease in body weight gains at 70 mg/kg/day (LOAEL).

Comments about Study/Endpoint: Decreases in body weight gain was seen during the first 5 days of dosing. The endpoint is indicative of general or systemic toxicity and is thus appropriate for the population (infants and children) of concern.

Intermediate-Term (7 Days to Several Months) Incidental Oral Exposure

Study Selected: Developmental toxicity in rats §83-3a

MRID No.: 40566302

Executive Summary: Same as for short term-incidental oral

Dose and Endpoint for Risk Assessment: 10 mg/kg/day based on statistically significant decrease in bodyweight gain at 70 mg/kg/day (LOAEL).

Comments about Study/Endpoint: Decreases in body weight gain was seen during the first 5 days of dosing. The endpoint is indicative of general or systemic toxicity and is thus appropriate for the population (infants and children) of concern.

.1 **Dermal Absorption**

Dermal Absorption Factor: The HIARC considered a rat (MRID 43314302) and human dermal penetration study (MRID 44152144) in determining the appropriate dermal absorption factor. A comparison of the two studies reveals a similar dose used in each study. The rat study had dose of 0.1 mg/kg left on the skin for 10 hours with measurement of the percent absorbed at 82 hours following the start of exposure (10/82). The human study had a similar dose of 0.067 mg/kg/ left on the skin for 10 hours with measurement 168 hours following the start of exposure (10/168) .
The dermal penetration seen at 0.1 mg/kg with 10/82 hours was 13.32%. Were dermal penetration between the rat and human similar then the human percent absorbed at 0.067 mg/kg with 10/168 hours would be expected to somewhat greater since, in the human study, the dose is lower and the time before measurement is longer (both factors would be expected to increase the percent absorbed) . The percent absorbed in the human study is lower (less than half) the percent absorbed in the rat study though. Higher absorption in the rat compared to the human is not uncommon as the stratum corneum is known to absorb many compounds and serve as a sink from which compound steadily diffuses across the epithelium into the dermis. Rat stratum corneum is much thicker than human stratum corneum resulting in this sink being a much greater factor to the rat than to the human. Thus, rat dermal absorption frequently is much greater than human dermal absorption.
Comparison of the penetration achieved following these two doses indicates that the absorption factor seen at this dose in the human study (6%) is an appropriate value to use for risk assessment. Measurement of percent absorbed was not conducted until 168 hours following exposure in the human study. This is ample time to allow any atrazine associated with unexfoliated skin to be absorbed. The higher absorption seen in a rat study with a similar dose is what would be expected and indicates that the 6% value seen in the human study is a reasonably accurate estimate of human dermal absorption at this dose.

$$\text{Rat:Human Dermal Penetration Factor} = \frac{22}{6} = \mathbf{3.6}$$

The committee recommended a dermal absorption factor of 6% (rounded up from 5.6%). This factor is based on a human study (MRID 44152114) in which 10 human volunteers were exposed to a single topical dose of [triazine ring-U-^{14}C]atrazine (94.3-96.3% a.i., 98.0-98.4% radiochemical purity) at 6.7 (4 volunteers) or 79 µg/cm^2 (6 volunteers) for 24 hours; equivalent to 0.1667 and 1.9751 mg of [14C] atrazine for the low and high doses, respectively. After 24 hours the atrazine was removed and determination of percent absorbed occurred was determined 168 hours (7 days) after the commencement of exposure. The maximum percent absorbed in this study was 5.6% of the dose in the lower dose group. Because the maximum percent absorbed is being used and because an ample amount of time (168 hours) was allowed for absorption to occur, 6% is deemed to be a protective estimate of dermal exposure.

Because both rat and human dermal absorption studies are available, a "Rat:Human Dermal Penetration Factor" can be calculated. As noted above, humans would be expected to have lower skin permeability of many compounds. The available data indicates that skin permeability of atrazine is indeed lower in humans than in rats. To account for this species difference, dermal NOAELs may be multiplied by a rat:human dermal penetration factor. The rat:human dermal penetration factor is calculated by dividing the dermal absorption in the rat by the dermal absorption in the human. In this case the dermal absorption in the rat is taken to be 22% (the 21.6% from the 0.01 10/82 hours dose in the above described rat dermal penetration study [MRID 43314302] rounded up to 22%). This value is selected because it represents the highest dermal absorption value seen following a 10 hour exposure (approximating a tyical workday) in the rat study. The value selected for the denominator for the ratio (the human portion) was 6%. This was the highest percent absorbed in this study (MRID 44152144).

This factor is multiplied by the NOAEL for exposure scenarios in which endpoints from a dermal study are used. Exposure scenarios whose endpoints are derived from oral studies will use a 6% dermal absorption factor.

.1 **Short-Term Dermal (1-7 days) Exposure**

Study Selected: 21-day dermal toxicity in the rat §82-2
MRID No.: 42089902
Executive Summary:

Atrazine technical (97.6%) was administered dermally to 30 New Zealand White rabbits for 6 hours/day for 25 days. Dose levels were 0, 10, 100 or 1000 mg/kg/day. (5 rabbits/sex/dose). The increased absolute and relative spleen weights in high-dose animals were not accompanied by histological findings; however, statistically significant reductions in red blood cell counts and hematocrit levels were noted in high dose females. Further findings included statistically

significant (p<0.01) reductions in total protein and chloride in males and significantly increased cholesterol and triglyceride levels in females.

Dermal application of the test material resulted in minimal to moderate acanthosis, hyperkeratosis, and focal subacute inflammation of treated skin in high-dose females. Dermal irritation included limited to slight (grade 1) erythema and scaling in one high dose female at days 17 - 25. erythema (grade 1) was observed in one high dose male at day 18.

The study authors reported a NOAEL of 10 mg/kg/day and a LOAEL of 100 mg/kg/day, based on slight transient reductions in mean percent body weight gain in mid-dose females at days 7 and 14. Because the reductions in female body weight gain at 100 mg/kg/day were slight, not statistically significant, transient, and without significant reductions in food consumption and absolute body weight, the reviewers assessed that the changes were of equivocal biological importance.
Thus, the NOAEL for systemic toxicity is 100 mg/kg/day. The LOAEL is 1000 mg/kg/day based on statistically significant reductions in food consumption, mean body weight, and percent weight gain in both sexes, statistically significantly increased absolute and relative spleen weights in both sexes, and slight changes in excretion (i.e. few and/or mucoid feces). This study is classified **Acceptable- Guideline**. This study satisfies the Guideline series 82-2 requirements for a 21-day dermal toxicity study in the rabbit.

Rat:Human Dermal Penetration Factor: 3.6.

Dose and Endpoint for Risk Assessment: **360 mg/kg/day**.
This value is derived by multipliying the study NOAEL of 100 mg/kg/day (based on reduced food consumption, mean body weight, body weight gain, increased spleen weights at the LOAEL of 1000 mg/kg/day) by the dermal penetration factor of 3.6.

Comments about Study/Endpoint: This study is appropriate because the duration and route of exposure (21-day, dermal) match the duration and route of exposure (up to one week, dermal) in the short-term dermal risk assessment.

.1 **Intermediate-Term Dermal (7 Days to Several Months) Exposure**

Study Selected:
Six-month LH
surge study §
Special study

MRID No.: 44152102
Executive Summary: See above under "Chronic RfD"
Dose/Endpoint for Risk Assessment: 1.8 mg/kg/day based on estrous cycle alterations and LH surge attenuation at 3.65 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The endpoint of concern was seen after 6 months of exposure and is appropriate for this exposure period of concern. The 21-day dermal study was not selected since estrous cycle evalutions and LH measurements (both of which have been shown to be very sensitive endpoints following atrazine exposure) were not performed in this study. Since an oral NOAEL was selected, the 6% dermal absorption factor should be used in route-to-route extraploation.

.1 **Long-Term Dermal (Several Months to Life-Time) Exposure**

Study Selected:
Six-month LH
surge study §
Special study

MRID No.: 44152102

Executive Summary: See above under "Chonic RfD"

Dose/Endpoint for Risk Assessment: 1.8 mg/kg/day based on estrous cycle alterations and LH surge attenuation at 3.65 mg/kg/day (LOAEL).

Comments about Study/Endpoint: This study was also used to derive the Chronic RfD. Since an oral NOAEL was selected, the 6% dermal absorption factor should be used in route-to-route extraploation.

.1 **Inhalation Exposure (All Durations)**

With the exception of an acute inhalation study, no inhalation studies are available for evaluation. Therefore the HIARC selected the oral NOAELs for inhalation risk assessments. Since an oral dose is used, risk assessment should follow the route-to-route extrapolation as below:

Step I. The inhalation exposure component (*i.e* µg a.i./day) using 100% absorption rate (default value) and application rate should be converted to an equivalent oral dose (mg/kg/day).

Step II. The dermal exposure component (mg/kg/day) using a 6% dermal absorption rate and application rate should be converted to an equivalent oral dose. This dose should then be combined with the oral equivalent dose in Step I.

Step III. The combined oral equivalent dose from Step II should then be compared to the oral NOAELs to calculate MOEs. The NOAELs are as follows:

For intermediate term: 1.8 mg/kg/day
For chronic exposures: 1.8 mg/kg/day

NOTE: Route-specific MOE should be used for short-term exposure. Inhalation exposure can not be combined with dermal due to the lack of a common

toxicity endpoint via the dermal (decreased body weight gain, and food consumption) and inhalation (attenation of LH surge routes).

1. **Margins of Exposure for Occupational/Residential Risk Assessments**

the level of concern for dermal and inhaltion occupational exposure is an MOE of 100.
The MOEs for residential exposure risk will be determined by the FQPA SF committee.

2.3.9 Reccommendation for Aggregate Exposure Risk Assesments

For acute aggregate exposure, the high end value from food plus water should be combined and compared to the RfD.

For short-term aggregate exposure, the oral, dermal and inhalation exposures can not be combined due to the differences in toxicity endpoints via these routes; oral - developmental; dermal - decreased body weight gain, food consumption and spleen weights; and inhalation - attenuation of the LH surge.

For intermediate and long-term aggregate exposures, the three routes can be combined because the dermal and inhalation exposures are corrected to oral equivalent doses and are based on the same endpoint as the RfD

1 CLASSIFICATION OF CARCINOGENIC POTENTIAL

.1 Combined Chronic Toxicity/Carcinogenicity Study in Rats

MRID Nos.: 00158930; 42085001; 42204401; 44544701

Discussion of Tumor Data: Several chronic bioassays in the Sprague-Dawley rat (MRIDs shown above) have demonstrated that chronic atrazine exposure is associated with an increased incidence and/or an earlier onset of mammary tumors. There is also limited evidence (primarily from a single chronic bioassay) that atrazine exposure may be associated with an earlier onset of pituitary adenomas.

Adequacy of the Dose Levels Tested: The dose levels tested were adequate to determine the carcinogenic potential of atrazine.

.1 Carcinogenicity Study in Mice

MRID No. : 40431302

Executive Summary: In an oncogenicity study (MRID 40431302), atrazine, (purity not given) was administered to CD-1 mice, 59-60/sex/dose, in the diet at dose levels of 0, 10, 300, 1500 and 3000 ppm (male/female mean daily dose 0/0, 1.4/1.6, 38.4/47.9, 194.0/246.9, 385.7/482.7 mg/kg/day) for 91 weeks. Female mice in the 300, 1500 and 3000 ppm groups received a daily atrazine dose about 25% higher than their counterpart males. No dose-related increases in neoplasms were observed. The dose response curve seemed adequate since toxic effects, such as a decrease in mean body weight of both sexes and an increase in cardiac thrombi in the females, are seen at both 1500 and 3000 ppm, while no dose-related toxic effects are seen at 10 and 300 ppm. In addition to the toxic effects just mentioned, the 3000 ppm animals of both sexes also displayed decreases in food consumption and decreases in RBC, hematocrit, and hemoglobin concentration. Female mice, but not males, at 3000 ppm showed decreased mean group brain and kidney weights and decreased percentages of neutrophils and lymphocytes. There was also an increase in mortality ($p < 0.05$) in 3000 ppm females, but not males, with only 25% of the females surviving *vs* 39-43% of the females surviving in the other female dose groups.
The cardiac thrombi found at both 1500 and 3000 ppm may have contributed to unscheduled female deaths during the course of the study. The incidence of unscheduled death in mice with cardiac thrombi is statistically significantly different from the incidence of unscheduled death in mice from control groups. The occurrence of cardiac thrombi must be considered a severe effect.

The LOEL is 1500 ppm (222.0 mg/kg/day), based on decreased body weight gain in both sexes and increased cardiac thrombi in the females. The NOEL is 300 ppm (43 mg/kg/day).

At the doses tested, there was not a treatment-related increase in tumor incidence when compared to controls. Dosing was considered adequate due to the occurrence of decreased body weight gain and cardiac thrombi.

This carcinogenicity study in the mouse is **Acceptable-Guideline,** and does satisfy the guideline requirement for a carcinogenicity study in the mouse.

Discussion of Tumor Data: There was no evidence of carcinogenicity in CD-1 mice following exposure to atrazine for 91 weeks.

Adequacy of the Dose Levels Tested: Dose levels tested were adequate to determine carcinogenic potential

.1 **Classification of Carcinogenic Potential**

In 1987, the HED Cancer Peer Review Committee (CPRC) classified atrazine as a Group "C" carcinogen (possible human carcinogen) and recommended a linear low dose approach (Q_1*) for human risk characterization. The CPRC met again on June 6, 1988 and September 29, 1988 and reaffirmed the Group C classification.

In 1997, the HED Cancer Assessment Review Committee (CARC) evaluated the carcinogenic potential of atrazine and discussed mode of action data submitted by the Registrant in regards to the ability of atrazine to produce mammary tumors in Sprague-Dawley rats. A

document from that CARC meeting has been prepared, was evaluated by CARC in the Fall of 1999, and was presented at the Science Advisory Panel (SAP) in June 27, 28 and 29th, 2000. This document may be accessed at *http://www.epa.gov/oscpmont/sap/*. A final Cancer Peer Review Document will not be ready until after receipt of the written SAP evaluation and final approval by CARC.

1 **MUTAGENICITY**

Atrazine has not been found to be mutagneic in bacteria and does not cause unscheduled DNA synthesis in primary rat hepatocytes. Atrazine did not induce clastogenicity in the mouse micronucleus assay. Atrazine was negative in a mouse Dominant-Lethal Assay.

An extensive review of more than 50 mutagenicty studies using atrazine, and atrazine metabolites is included as a chapter in the most recent cancer peer review document. This document can be accessed at the internet address shown in the previous paragraph.

(I) Gene Mutation

In a reverse gene mutation assay in bacteria (MRID 40246601), strains TA 98, 100, 1535 and 1537 of S. typhimurium were exposed to atrazine (98.2% a.i.), in dimethylsufoxide, at concentrations of 0, 20, 78, 313, 1250, and 5000 g/plate. Tests were conducted in the presence and absence of mammalian metabolic activation S9 fraction of Tif:RAIf rats treated with Aroclor 1254. Atrazine was tested up to the limit concentration, 5000 g/plate. The positive controls did induce the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable-Guideline**. It does satisfy the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

(ii) Structural Chromosomal Aberrations

A mouse bone marrow micronucleus test was conducted using Tif:MAGF mice (MRID 40722301). The test consisted of two parts. The first portion consisted of 24 male and 24 female mice being dosed with 2250 mg/kg atrazine (98.2% a.i.). Eight animals of each sex were then sacrificed at 16, 24 or 48 hours following treatment. The second portion of the study 24 mice, 8/sex/dose, were treated with atrazine (98.2% a.i.) at doses of 562.5, 1175, 2250 mg/kg. Bone marrow cells were harvested at 24 hours post-treatment. The vehicle in both portions of the study was carboxymethyl cellulose. Exposure in both portions of the study was accomplished by a single gastric intubation. There were no signs of cytotoxicity in bone marrow erythropoiesis seen either portion of the study. However, the high dose was clearly toxic since 7 of the 32 females which received the high dose died prematurely. Atrazine was tested at an adequate doses being that these were doses that induced death in mice. The positive control induced the appropriate response. **There was not a significant increase in the frequency of micro nucleated polychromatic erythrocytes in bone marrow after any treatment time or dose.** This study is classified as **Acceptable-Guideline**. It does satisfy the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data.

(iii) Other Genetic Effects

In an unscheduled DNA synthesis assay (MRID 42547105), primary rat hepatocyte cultures were exposed to atrazine, (97.1% a.i.), in dimethyl sulfoxide at concentrations of 15, 46, 139, 417, 835, and 1670 g/ml for 16-18 hours. Atrazine was tested up to precipitating concentrations, 139 g/ml. The positive controls did induce the appropriate response. **There was no evidence that unscheduled DNA synthesis, as determined by nuclear silver grain counts, was induced.** This study is classified as **Acceptable-Guideline**. It does satisfy the requirement for FIFRA Test Guideline 84-2 for other genotoxic mutagenicity data.

In a mouse dominant lethal assay (MRID 42637003), groups of 30 male Tif: MAGf (SPF) mice were treated orally by gavage with Atrazine technical (97.1% a.i., batch #SG8029BA10) at doses of 0, 500, 1000, 2000, or 2400 mg/kg body weight in a volume of 10 mL/kg. The vehicle was corn oil. Starting immediately after dosing, each male was mated with 2 untreated females per interval for days 1-4, days 4-8, and days 8-12. Each male was then mated with 2 untreated females per week for weeks three through eight.

Atrazine technical was tested at an adequate dose. There were signs of toxicity after dosing as evidenced by piloerection and decreased locomotor activity. The females were sacrificed on gestation day 13-15 and the uteri examined for the number of alive, early, and late dead embryos and resorptions. Cyclophosphamide served as the positive control. There was no significant difference between the control group and treated groups with respect to post-implantation mortality of embryos. Under the conditions of this study atrazine technical did not induce dominant lethal mutations in male mice at doses as high as 2400 mg/kg.

This study is classified as **Acceptable-Guideline**. It does satisfy the requirement for FIFRA Test guideline 84-2 for rodent dominant lethal data

1 FQPA CONSIDERATIONS

1. Adequacy of the Data Base

The toxicology database for atrazine was considered adequate by the HIARC for consideration of factors under FQPA.

.1. Neurotoxicity

Acute and subchronic neurotoxicity studies are not available for atrazine and are not required as atrazine is neither a carbamate nor an organophosphate compound. Indications of possible neurotoxicity were not evident in the submitted guideline studies. Special studies submitted by the registrant (MRIDs 44152102 and 43934406) and published in the open literature (Cooper, *et al.* 2000. Atrazine disrupts the hypothalamic control of pituitary- ovarian function. Tox. Sci. 53: 297-307 [MRID 45166902]) provide evidence of atrazine-associated neurotoxicity. The neurotoxicity

seen in these studies was a central nervous system (CNS) toxicity (specifically, neuroendocrine alterations at the hypothalamus).

.1 **Developmental Toxicity**

One rabbit and two rat developmental studies are available for evaluation. The executive summaries for these three studies are shown above under "Acute RfD: Study #'s 1, 2, and 3".

5.4
Reproductive Toxicity

A two-generation study is available for evaluation.
MRID: 40431303 Guideline no.: §83-4

In a 2-generation reproduction study (MRID 40431303) atrazine, (purity not specified but said to be technical grade) was administered to 240 Charles River (CRCD, VAF/PLUS) rats 30/sex/dose in the diet at dose levels of 0, 10, 50, and 500 ppm. There was very little variation in test article consumption between generations; the F_0 and F_1 males had similar test article consumption during the 70-day premating period as did the F_0 and F_1 females. The average values for the two generations are 0, 0.75, 3.78, 39.0 mg/kg/day for males and 0, 0.86, 3.70, 42.8 mg/kg/day for females. Test article consumption for the F_0 and F_1 generation females during their gestation period did not vary greatly between generation. Mean compound consumption for both generations were 0, 0.66, 3.33 and 35.43 mg/kg/day.

Parental body weights, body weight gain, and food consumption were statistically significantly reduced at the 500 ppm dose (HDT) in both sexes and both generations throughout the study. Compared to controls, body weights for F_0 HDT males and females at 70 days into the study were decreased by 12% and 15%, respectively while F_1 body weight for the same time period was decreased by 15% and 13% for males and females, respectively. The only other parental effect which may have been treatment related was a slight, but statistically significant, increase in relative testes weight which occurred in both generations of the HDT. **The parental LOAEL is 500 ppm (39 mg/kg/day in males, 42.8 mg/kg/day in females) based on decreased body weights, body weight gains and food consumption. The NOAEL is 50 ppm (3.78 mg/kg/day in males, 3.7 mg/kg/day in females).**

There did not appear to be any offspring toxicity in females from compound exposure. Male offspring pup body weight was significantly decreased ($p<0.05$) in males at day 21 in both generations. **The offspring toxicity LOAEL is 39 mg/kg/day based on decreased body weights in both generations of males at PND 21. The offspring toxicity NOAEL is 3.78 mg/kg/day.**

This study is classified **Acceptable-Guideline** and satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800, §83-4) in the rat.

5.5 Additional Information from Literature Sources

An open literature publication (MRID 45166902, discussed above under "Acute RfD, Study #4") has demonstrated that exposure of a lactating dam to atrazine during the days shortly after parturition may result in increased incidence and severity of prostate inflammation in male offspring. Other work from the NHEERL laboratories at EPA has indicated that atrazine exposure to immature rats may delay the onset of puberty (Stoker *et al.*, 2000. The Effects of Atrazine on Puberty and Thyroid Function in the Male Wistar Rat: An Evaluation in the Male Pubertal Protocol. Submitted.; Laws, *et al.* 2000. The effects of atrazine on puberty in female Wistar rats: An evaluation in the protocol for the assessment of pubertal development and thyroid function. Submitted).
The mode of action for these two effects (prostate inflammation and delayed puberty) is believed to be similar to the mode of action described for atrazine-associated cancer and involves the CNS neuroendocrine alterations described in the HED CPRC document (specifically, neuroendocrine alterations at the hypothalamus).

5.6 Determination of Susceptibility

HIARC concluded that an increased quantitative or qualitative susceptibility was not seen in the developmental or two generation reproduction studies. However, the studies mentioned above under section 5.5 provide evidence of increased susceptibility.

Recommendation for a Developmental Neurotoxicity Study

5.6.1 Evidence that suggest requiring a Developmental Neurotoxicity study:

Special studies and an open literature study mentioned above under section 5.2 indicate a neuroendocrine toxicity in the CNS of rats following atrazine exposure.

5.6.2 Evidence that **do not** support a need for a Developmental Neurotoxicity study:

The avaible studies have not indicated any disruption of thyroid function following atrazine exposure. Evidence of neurotoxicity was seen following atrazine exposure. Evidence of neurotoxicity following exposure to a compound is frequently seen as evidence that supports the need for a Developmental Neurotoxicity study (DNT). However, numerous studies have been conducted which have described and defined this atrazine-associated neurotoxicity. The neurotoxicity seen following atrazine exposure is CNS neuroendocrine toxicity. This CNS neuroendocrine toxicity has been well-defined through a series of registrant submitted studies and studies performed by EPA scientists at NHEERL. Many of the parameters measured in the DNT are behavioral in nature, or are effects on the peripheral nervous system (PNS). Tests conducted in a DNT are those such as motor activity tests, auditory startle tests, and learning and memory tests. The neurotoxicity associated with atrazine exposure has

been well-defined, and does not involve these sorts of behavioral alterations or PNS alterations.
Certain measures performed in the DNT (such as determination of onset of developmental landmarks and neuropathology) would be useful in examining this CNS neuroendocrine toxicity. However, special studies designed specifically to examine these endpoints would be much more useful in this regard as protocols could be designed around the desired endpoints.

Therefore, HIARC determined that a DNT is not required. Instead, the HIARC reccommended that studies examining the specific CNS alterations described in the studies conducted by the registrant and the Agency's NHEERL labs, be performed.

1 **HAZARD CHARACTERIZATION**

Atrazine is herbicide most commonly used on corn and sorghum to control broadleaf grasses. The toxicological database for atrazine is complete, and acceptable. Atrazine has low acute toxicity and is not a dermal sensitizer.

Guideline subchronic, dermal, chronic, developmental, and reproduction studies did not indicate any particular target organ for toxicity though several studies have indicated that atrazine is associated with mammary and pituitary tumors in females of the Sprague-Dawley strain of rat. Special studies designed to elucidate a mode of action of for this carcinogenic effect have demonstrated that the hypothalamus appears to be a target organ. Neuroendocrine alterations of the hypothalamic-pituitary axis of rodents following atrazine exposure have been well-described both in studies submitted by the registrant and in studies conducted by EPA labs. These alterations are seen in chronic studies at low doses and in shorter term studies at higher doses.

The mutagenicity database for atrazine is extensive and has indicated that atrazine is not mutagenic. Special studies have also been conducted to determine the estrogenic potential of atrazine and these studies have demonstrated that atrazine lacks direct estrogenic activity.

The Cancer Assessment Review Committee (CARC) has evaluated atrazine and a draft report from that committee has been prepared. This draft report was presented to the Agency Science Advisory Panel June 28 - 30, 2000.

1 **DATA GAPS**

There are no datagaps for atrazine according to the Subdivision F Guideline requirements. HIARC reccommends, *but does not require*, that special studies examining atrazine's associations with delayed puberty and prostatitis in offspring of dams exposed shortly after partution, be conducted. Should such studies be conducted, it is reccommended that study protocols be approved by HED prior to commencent of any such study.

In addition, HIARC recommends, but does not require, that special studies examining the CNS alterations following atrazine exposure, be performed.

ACUTE TOXICITY

Acute Toxicity of Atrazine

Guideline No.	Study Type	MRIDs #	Results	Toxicity Category
81-1	Acute Oral	Acc 230303	LD_{50} = 1,869 mg/kg (M+F combined)	III
81-2	Acute Dermal	Acc 230303	LD_{50} > 2,000 mg/kg (M+F combined)	III
81-3	Acute Inhalation	430165-02	LC_{50} > 5.8 mg/L (M+F combined)	IV
81-4	Primary Eye Irritation	Acc 230303	PIS= 0.0/110	IV
81-5	Primary Skin Irritation	Acc 230303	PIS= 0.2/8.0	IV
81-6	Dermal Sensitization	001051-31	Non-sensitizing	IV
81-7	Acute Neurotoxicity	none	Not Applicable	—

1 SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	NOAEL= 10 UF = 100	Delayed ossification of certain cranial bones	Developmental toxicity study in the rat
	Acute RfD = 0.1 mg/kg/day		
Chronic Dietary	NOAEL = 1.8 UF = 100	Attenuation the pre-ovulatory lutenizing hormone (LH) surge	Six-month LH surge study in the rat
		Chronic RfD = 0.018 mg/kg/day	
Incidental Oral, Short-Term	NOAEL= 10	Decreased body weight during the first five days of dosing in the dams	Developmental toxicity study in the rat
Incidental Oral, Intermediate-Term	NOAEL= 10	Same as short term	Same as short term
Dermal, Short-Term[a]	NOAEL= 360 (NOAEL from study was 100 mg/kg/day. Multiplied by the rat:human dermal pentration factor of 3.6 = 360 mg/kg/day)	reductions in food consumption, mean body weight, and percent weight gain in both sexes, statistically significantly increased absolute and relative spleen weights in both sexes, and slight changes in excretion (*i.e.* few and/or mucoid feces).	21-day dermal toxicity study
Dermal, Intermediate-Term[b]	NOAEL= 1.8	Attenuation the pre-ovulatory lutenizing hormone (LH) surge	Six-month LH surge study in the rat
Dermal, Long-Term [b]	NOAEL= 1.8	Same as intermediate term	Same as intermediate term
Inhalation, Short-Term [c]	NOAEL= 10	Delayed ossification of certain cranial bones	Developmental toxicity study in the rat
Inhalation, Intermediate-Term[c]	NOAEL= 1.8	Attenuation the pre-ovulatory lutenizing hormone (LH) surge	Six-month LH surge study in the rat
Inhalation, Long-Term [c]	NOAEL= 1.8	Same as intermediate term	Same as intermediate term

a The rat:human dermal penetration factor of 3.6 is applied to this scenario only.
b Dermal absorption rate = 6% c Convert from oral dose using an inhalation absorption rate= 100% default

Department of Pesticide Regulation

Gray Davis

January 8, 2001

To: **Karl P. Baetcke, Ph.D., Senior Scientist**
Health Effects Division, Office of Pesticide Programs, USEPA
1200 Pennsylvania Avenue NW,
Washington DC 20460

From: **Gary Patterson, Ph.D. Branch Chief**
Medical Toxicology Branch, Dept. of Pesticide Regulation,
California EPA,
1001 I St, Sacramento, CA 95814

Subject: Atrazine draft final RCD: responses to issues raised by USEPA

Many thanks for sending USEPA's comments on DPR's draft final RCD for atrazine on 12-10-2000. In the attachment with this letter, we have addressed these comments along with making some additional comments on the recently completed SAP report. Several of the points overlap.

In the reponses that follow, the page numbers in parenthesis, in bold, correspond to page numbers in the draft final RCD. The comments on the SAP report are in parenthesis, in bold. The (5) bolded statements in the responses to USEPA comments on the RCD are taken from the memo of 12/10/00 from Karl Baetcke and Vicki Dellarco to Gary Patterson.

c.c. Vicki Dellarco (USEPA),
Keith Pfeifer, Joyce Gee, Chuck Aldous, Derek Gammon (DPR).w/ attach.

SAP Report No. 2000-05 FIFRA SAP Meeting, June 27-29, 2000

On p.8, "...a compound that does not bind to an estrogen receptor." ? **(see pp. 73-4).**

On p.10, Mode of action analysis, "...altered levels of hydroxylases, aromatase or estrogen receptors." ?**(see p. 96).**

On p.11, "Instead of a direct toxic hypothalmic/pituitary action of atrazine, it was suggested that atrazine acts to reduce food intake and weight as a potentially benign appetite suppressant, resulting in the reduced LH surge." ? **(see p. 76 and below).**

On p.13, "there is compelling evidence to support the conclusion that the mode of action of atrazine is not relevant to humans"... ? **(and yet LH surge suppression is used as the endpoint for chronic toxicity risk assessment).**

On p.14, "The panel concluded that there is no established or even likely neuroendocrine pathway through which atrazine could induce cancer in humans by a mode of action similar to that in female SD rats or by alternative modes of action such as mutagenicity or direct estrogenic activity." ? **(see pp. 73-4; also, what about impurities, such as potentially genotoxic dichloroaminoalkyltriazines?)**

On p.15, "...mammary tumors and developmental toxicity in rats, namely, reduced LH secretion, possibly induced by decreased food consumption." ? **(see p. 76 and below).** "puberty is delayed as a result of atrazine consumption." **(see p. 76 and below).**

On p.21, "...tumorigenesis in female F-344 rats may be obscured by the reduction in body weight." ? **(why not in the SD rats, also?)**

On p.26, "Thus, the evidence is consistent with the view that appetite suppression and reduced food intake is the primary mechanism of the effect of atrazine on LH in all rats." **but,** atrazine reduced the reproductive lifespan in SD rats whereas dietary restriction increases it. ? **(see p. 76 and below).**

On p.34, "...rats, where reproduction is under pituitary control, and humans, where reproductive senescence is controlled by the ovary." (**This refers to SD rats, I presume?)**

On p.37, "Moreover, if reduced food intake does underlie the atrazine effect in female SD rats, then it could be relevant to humans at doses that induced anorexic conditions..." ? **(see p. 76).** "These differences...tend to make the fetus, infant, and young child more vulnerable to insults, whether chemical, radiological, infectious or physical." ? **(Why?)**

On p.40, "As noted, it is well established that food restriction reduces LH and delays puberty." **(see p. 76 and below).**

Conclusions

Dietary restriction of SD rats resulted in a slight delay in puberty but a prolonged increase in the reproductive lifespan; 40% restriction delayed persistent estrus by four months (McShane & Wise, 1996). Therefore, it is unlikely this effect of atrazine (delay in puberty with **early** reproductive senescence) could be secondary to body weight loss **(see p. 76).**

There is evidence that aromatase is induced in human cells by triazine herbicides (Sanderson *et al.*, 2000a,b **see p. 76**). This seems a plausible explanation for many of the endocrine effects but is only briefly discussed in the SAP report.

It is widely accepted that dietary restriction of SD rats results in the abolition of tumors caused by several carcinogens *e.g.* DMBA, MNU **(see p. 81).** The fact that atrazine results in mammary tumors accompanied by a marked reduction in food intake/body weight suggests either that atrazine is a particularly effective carcinogen or else that it is acting through a different mechanism (to DMBA and MNU) **(see 5, below).**

Points in response to USEPA's review of the draft final RCD for atrazine

"…some mutagenicity/genotoxicity testing yielded positive results but…….there is not much support for a role of mutagenicity….."

Klingerman et al., 2000, have suggested that atrazine lacks genotoxicity. However, a question has been raised about the possible presence of genotoxic impurities, especially in some of the earlier studies, which could account for the observed genotoxic effects. From looking over Novartis product chemistry reports, it appears that, in 1991, technical atrazine contained ≤0.25% of G28513 and GS11748 *i.e.* ≤2500 ppm of each. [These are the aminoethyl and aminoisopropyl dichlorotriazines, potentially genotoxic manufacturing intermediates.] From 1994 onwards, it appears that these impurities were down to <0.03% *i.e.* <300 ppm of each. What is the genotoxicity of these triazines? If positive, it would then be important to know the absolute concentrations of these impurities in the atrazine end product(s).

"…the absence of a tumor response in ovariectomized rats…...linkage of sustained estrogen levels with tumor responses in intact female rats"

Mammary tumors were eliminated by ovariectomy whether they arose in atrazine-treated or control animals. However, ovariectomy does not eliminate mammary tumors following the administration of a genotoxic (MCA) or endocrine (estradiol) carcinogen **(p.77)**. It is therefore unclear how to interpret this aspect of the (atrazine) ovariectomy experiments.

"…(HIARC) recently selected for chronic dietary assessment is the suppression of the LH surge and estrus cycle alterations reported in a 6 month study…"

USEPA considers that a good case for a threshold mechanism exists for mammary tumors in the female SD rat. This is based on a suppression of the LH peak, leading to sustained elevated estradiol in the blood, that is of little or no relevance in humans. However, USEPA nevertheless considers it appropriate to use the LH endpoint for establishing a NOEL for chronic toxicity. If the LH effect is not relevant to humans for mammary tumors, then it is difficult to see why it would be relevant for other aspects of chronic toxicity in humans.

Several reviewers at DPR concluded that the chronic (1 yr.) dog study **(p. 43)** reveals cardiotoxicity at lower dose levels than the LH surge suppression in the SD rat. It could be argued that the low NOEL in this study (15 ppm; ~0.5 mg/kg/day) is artificially low, being partly a result of the dose selection (0, 15, 150 and 1000 ppm). However, cardiotoxicity in the dog of a similar nature was also detected in 3 other experiments conducted with atrazine degradates **(p. 80).** These were DACT over 1 yr., DIPA 14 wks. and DEA 13 wks. Furthermore, the study authors failed to mention the dramatic EKG effects of DIPA or DEA **(pp. 25-26)** in their reports.

4. **"…HIARC selected 10 mg/kg/day as the NOAEL for acute dietary risk assessment."** It was considered that the rabbit could be more susceptible to the acute effects of atrazine (than the rat). Therefore, a NOEL of 5 mg/kg/day from a rabbit developmental toxicity study was used for acute risk assessment **(p. 64)**.

5. **"…mammary fibroadenoma and carcinoma incidences should not be combined…"** Cancer potency factors (Q_1 and Q_1*) have been calculated for both rats with adenocarcinoma as well as for rats with any tumor originating in the mammary glands **(p. 82)**. Some pathologists use this grouping. The latter calculation gave rise to an approximate doubling of the cancer potency factors. Considering advances made in the field of molecular biology over the past 15 years, it should be possible to determine the precise genetic mutation which predominates in each tumor of every animal in a study. With knowledge of the specific mutation in each oncogene or tumor suppressor gene, it would be possible to address two questions with considerable confidence:

a/ is the mutation(s) occurring in atrazine-dosed animals the same as in untreated controls (a pre-condition for justifying the use of the linearized multistage model)?

b/ does the mutation(s) (in atrazine-dosed animals) resemble that of other, known agents causing mammary tumors in SD rats, by known mechanisms?

VOLUME ____ OF ____ OF SUBMISSION

ATRAZINE: RESPONSE REPORT

TITLE

Response to the California Department of Pesticide Regulation's Draft Risk Characterization Document for Atrazine Dated September 29, 2000

DATA REQUIREMENT

Not Applicable

AUTHORS

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COMPLETION DATE

November 29, 2000

PERFORMING LABORATORY

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LABORATORY STUDY IDENTIFICATION

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VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 16

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d) (1) (A), (B) or (C).

Company: Novartis Crop Protection, Inc.

Company Representative: Debra S. Stubbs

Title: State Regulatory Manager

Signature: Date:

These data are the property of Novartis Crop Protection, Inc. and, as such, are considered to be confidential for all purposes other than compliance with the regulations implementing FIFRA Section 10.

Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality, which may exist under any other provision of common law or statute or in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

A Good Laboratory Practices Compliance Statement is not appropriate for this volume because this volume is in response to the California Department of Pesticide Regulation's Draft Atrazine Risk Characterization document dated September 29, 2000.

_____ _____

Debra S. Stubbs *Date*
State Regulatory Manager

Submitter/Sponsor: *Novartis Crop Protection, Inc.*
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Department of Pesticide Regulation

Gray Davis

August 9, 2001

To: ***Debra S. Stubbs, State Regulatory Manager***
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Subject: ***Atrazine draft final RCD: responses to issues raised by Novartis***

Many thanks for sending Novartis' comments on DPR's draft final RCD for atrazine on 11-29-2000. In the attachment with this letter, we have addressed each of these comments.

In the following response, CDPR comments have been added to the original (Novartis) memorandum in bold, arial font. Changes or additions to the draft, final RCD have been made on the following pages (in the sequence of comments): 1, 97, 11, 96, 13, 8, 2. Additional changes to the draft final RCD have also been made to address comments made by US EPA and OEHHA. You may obtain a copy of the final RCD by requesting it through the usual channels.

Once again, thank you for taking the time to review the atrazine draft final RCD and it is hoped that your points are suitably addressed.

c.c. *Tobi Jones (DPR),*
Keith Pfeifer, Joyce Gee, Chuck Aldous, Derek Gammon (DPR).w/ attach

1.0 SUMMARY

Novartis has reviewed CDPR's Draft Risk Characterization Document for Atrazine and has determined that the exposure and risk calculated for atrazine is overly conservative. These estimates are conservative because CDPR did not utilize all of the currently available data developed by Novartis to more accurately characterize potential exposure to atrazine via the diet, drinking water or as a result of occupational exposure.

Novartis agrees with CDPR that a margin of exposure (MOE) approach may be the best method to characterize risk associated with atrazine exposure. Novartis believes that the no observed effect level for cardiotoxicity in the atrazine chronic dog study has been established at a dose of 5.0 mg/kg/day instead of 0.5 mg/kg/day stated by CDPR. Furthermore, Novartis believes that atrazine does not have any direct effects on the immune system in either the rodent or the dog. Novartis recommends that CDPR considers adopting the NOEL from the 2-generation rat reproduction study of 3.5 mg/kg/day as a basis for establishing the atrazine chronic reference dose as is currently done by EPA.

Although Novartis has previously provided CDPR with a comprehensive aggregate and cumulative risk assessment of atrazine and simazine, this document was not mentioned in CDPR's review. Furthermore, several key publications that have evaluated the epidemiological data on atrazine apparently have not been considered by CDPR. These and other documents have been identified in the reference list and those which we believe are not in CDPR's files have been provided as part of this submission.

2.0 Introduction

Novartis Crop Protection appreciates the opportunity to provide a response to the California Department of Pesticide Regulation's (CDPR) draft Risk Characterization Document for Atrazine dated September 29, 2000. Four main topics of the Department's document – Product Use, Environmental Fate, Toxicology Profile, and Risk Assessment will be considered.

3.0 Product Use

Paragraph 1 of the introduction to the Summary states that only one formulation of atrazine is registered in California. Novartis has 3 registered products in California [Aatrex 4L (100-497-AA, Aatrex Nine-O (100-585-AA) and BICEP II Magnum, (100-817-AA) and Drexel has one [Drexel Atrazine 4L (19713-AA)]. This statement is also inconsistent with information presented by CDPR in Section IIC (page 6). **CDPR: This has been corrected (pp.1 and 97).**

4.0 Environmental Fate

Comments are provided for those sections where Novartis believes that additional information may assist CDPR in their review, interpretation and conclusions.

4.1 Soil Metabolism

The second paragraph of this section refers to a study by McDonald et.al. (1990). This paragraph should be deleted because it is not the appropriate reference for atrazine soil metabolism. This report is actually an ID report in which samples from the (actual) soil metabolism study were sent to Novartis, as a result of an inquiry from CDFA, for further metabolite identification. The amounts of atrazine and metabolites cited in the second paragraph of the characterization report, are from the ABR, but are per cent of total radioactivity in the sample and not per cent of initial applied radioactivity to the sample. Rustum, (1988) is the more appropriate reference because it is the report for the actual soil metabolism study or the report by Nelson et. al. 1991 which summarizes the Hazleton soil metabolism study and two identification (ABR) reports. **CDPR: The paragraph referring to the McDonald study (on p. 11) has been deleted.**

4.2 Potential for Nitrosoatrazine Formation

Many nitrogen containing compounds, especially secondary amines, including atrazine, have the ability to form N-nitroso derivatives. Chemically the formation of nitroso-atrazine (in acidic medium) could occur as follows:

secondary amines (e.g. atrazine) + sodium nitrite → nitrosoamine (nitroso-atrazine)

A significant concentration of nitrite is required for this reaction. The nitrite level in a meal is fairly constant of the order of 6-10 ppm, which apparently is little influenced by the composition of the meal.

4.2.1 In Vitro Studies

It is theoretically possible to form of nitroso-atrazine under acidic conditions, provided that the nitrate is first reduced to nitrite, which commonly occurs in the intestine. However, there are several factors influencing this chemical process and controlling the outcome of such a reaction in the mammalian system.

It is known that dietary fiber inhibit the oxidation of ammonia to nitrate and nitrite since the fibers contain carboxyl groups that bind the ammonia. Moreover, the metabolism of any nitrite formed is quite efficient via glutathione-S-transferase and other microsomal enzymes. In addition, nutritional vitamin C and E can chemically reduce the formed nitrite (Mervish, 1994; Volume 2 of this submission).

N-nitroso-atrazine is very unstable under environmental conditions such as high temperature, pH, microbial activity, etc., and so its persistence in the human

environment is unlikely (Mücke 1990; Volume 2 of this submission). In vitro experiments have shown that this reaction was dependent on the medium pH and on the presence of food (Sumner & Cassidy, 1975a, Marco et. al., 1976, Mücke & Mathies, 1988; Volume 2 of this submission). Using artificial gastric fluid in vitro at pH 1.9 resulted in the following course of reaction: 21 ppm atrazine + 700 ppm nitrite -→ 6.4% nitroso-atrazine (within one hour). Addition of rat feed to the above mixture at, pH 1.9, lowered the amount of nitroso-atrazine formed to < 2%, while raising the pH to 3.9, the maximum amount of nitroso-atrazine did not exceed 0.9%.

Incubation of atrazine (21 ppm) and nitrite 700 ppm in sheep rumen fluid (pH = 6.8) gave rise to transient formation of N-nitroso-atrazine with a maximum yield of 0.6%-1.9% after 1 to 2 hours. After 4 hours, no N-nitroso-atrazine could be detected. Thus, it appears that microbial degradation is responsible for the disappearance of the compound, since the pH 6.8 of the solution is not low enough to cause hydrolysis (Marco et. al., 1976, Sumner & Cassidy, 1975b; Volume 2 of this submission).

4.2.2. In Vivo Studies

No N-nitroso-atrazine was detected in stomach contents, stomach tissue or in the excreta of rats fed atrazine at 0.5 mg/kg and nitrite at 17 mg/kg. In another experiment, there were no detectable amount of N-nitroso-atrazine in the liver, urine, or feces of rats dosed with ^{14}C-atrazine at daily doses equivalent to 5 ppm in the feed for 7 days and sodium nitrite at 175 ppm in the drinking water (Marco et. al., 1976, Geissbühler, 1975; Volume 2 of this submission).

The same findings were reported in the goat receiving 50 ppm of atrazine or hydroxy atrazine (the main plant metabolite of atrazine) simultaneously with 1700 ppm nitrite. No N-nitroso-atrazine was detected in liver, processed muscle, milk and excreta, even under the exaggerated conditions described (Sumner & Cassidy, 1975c; Volume 2 of this submission).

N-nitroso-atrazine is rapidly metabolized via the glutathione conjugate, which is ultimately degraded and excreted as the mercapturic acid derivative. The N-nitroso-hydroxyatrazine (nitroso derivative of the main plant metabolite of atrazine) is mainly metabolized by denitrosation by the liver microsomal enzymes.

4.2.3 Conclusion

These data demonstrate clearly that the formation of N-nitroso-atrazine occurs only under artificial/ non-biological conditions. The presence of food and the rapid metabolism of the N-nitroso-atrazine are the most important factors in preventing the presence of this derivative forming in living organisms.

CDPR: The potential for nitroso-atrazine formation has been described on p.16. The chronic toxicity is described on p.48 and the genotoxicity on p.53-54. It is further stated on p.96 that nitroso-atrazine is unlikely to be of toxicological concern under the present use conditions of atrazine. A

paragraph is being added to RCD p.96, describing the review by Sasaki et al., 2000.

4.3 Soil Mobility/Partition Coefficients

The soil-water partition values of atrazine and its primary metabolites were determined in studies sponsored by Novartis using the batch equilibrium technique (adsorption/desorption). Sorption coefficients, K_{oc}, and sorption constants, K_d, were determined in the laboratory for atrazine (G-30027), the three chloro-metabolites, and hydroxyatrazine (HA, G-34048) using four soil textures. Results are summarized in Table 1. Atrazine's three chloro-metabolites are deethylatrazine (DEA, G-30033), deisopropylatrazine (DIA, G-28279), and, diaminochloroatrazine (DAC, G-28273). Data for atrazine (Spare, 1989a; Volume 3 of this submission) demonstrated that atrazine has a medium to very high mobility classification (McCall, et al., 1980) based upon adsorption coefficients, K_{oc}. All three chloro-metabolites, DEA (Spare, 1989b; Volume 4 of this submission), DIA (Spare, 1989c; Volume 5 of this submission), and, DAC (Spare, 1989d; Volume 6 of this submission), demonstrated high to very high mobility. Hydroxyatrazine (Spare, 1989e; Volume 7 of this submission) demonstrated a medium to immobile classification.

CDPR: The Kd values for atrazine (from Spare, 1989) are given on p.13. Ranges of values are also given for the chlorinated metabolites as well as for hydroxy-atrazine. A statement has been added which summarizes the adsorption/desorption data. "In conclusion, the soil mobility of atrazine and its degradates can be summarized as follows: atrazine – medium to very high; chloro- metabolites – high to very high; hydroxy-atrazine – immobile to medium."

Table 1
Soil-Water Partition Values

	ATRAZINE		DEA		HA		DAC		DIA	
	G-30027		G-30033		G-34048		G-28273		G-28279	
SOIL TYPES	Kd	Koc	Kd	Koc	Kd	Koc	Kd	Koc	Kd	Koc
MD Clay OM = 4.8%; OC = 2.8% pH= 5.9	2.46	87.0	1.02	36.1	389.6	13797	1.56	55.0	2.73	97.0
MD Sand OM = 0.9; OC = 0.5% pH = 6.5	0.20	40.0	0.06	12.2	1.98	374	0.16	31.0	0.16	30.0
MD Sandy Loam OM = 1.9; OC = 1.1% pH = 7.5	0.79	70.0	0.36	31.8	6.52	583	0.65	58.0	0.51	45.0
CA Loam OM = 0.8%; OC = 0.5% pH = 6.7	0.73	155.0	0.21	44.9	12.11	2573	0.36	76.0	0.27	58.0

OM: Organic Matter
OC: Organic Carbon

Mobility: Ground and Surface Water Contamination

CDPR has not included a description of Novartis' ground and surface drinking water monitoring study for atrazine and simazine which has recently been updated (Tierney et. al., 2000) the summary of which is provided in Appendix 1. The summary Update VI for this study is included in Appendix 1 of this submission; the entire study is found in Volumes 8 through 17 of this submission. The results from the Novartis study indicate that atrazine was detected in only 3 of 10,510 finished water samples (99.97% non-detects) in California. Since 1993, analyses conducted in the State of California were performed using EPA Method 525.1. Limits of quantification ranged from 0.1 to 1.9 ppb for atrazine. The majority of the samples had LOQ's of 0.1 or 1 ppb.

Of the three samples in which atrazine was detected, 1 sample was from a ground water Community Water Supply (CWS), the second CWS provided surface water and the 3rd CWS provided blended water. The maximum detected concentration was 0.28 ppb. This evaluation accounted for 87.32% (> 27 million people) of the population of the state of California.

CDPR: DPR considered an earlier version of Tierney et al. (2000) but it failed to include atrazine's chlorometabolites when calculating potential atrazine residues in water. DPR therefore did not use this registrant study. Instead, DPR used California-specific well water data collected by the department which accounted for the parent atrazine and its toxicologically significant chlorometabolites (DPR, 1994). Refer to the DPR draft Atrazine RCD Risk Appraisal Section (V.), (C.) for additional information.

5.0 Toxicity Profile

5.1 Margin of Exposure Approach

Novartis agrees with CDPR's conclusion that a NOEL/MOE may be the most appropriate method for estimating risk associated with atrazine exposure and questions the rationale underlying CDPR's decision to present risk estimates based on Q_1.*

5.2 Cardiotoxicity

Novartis believes that the data and expert opinion (Detweiler, 1989, Appendix 2) support the view that the NOEL for atrazine in the chronic dog study on atrazine is 5 mg/kg/day (150 ppm) instead of 0.5 mg/kg/day (15 ppm) as stated by CDPR. In his report, Detweiler, who is a certified veterinary cardiologist, concluded that "the test agent produced electrocardiographic evidence in the highest dose group." EPA concurred with Dr. Dietweiler's conclusion (See Appendix 3).

Detweiler goes on to state that … the occasional occurrence of statistically significant PII wave amplitude decrease in Groups II (sic 15 ppm) and Group III (sic 150 ppm) … should not be considered evidence of cardiotoxicity for several reasons:

The P waves vary spontaneously with foreleg position change and as the result of respiratory sinus arryhthmia.

The P waves in these animals remain distinct.

The findings were inconsistent among individuals.

The amplitudes of P waves in groups of dogs are not Gaussian distributed but usually skewed excessively to the left and comparison of such data from different groups is always suspect.

Body and foreleg position are additional factors causing apparent (spurious) statistically significant amplitude changes in serial records. Most important is rigorous control of foreleg position, which was not done in this study.

Visual measurement of a 1.0 mm change in amplitude is measuring a very small difference to consider significant since many people cannot resolve more than about 0.25 mm, leaving a large margin for error. Electronic measurement is more reliable and when done electronically, demonstrates that P waves often vary by more than 1.0 mm (0.1 mV) spontaneously.

This conclusion is also supported by the NOEL established for cardiotoxicity in the dog for the dealkylated metabolites of atrazine (See CDPR Text Table 27).
CDPR: There is clearly cardiotoxicity at the high dose (1000 ppm) and no effect at the low dose (15 ppm), as described on pp.43-46. The effect at the mid-dose (150 ppm) is equivocal: one male died early in the study and two of the survivors showed little or no effect. However, the other male at 150 ppm (male, #12) showed some of the same effects as those observed at 1000 ppm, although these were not as severe. Therefore, it was considered inappropriate to ignore effects that occurred in one of the three surviving dogs and thus, 150 ppm was the LOEL rather than the NOEL. As pointed out on pp.95-96, it is probable that the "true" NOEL for cardiotoxicity in the dog is closer to 150 ppm than to 15 ppm. However, partly because of the dose selection, it is difficult to ascribe a value to it other than 15 ppm.

It is agreed that the effects on PII were slight at 150 ppm. However, dog #12 also exhibited moderate atrial dilation, an effect that was otherwise observed only at the high-dose (Table 16). Increases in heart weight were also noted, in both sexes, at 150 ppm (Table 17). The increases were significant ($p<0.05$) for females. Additional data have been included in Table 17, showing mean relative heart to body weight changes with dose. At 150 ppm, this parameter increased by 11% (M) and 14% (F). For dog #12, there was a 17% increase in absolute heart weight and a 14% increase in relative (heart:body) weight.

In an earlier study (Woodard, 1964), which was submitted to DPR only in summary form (RCD pp. 43-44), increases in heart and liver weights were

noted by USEPA at 150 ppm, in females. Decreased food consumption was also noted at this dose.

Table 27 was included to show that cardiotoxicity was also apparent for the dealkylated metabolites of atrazine. The type of effect was qualitatively similar and it is agreed that the NOEL values were higher than for parent. However, for various reasons, it is not considered appropriate to use one of these NOEL values for chronic risk assessment purposes for atrazine. It should be noted that the study authors did not comment on, or appear to even notice, cardiotoxicity of the dealkylated metabolites.

It is also noted in Section III.J. in a (human) epidemiological study of birth defects in southern Iowa, Munger et al., 1992 found that cardiac defects were the most common type of birth defects reported. An association was found with the drinking water source, which was contaminated with atrazine, giving RR values in the range of 4.4 to 5.7. Although the study had several possible biases and confounding factors, it remains possible that the cardiotoxicity of atrazine played a causal role in these birth defects.

<u>Summary</u>: Taken together, the possible effects on the PII wave, the histological effects on the heart, the increases in heart weight, both in the 1987 and 1964 studies, the cardiotoxicity of metabolites and the human birth defect epidemiology study lead to a choice of LOEL/NOEL values of 150 ppm and 15 ppm, respectively.

5.3 Immunotoxicity

Novartis believes that marginal statistical differences in hematological parameters in short term rodent and dog studies do not constitute evidence that atrazine affects the immune system. A more plausible scientific explanation is that at high doses, delayed development of the immune system is associated with generalized toxicity. This is supported by the absence of histopathologic or functional evidence of immune system effects in chronic studies.

CDPR: As noted on p.69 (section III.I), clear-cut effects on the rodent immune system were only observed at relatively high doses in short-term experiments, with LOEL values ~100 mg/kg and a NOEL of 27 mg/kg. However, EMH was noted in the spleen at much lower doses in subchronic and chronic studies in the rat, in a dose-related manner. Because of the critical immunological role of the spleen, it was considered that a possible effect on the immune system could not be excluded. However, it was also conceded that the EMH effect could also have been secondary to the increased incidence of mammary tumors or have been a result of (natural) aging (p.95).

5.4 Human Data

CDPR's human exposure assessment indicates that a low incidence of eye irritation and contact dermatitis have been with associated atrazine exposure. CDPR's review of the epidemiological literature is incomplete and CDPR is referred to EPA's SAP briefing document and the publications of Loosli, 1995 (Appendix 4), Neuberger, 1996 (Appendix 5), and Sathiakumar et. al., 1997 (Appendix 6) for a more comprehensive treatment.

CDPR: Because of the nature of epidemiological studies, along with the difficulties usually inherent in their interpretation, they are generally of interest only when they are positive. Nonetheless, the following has being added to the RCD at the end of p.8:

"Similar conclusions were reached in a follow-up study of workers at the same plants, 1975-1993 (Ciba-Geigy, 1997a,b). Again, a slight increase in deaths from NHL was observed which was considered by the report's authors to be a chance event since NHL deaths did not correlate with degree or length of exposure. However, it should be noted that in the Hoar et al., 1986 study (above), a significant association was noted between triazine exposure and NHL but was discounted because the a priori hypothesis focussed on 2,4-D as the causative agent.

In a review of 11 epidemiological studies of triazine herbicides and cancer (Sathiakumar & Delzell, 1997), ten case-control and one follow-up study were analyzed, including four of the above studies. The authors noted that the case-control studies were subject to bias (inaccurate recall) and that all studies were subject to confounding factors such as: exposure to other agricultural chemicals, poor dose-response patterns and induction time inadequacies. Limited numbers of cases added further to the difficulties of drawing conclusions from the studies, with the possible exception of non-Hodgkins lymphoma. However, it was concluded that none of the studies, in themselves, was persuasive of the presence or absence of a causal association between triazine herbicides and cancer."

6.0 Risk Assessment

6.1 Occupational Exposure & Risk

The review of worker exposure to atrazine by Sanborn is thoughtful but the calculated unacceptable acute short-term risk (MOE = 55 at the 95th percentile) is extremely conservative because 1) only 6 to 16 workers were evaluated, 2) the MOEs were 491, 391 and 180, for the applicator, mixer/loader/tender, and mixer/loader/tender/applicator, respectively based on biomonitoring data and 3) even when passive dosimetry data is used, the MOE at the 95th percentile for the mixer/loader/tender/applicator (6 individuals who actual reported doing all these functions) was 582 in contrast to an MOE of 55 calculated by combining the data from workers who either applied atrazine or mixed/loaded or were truck tenders.

Novartis also wishes to point that using upper percentile estimates of risks in probablistic assessments only becomes meaningful if distributional data is used for all major parameters that may effect exposure (e.g. amount handled, absorption, protective clothing) or hazard (distribution of NOELS) are used. It is noted that in California, the average amount of atrazine handled/day was reported by CDPR to be 150 pounds ai/day whereas the amount handled by workers in the occupational exposure study conducted in the mid-western states ranged from less than 100 up to 2500 pounds ai/day.

CDPR: The calculation of acute exposure at the 95th. percentile is standard practice at CDPR. As such, it provides an estimate of possible worst-case short-term exposure, which may be important for pesticides where acute toxicity is a major concern. As pointed out on p.95 (Risk Appraisal), the acute NOEL used in this calculation was 5 mg/kg/day, taken from a rabbit developmental toxicity study where the LOEL was 75 mg/kg/day i.e. 15x the NOEL. It is possible therefore, that the "true" NOEL in the rabbit is greater than 5 mg/k/day.

The calculation of the 95th.UB of exposure (ADD) used the upper end of the exposure range, at the highest percentage of dermal absorption, using the most sensitive measurement method (passive dosimetry vs. urinary monitoring). Thus, the range of exposures (1.8 to 6.1 ug/kg/day) was appreciably lower (15 to 50x) than the 95th.UB of 91 ug/kg/day.

Both of the choices of NOEL and exposure will tend to make the high-end MOE extremely health-protective. However, because atrazine is not considered to present acute toxicity concerns, it is unlikely that CDPR would regulate exposure based on the 95th. percentile of acute exposure and the corresponding MOE (55).

6.2 Dietary Exposure & Risk

The residue information for corn and sorghum in CDPR's exposure assessment was generated from older field trials conducted at higher rates than the maximum rates stipulated by the current label. The current maximum label rate is 2.0 lb a.i./A post-emergence or a maximum of 2.5 lb a.i./A (pre- + post-application). Recent residue information for corn and sorghum has been developed from side-by-side field trials conducted at the current maximum rates. These residue studies have been submitted to CDPR and are identified as the following DPR Document No.: Corn residue studies – 220-472, 220-500, and 220-522; Sorghum residue studies – 220-473, 220-501 and 220-523. Residues were generated from trials conducted by pre-emergence application of 2.0 lb a.i./A, post-emergence application (0.5 lb a.i./A pre-application followed by a 2.0 lb a.i./A post-application), as well as a split, more typical application. The split application for corn was conducted by a 1.5 lb a.i./A pre-treatment followed by a 1.0 lb a.i./A post-application. For sorghum, atrazine was applied using 1.3 lb a.i./A pre-emergence followed by a 1.2 lb a.i./A post-emergence treatment. The CDPR's dietary assessment utilized field trial values for

field and sweet corn generated by post-emergence application of 3.0 lb a.i./A (1.5X the current maximum post-emergence rate), resulting in an over estimation of exposure to corn. (The 1997 Novartis dietary assessment utilized field trial values from pre- and post-emergence treated corn and sorghum at the current label rate.)
CDPR: DPR considered data volumes 220-472, 220-500 (corn) and 220-473, 220-501 (sorghum). These data were not used because total atrazine residues were reported only from corn/sorghum forage. Instead, DPR used total atrazine residues derived from either composited corn ears or grain or sorghum grain composites. Volumes 220-522 (corn) and 220-523 (sorghum) were not available prior to the last draft dietary exposure assessment. These registrant field trial data do reflect the use of the new, lower atrazine application rates for both corn and sorghum. However, the use of these (registrant) data would not reduce the calculated dietary exposure because the analytical methods are less sensitive. The LODs for both grain and ears in the newest studies are higher (0.05 ppm vs. 0.01 ppm) than the values used in the DPR draft dietary exposure assessment. The combined total atrazine LOD cited in the DPR document is 0.04 ppm (0.01 ppm; parent, 0.01 ppm; each of the three chlorometabolites) versus 0.2 ppm combined LOD in the newest registrant submissions. A switch from a 0.04 ppm combined atrazine LOD to a 0.2 ppm combined LOD method reported in the most recent studies would result in a 5-fold increase in apparent residues for no reason. Therefore, DPR will continue to use the residues obtained from the corn and sorghum studies referenced in the DPR draft dietary exposure assessment.

CDPR's risk characterization document discusses two open literature studies in which atrazine was detected in commercial milk. A publication in the Journal of Food Protection was cited (Pylypiw and Hankin, February 1991) which described the detection of atrazine in raw pooled milk by immunoassay. In June of 1991, a letter to the editor of the same journal stated that the cited study had serious "scientific shortcomings" with regard to unrealistic sensitivity limits and missing data.
CDPR: The immunoassay articles (Pylypiw & Hankin, 1991 and Bushway et al., 1992) were merely cited to support the registrant theoretical atrazine milk residue value (0.005 ppm) used by DPR. Thus, atrazine residues have been detected of this order from commercial raw milk samples using an immunoassay detection method (Bushway et al., 1992, Pylypiw and Hankin, 1991). The referenced letter to the journal editor was from Bushway criticizing Pylypiw & Hankin's reported sensitivity limits and missing information. The Pylypiw & Hankin response included the missing data and defense of their atrazine sensitivity limit. There was no indictment of the accuracy of the atrazine immunoassay method.

CDPR uses a calculated milk residue of 0.005 ppm in Novartis' 1997 dietary evaluation for atrazine. This value arose from a theoretical dietary burden (dairy cattle diet) and transfer information (feeding studies) for the total triazines minus hydroxy triazines residue subset. The total triazines minus hydroxy triazines are comprised of only a minor amount of chloro triazines (<10%) with the majority being

conjugates derived from glutathione. These conjugates are formed by elimination of the chlorine from parent atrazine and/or corresponding products of N-dealkylation and are by nature more water soluble and more easily eliminated than parent and corresponding chloro triazines. The conjugates are not expected to have the same toxicological profile as the chloro triazines. In the Novartis 1997 dietary assessment, the calculated milk residue for atrazine and chloro triazine residue subset was 0.0004 ppm (0.4 ppb) and is a magnitude lower than the milk value calculated for the total triazine minus hydroxy triazine residue subset (0.005 ppm).

CDPR: The DPR milk residue value was taken from the Novartis 1997 atrazine dietary evaluation, which was derived from the theoretical dairy cow dietary burden expressed as a TRR residue. This method was consistent with other TRR-derived values used to represent meat and egg dietary residues in the DPR draft dietary exposure assessment. It is recognized that this may be a conservative milk residue estimate.

6.3 Exposure via Drinking Water

CDPR utilized an extremely conservative estimate of the total chlorotriazine concentration in drinking water of 2.73 ppb in their acute analysis and 0.631 ppb as their chronic estimate. In contrast, Novartis has determined from data on potable water in the State of California that 99.97% of the samples reported by CWS's in the State were non-detects.

CDPR: For the reasons given above (4.3), DPR did not use the registrant study (Tierney et al., 2000). This study did not include atrazine's chloro-metabolites when calculating potential total atrazine residues in water. Refer to the DPR draft Atrazine RCD Risk Appraisal Section (V.), (C.) for additional information.

7.0 Aggregate and Cumulative Risk Assessment Under FQPA

CDPR has not considered Novartis' comprehensive probabilistic aggregate (atrazine multiple pathways) and cumulative (atrazine plus simazine) risk assessment. Novartis encourages CDPR to review this report (Sielken et.al, 1996).

CDPR: The Sielken, Jr. et al. (1998) article contains some interesting information. DPR considered the RCD risk estimate of dietary exposure to be more appropriate, for the following reasons: 1./ Unlike Sielkin et al., DPR included chlorometabolites of possible toxicological importance. 2./ As stated on p. 451 of Sielken, Jr. et al., "Residue concentrations in human foods are all non-detects." whereas DPR, using registrant data, determined that there were indeed some detections in the database (Table 30, RCD), albeit well below tolerance levels. 3./ Sielken et al. developed a probabilistic risk assessment, whereas DPR used a (slightly) more conservative deterministic method. DPR considers that because the residue data were collected from registrant field trials, there is zero probability that the crop was <u>not</u> treated, making the percent crop-treated adjustment factor for <u>acute</u> dietary exposure inappropriate. In general, DPR considers that the percent crop-treated adjustment is appropriate only for <u>chronic</u> exposure.

8.0 Conclusions

Novartis has reviewed CDPR's Draft Risk Characterization Document for Atrazine and has determined that the exposure and risk calculated for atrazine is overly conservative. CDPR did not utilize all of the currently available data developed by Novartis to more accurately characterize potential exposure to atrazine via the diet, drinking water or as a result of occupational exposure or several key publication that have evaluated the epidemiological data on atrazine.

Novartis has provided CDPR with a comprehensive aggregate and cumulative risk assessment of atrazine and simazine which shows that acceptable margins of exposure exist for all exposure scenarios for both aggregate or as a result of cumulative exposure to these chlorotriazines.

Novartis has performed an extensive evaluation of the mode of action underlying atrazine effects in the female Sprague-Dawley rat (Breckenridge et. al, 2000, Appendix 7). An independent expert panel has determined that the effects of atrazine on the development of mammary tumors in the female Sprague-Dawley rat is not relevant to humans (Simpkins et. al., 2000, Appendix 8); EPA's Scientific Advisory Panel concurred with this conclusion (FIFRA SAP, 2000; Transcripts available, final report not issued).

CDPR: With respect to the mammary tumors, the following will be added on p. 2 (Summary):

"Indeed, using a weight-of-the-evidence approach, the massive amount of data generated over the past few years make an endocrine mechanism almost inescapable, although the precise mechanism(s) remains to be determined."

Also, the following will be added on p. 99 (Risk Appraisal):

"DPR does not believe that the exact mechanism has yet been resolved. However, the preponderance of evidence is that an endocrine mechanism exists. Such mechanism(s) could include aromatase induction, an effect on the hypothalamic/pituitary axis and/or an effect on an endocrine hormone receptor. Although there is limited evidence for each of these three mechanisms, critical experimental evidence is lacking."

Novartis agrees with CDPR that a margin of exposure (MOE) approach may be the best method to characterize risk associated with atrazine exposure. Novartis believes that the no observed effect level for cardiotoxicity in the atrazine chronic dog study has been established at a dose of 5.0 mg/kg/day instead of 0.5 mg/kg/day stated by CDPR. Novartis believes that atrazine does not have any direct effects on the immune system in either the rodent or the dog. Novartis recommends that CDPR considers adopting the NOEL from the 2-generation rat reproduction study of 3.5 mg/kg/day as a basis for establishing the chronic reference dose for atrazine as is currently done by EPA (Appendix 9).

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19 *Spare, William C., "Adsorption/Desorption of 14C-G-28273," Agrisearch Incorporated, Frederick, MD, Project Number 12173, July 6, 1989(d), MRID 41257904 (Volume 4 of this submission).*

20 *Spare, William C., "Adsorption/Desorption of 14C-G-28279," Agrisearch Incorporated, Frederick, MD, Project Number 12170, July 27. 1989(c), MRID 41257905 (Volume 5 of this submission).*

21 *Spare, William C., "Adsorption/Desorption of 14C-G-30033," Agrisearch Incorporated, Frederick, MD, Project Number 12169, August 23, 1989(b), MRID 41257906 (Volume 6 of this submission).*

22 *Spare, William C., "Adsorption/Desorption of 14C-G-34048," Agrisearch Incorporated, Frederick, MD, Project Number 12171, August 23, 1989(e), MRID 41257902 (Volume 7 of this submission).*

23 *Sumner, D., and Cassidy, J, 1975a, The formation of atrazine derivative in simulated gastric fluid - A preliminary study, Ciba-Geigy Corp., Greensboro, NC, Rep. N°: GAAC-75072R, July 31, 1975 (Volume 2 of this submission).*

24 *Sumner, D., and Cassidy, J., 1975b, The formation of atrazine derivative in rumen fluid - A preliminary study, Ciba-Geigy Corp., Greensboro, NC, USA, Rep. N°:GAAC-75071R, July 31, 1975 (Volume 2 of this submission).*

25 *Sumner, D., and Cassidy, J., 1975c, Metabolism and distribution of ^{14}C-atrazine derivatives in a lactating goat, Ciba-Geigy Corp., Greensboro, NC, USA, Rep. N°;GAAC-75086R, 31.10.1975 (Volume 2 of this submission).*

26 *Tierney, D.P., Christensen, B.R., Dando, C.L., and Marut, K.M., 2000. Human exposure to atrazine and simazine Via Ground and surface drinking water: Update VI. En-Fate, Plymouth, Minnesota. En-Fate No. 00800. October 6, 2000. (Appendix 1; Volumes 8 through 17 of this submission).*

27 *Tinsworth, E.F., 1989. Data Call-in Notice dated August 4, 1989. Special Review and Registration Division. USEPA. Washington, DC (Appendix 3).*

28 *USEPA IRIS Substance File for Atrazine:http://www.epa.gov/iris/subst/0209.html Accessed on 11/28/2000 (Appendix 9).*

10.0 Appendices

M E M O R A N D U M

TO: *Anna M. Fan, Ph.D., Chief*
Pesticide and Environmental Toxicology Section
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FROM: *Gary Patterson, Ph.D., Chief*
Medical Toxicology Branch
Department of Pesticide Regulation
P.O. Box 4015
Sacramento, California 95812-4015

DATE: *August 9, 2001*

SUBJECT: *COMMENTS ON THE DEPARTMENT OF PESTICIDE REGULATION´S DRAFT RISK CHARACTERIZATION DOCUMENT FOR ATRAZINE*

Response to OEHHA comments of May 11, 2001 on Final Draft RCD for atrazine

Thank you for your comments on the draft final RCD for atrazine. Each of the points has been addressed but, because there is so much overlap, many of the DPR responses answer several OEHHA questions.

In the seven months that elapsed between submitting the RCD to OEHHA and your response, there has been considerable regulatory activity regarding atrazine. USEPA issued a revised risk assessment, dated 1/19/01, on 2/8/01. This report incorporated the review comments of the SAP, following on from the meeting in Washington in June, 2000 (attended by representatives of DPR and OEHHA). This was anticipated because, according to the draft risk assessment (reviewed in June, 2000), USEPA accepted the Novartis argument that an endocrine mechanism was responsible for the mammary tumors in the atrazine-treated female SD rat. Furthermore, USEPA concluded that this mechanism was not relevant in humans. Nonetheless, according to the USEPA draft risk assessment reviewed by the SAP, this made atrazine a "likely" human carcinogen. The original Class C carcinogen rating, which was arrived at in 1986 without any of the mode of action work, describes atrazine as a "possible" human carcinogen. It thus appeared in June 2000 that all of the work to establish a non-genotoxic mechanism for the mammary tumors had resulted in atrazine being rated as more likely than before to be a human carcinogen.

In the following response, DPR comments have been added to the original (OEHHA) memorandum in ***(bold, italics).*** *Changes or additions to the draft, final*

RCD have been made, to address the OEHHA comments, on the following pages (in the sequence of comments): 2, 8, 81, 99, 28, 34, 19, 22, 54, 59, 81, 99. In the final RCD, additional changes have been made on pages 1, 2, 8, 11, 13, 73, 78-80 and 96-97. These changes address issues raised by Syngenta as well as issues emerging from USEPA's atrazine risk assessment activities (above).

cc: Tobi Jones, Ph.D.
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Department of Pesticide Regulation.

Keith Pfeifer, Senior Toxicologist,
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Department of Pesticide Regulation.

Charles M. Andrews, Chief
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M E M O R A N D U M

TO: *Gary Patterson, Ph.D., Chief*
Medical Toxicology Branch
Department of Pesticide Regulation
P.O. Box 4015
Sacramento, California 95812-4015

FROM: *Anna M. Fan, Ph.D., Chief*
Pesticide and Environmental Toxicology Section, OEHHA

DATE: *May 11, 2001*

SUBJECT: *COMMENTS ON THE DEPARTMENT OF PESTICIDE REGULATION'S DRAFT RISK CHARACTERIZATION DOCUMENT FOR ATRAZINE*

We have completed our review of the draft risk characterization document (RCD) for 6-chloro-N-ethyl-N′-(1-methylethyl)-1,3,5-triazine-2, 4-diamine (atrazine) prepared by the Department of Pesticide Regulation (DPR). Atrazine is a pre- or post-emergence herbicide used in the United States for weed control in corn, sorghum, milo, wheat, Sudangrass, macadamia orchards, forest trees, pineapples, sugar cane, and other crops. In California, atrazine is used to control broadleaf weeds in forest trees, corn, Sudangrass, Bermuda grass, sorghum, and in landscape maintenance. There are currently ten different formulations registered in California with atrazine as an active ingredient. Atrazine may be formulated with other active ingredients including alachlor, propachlor, metolachlor, sodium chlorate, sodium metaborate or prometon for a variety of uses.
DPR: The tolerance for pineapples was cancelled in 1998 (see Table 35 of RCD).
The package received by the Office of Environmental Health Hazard Assessment (OEHHA) consists of the draft RCD dated October 13, 2000, and the following supporting documents:

Appendix I. Table A1. Historical Control Mammary Tumor Data for Female SD Rats in 2-Year Studies at EPL Inc.
Appendix II. Global 86: file for mstage calculation
Appendix III. Table B1 The MLE and 95% Upper CL for Mammary Gland Tumors in Female Sprague-Dawley Rats Dosed with Atrazine for 2 years
Appendix IV. Morseth, 1998: Weibull File
Summary of Toxicology Data for Atrazine by Medical Toxicology Branch
DPR; Dietary Exposure and Acute Tolerance Assessments by Wesley C. Carr, Jr. Medical Toxicology Branch, DPR

1. *Human Exposure Assessment for Atrazine by James R. Sanborn, Worker Health and Safety Branch, DPR*

To assist us in our review, we consulted our public health goal (PHG) documents for atrazine (OEHHA, 1999) and simazine (OEHHA, draft version of October, 1999). We have also conducted a brief review of the pertinent summary data provided by the United States Environmental Protection Agency Integrated Risk Information System and the Hazardous Substance Database on atrazine available on the Internet.

In general, we do not support the approaches and procedures used in the draft RCD for atrazine. We recommend that the draft RCD be revised for the reasons listed below and presented in detail in our attached report.

1. *We recommend that DPR´s RCD for atrazine contain a brief discussion and comparison of risk assessment approaches taken by various agencies and organizations including OEHHA´s PHG document on atrazine issued in February 1999. The latter was peer reviewed by DPR´s Medical Toxicology Branch. The atrazine PHG and the draft RCD differ in their approaches to risk assessment for atrazine. The reason(s) for this difference and supporting data should be clearly stated in the RCD.*

DPR Response:
Much is made in the attached comments of DPR´s failure to acknowledge OEHHA´s public health goal (PHG) documents for atrazine (OEHHA, 1999) and simazine (OEHHA, draft version of October, 1999). Reviews of these reports were written by DPR in October, 1998 and 1999, respectively. It is unclear whether DPR´s comments were addressed by OEHHA. Be that as it may, at the time that the atrazine PHG report was reviewed, nearly 3 years ago, the demonstration of an endocrine (threshold) mechanism for the mammary tumors (by Novartis) was incomplete. DPR was therefore prepared to overlook obvious inconsistencies in the PHG, such as, on p.25, in the summary of hormonal effects "Overall the in vivo and in vitro study data suggest that atrazine disrupts the ovarian cycling and induces mammary tumors." and, on p.32, in discussing the possible mode of action for these tumors: "Since the mechanism of action of atrazine is unknown, OEHHA has adopted the default option of linearity for this chemical and has used cancer slope factor (CSF)...." !? Please instruct on precisely how OEHHA would like DPR to reference the mechanism of action for atrazine in the PHG.

Since the completion of the OEHHA PHG report, almost 3 years ago, there have been innumerable publications/reports in the field. During this period, DPR has become convinced that, although the precise mechanism may be unclear, it is impossible to ignore the fact that an endocrine mechanism (rather than a genotoxic one) is almost certainly responsible for the mammary tumors in the female SD rat. Perhaps this has not been made transparently clear in the text and

efforts are underway to correct this. The following will be added at the bottom of p.2 (Summary): "Indeed, using a weight-of-the-evidence approach, the massive amount of data generated over the past few years make an endocrine mechanism almost inescapable, although the precise mechanism(s) remains to be determined."
See also the paragraph on p.4 of the attachment, to be added.

2. *OEHHA recommends that both oncogenic and non-oncogenic risks be evaluated. We also recommend that the RCD utilize the cancer risks already calculated from the cancer potency to evaluate oncogenic risks from exposure to atrazine, rather than relying on the margin of exposure approach for the evaluation of all chronic exposure risks including oncogenic risks.*

DPR: We recommend reading RCD pp. 79-83 and RCD pp. 91-94.

3. *OEHHA recommends that a summary and critical evaluation of the mechanistic data related to the proposed endocrine mechanism of carcinogenicity be added to the risk assessment section of the RCD.*

DPR: See answer to point #1, above and to comment on page 4 (attachment)

4. *The draft RCD for atrazine does not include a seasonal exposure assessment. No clear reason for this is provided. We believe that there are short periods of intensive agricultural use of the chemical during the year. Therefore, we recommend that health risks from such short-term exposures be assessed.*

DPR: the decision not to consider seasonal exposure was made by WH&S Branch; seasonal dietary exposure was also therefore not included in the RCD.

5. *We recommend that the potential for atrazine secretion into milk be addressed in the section on "Pharmacokinetics." We also recommend including a discussion on the role of atrazine metabolites in the overall toxicological response arising from exposure to the parent compound.*

DPR: Worst-case values (5 ppb, p.88), based on field studies, were used for determining MOE values (acute and chronic) from consuming contaminated milk, see Appendix VI, pp.6-7. The 5 ppb value includes contributions from atrazine plus chloro-metabolites, but excluding hydroxyatrazine because this appears to be toxicologically different.

6. *The tolerance assessment presented in the draft RCD does not take into account endocrine effects of atrazine. Neither does it consider cumulative exposures to other triazine compounds. Under the Food Quality Protection Act, both of these considerations call for applying additional uncertainty factors in assessing the degree of protection afforded by the current tolerances for children. OEHHA recommends addressing these issues in the RCD.*

DPR: The most sensitive endpoint in chronic studies was cardiotoxicity in the dog, 1-yr. study, with a NOEL of 0.5 mg/kg/day. As pointed out on p.102, there is no evidence supporting an endocrine mechanism for this effect. Endocrine effects occurred (in the SD rat) only at higher dosages, with a chronic NOEL of 1.8 mg/kg/day for attenuation of the LH surge (pp.72-78, 99), as used by USEPA for chronic risk assessment. It is unclear whether this effect has relevance in humans. It was concluded that the use of the lower NOEL by DPR (0.5 mg/kg/day) provides additional protection for such possible (yet unknown) endocrine effects.

7. *We recommend identifying groups particularly susceptible to atrazine effects. Data presented in the draft RCD indicate that these would include infants and children, especially those under six years old.*

DPR: These population sub-groups are <u>not</u> more susceptible to atrazine effects, but instead have greater (theoretical) dietary exposure than other sub-groups. However, crop residues are not found at harvest. In any event, dietary exposure is described in Table 31 (p.90) and Table 34 (p.93) for the US population as a whole, as well as for 20 sub-groups.

8. *Available guidance documents and health advisories such as PHG, Reference Exposure Levels and Threshold Limit Values should be addressed in the document.*

DPR: DPR does not consider this to be the appropriate place to address inadequacies in the PHG report for atrazine (see answer to point #1, above.)

9. *The document presents inconsistent views regarding the relevance of the rat mammary tumors. The document provides some evidence and justification for genotoxicity and a linearized approach to risk assessments. The document also provides evidence for a endocrine-based mechanism that may be irrelevant to humans. Clearly, the actual mechanism involved is uncertain. Yet, the document concludes by taking the endocrine-based approach without sufficient justification. Our concern is that breast cancer is the leading cause of death among American*

women regardless of race and ethnicity. In 2001, one can expect about 21,000 new breast cancer in California, of which about 4,205 will be fatal (ACS 2000). The United States age adjusted mortality rates per 100,000 person-years for breast cancer ranged from 27 among white females to 29 among black females. Neither the main cause nor the mechanism of breast cancer in humans is known. Therefore, OEHHA recommends that if the less health protective approach is taken in assessing risks from rat mammary tumor that it be fully justified by the available data.

DPR: Yes, the precise mechanism is uncertain, but see answer to point #1, above. It should be noted that, during the USEPA meeting with the Scientific Advisory Panel in June, 2000, a SAP panel member stated that LH surge suppression (as described in the female SD rat) in humans would lead to reduced rather than elevated estrogen hormone levels and would therefore <u>not</u> result in an elevated risk of breast cancer (in humans).

Thank you for providing the document for our review. We are available to meet in Sacramento on Monday, May 21, 2001, in the afternoon if you would like to discuss our comments. If you wish to meet or have any questions about our comments, please contact me or Dr. Michael DiBartolomeis at (510) 622-3170.
Attachment

cc: Joan E. Denton, Ph.D.
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ATTACHMENT

COMMENTS ON THE DRAFT RISK CHARACTERIZATION DOCUMENT FOR ATRAZINE

As part of our review of the draft risk characterization document (RCD) for atrazine, we conducted an independent, brief review of the summary data on atrazine available from Internet provided by the United States Environmental Protection Agency (U.S. EPA) Integrated Risk Information System and the Hazardous Substances Database. We also consulted our public health goal (PHG) documents for atrazine (OEHHA, 1999) and simazine (OEHHA, draft version as of November 2000).

The package received by the Office of Environmental Health Hazard Assessment (OEHHA) consisted of the draft RCD dated October 13, 2000, and the following supporting documents:

1. *Appendix I. Table A1 Historical Control Mammary Tumor Data for Female SD Rats in 2-Year Studies at EPL Inc.*
2. *Appendix II. Global 86: file for mstage calculation*
3. *Appendix III. Table B1 The MLE and 95% Upper CL for Mammary Gland Tumors in Female Sprague-Dawley Rats Dosed with Atrazine for 2 years*
4. *Appendix IV. Morseth, 1998: Weibull File*
5. *Summary of Toxicology Data for Atrazine by Medical Toxicology Branch*
6. *DPR; Dietary Exposure and Acute Tolerance Assessments by Wesley C. Carr, Jr. Medical Toxicology Branch, DPR*
7. *Human Exposure Assessment for Atrazine by James R. Sanborn, Worker Health and Safety Branch, DPR*

Our comments are listed below according to topics and are not necessarily presented in the order that they appear in the draft RCD

GENERAL COMMENTS

Other Health Risk Assessments for Atrazine

1. *Atrazine is currently under Special Review by U.S. EPA. This review was initiated for atrazine, simazine and cyanazine in 1994 to address the potential carcinogenic*

risks from exposure to triazine compounds. To this end, U.S. EPA has recently (May 22, 2000) reviewed all available data in a draft document entitled, "Hazard and Dose Response Assessment and Characterization: Atrazine." This document has a very coherent and well-written summary and discussion of the proposed neuroendocrine mode of action. We recommend including a brief discussion of this U.S. EPA document in the draft RCD.

DPR: However, in #3, below, OEHHA disagrees with this determination. On February 8, 2001, USEPA issued a report entitled "Revised Preliminary Human Health Risk Assessment Atrazine" dated January 19, 2001. This document results from modifications to the preliminary risk assessment of May, 2000, after the meeting with the FIFRA SAP in June, 2000. Because it was written after the draft RCD was completed and sent out for review (October 13, 2000), it has not been referred to in the RCD. However, a summary paragraph will be added to the RCD in section II.B. Regulatory History.

2. *OEHHA developed the atrazine PHG in 1999, which was reviewed by DPR. There is no mention of this document in the draft RCD. A summary of the atrazine PHG document and its major findings should be included in the RCD. It is apparent that there are inconsistencies both within the RCD itself and between the RCD and PHG (see section on carcinogenicity) with regard to the interpretation of the available data on the mechanism of carcinogenicity and approach to risk assessment. Therefore, it is important that a summary and critical evaluation of the mechanistic data related to the differences be added to the risk assessment section of the RCD.* ***DPR: See page 1, above.***
3. *Breast cancer is the leading cause of death among American women regardless of race and ethnicity. In 2001, one can expect about 21,000 new breast cancer in California, of which about 4,205 will be fatal (DHS 2001). The United States age adjusted mortality rates per 100,000 person-years for breast cancer ranged from 27 among white females to 29 among black females. Neither the main cause nor the mechanism of breast cancer is known. Ovarian hormones, modulated by atrazine exposure, may play a major role in the growth and differentiation of normal breast tissues and the development and progression of breast cancer. It is significant that epidemiologic studies suggest an association between atrazine exposure and hormone responsive cancers such as breast, ovary, and prostate cancer. There is also suggestive evidence for an association of Non-Hodgkin's lymphoma (NHL) with atrazine exposure. A recent study suggests a possible association between NHL and breast cancer in women (Wiernik et al., 2000). Besides the direct action of hormone on hormone responsive organs, genetic alterations induced by estrogen may be involved in the induction of breast cancer. Therefore, in the absence of a possible mechanism and a lack of dose and time correlations for many of the*

essential elements for the proposed hormonal hypothesis, OEHHA recommends that the RCD also take a more health protective linear dose-response approach for the risk assessment of atrazine.

DPR: .Follow-up manufacturing plant epidemiological studies indicate that there is no association between triazine exposure and any particular cancer or any form of death. A section is being added to p.8 to describe some recent studies. See also p. 1.

3. ***Health Risks from Chronic Exposure to Atrazine***

Risk estimates from chronic exposures to atrazine were calculated in the draft RCD for non-carcinogenic effects and for potential carcinogenic outcomes. Non-carcinogenic effects were evaluated by margins of exposure (MOE) and carcinogenic effects were characterized as excess lifetime cancer risks. An MOE of at least 100 was considered in the draft RCD as adequate to protect people from the toxic effects of chronic exposures to atrazine, and an excess cancer risk of below 10^{-6} (1 in 1,000,000) was considered negligible for the potential carcinogenic effects resulting from chronic exposures. While both methods were used to calculate the chronic toxicity risk for atrazine exposure, the draft RCD used the "MOE" approach for estimating risk associated with atrazine exposure. Further, the MOE approach was supported in the draft RCD by the endocrine mechanism for atrazine-induced carcinogenicity, which would be expected to have a practical threshold.

The draft RCD did not provide sufficient data to support a threshold for atrazine-induced carcinogenicity and there are theoretical reasons that it may not be a threshold dose response: for example, extreme genetic heterogeneity and differences in physiological states associated with age, sex, reproductive activities, nutrition, and exposure to environmental/occupational stresses including other carcinogens. ***DPR: This may or may not be true but DPR fails to see the possible relevance of this statement.*** *The Department of Health Services (DHS, 1985) in their guidelines for chemical carcinogens concludes that:*

DPR: The 1985 Guidelines are somewhat "dated" with respect to current scientific understanding of thresholds, in general, and specific to atrazine and mammary tumors, in particular.

1. *"It is not appropriate to apply the concept of "thresholds" to carcinogenesis unless dose-response data are available that are inconsistent with a nonthreshold model."*

2. *"The effect of a carcinogen can generally be assumed to be additive to that of ongoing processes or other agent that give rise to "background" incidence of*

cancer. Exceptions to this general assumption are appropriate when the carcinogen under discussion can be shown to operate by a mechanism that is distinct from those leading to background incidence or to act synergistically with other carcinogenic exposures."

The role of ovarian hormone in the development and progression of mammary tumors is well documented, but the draft RCD does not provide data to support a nonthreshold dose response. Therefore, OEHHA recommends that a linear dose response approach also be used with the carcinogenicity data in assessing the chronic toxicity risk for atrazine.

The chronic no-observed-adverse-effect level (NOAEL) selected in the draft RCD was 0.5 mg/kg-day (rounded from 0.48) based on cardiotoxicity in dogs (O´Connor et al., 1987). However, the lowest NOAEL for chronic effects is 0.41 mg/kg-day for carcinogenicity in the study by Mayhew (1986). ***DPR: The OEHHA PHG for atrazine quotes a dosage of 0.5 mg/kg/day for this (low-dose) group in the Mayhew study??*** *The draft RCD does not provide a suitable explanation why a higher NOAEL is scientifically justified and protective of public health. In addition, if one were to assume a threshold and use an MOE approach, an additional 1 to 10-fold uncertainly factor should have been considered for the limited evidence of carcinogenicity and cardiotoxicity of atrazine in human. This is appropriate since U.S. EPA recently noted that atrazine is likely to cause cancer in humans (U.S. EPA 1999 Draft Documents on Atrazine: Carcinogenicity Hazard Assessment and Characterization). Accordingly, OEHHA recommends that either an additional uncertainty factor be included, or that the scientific basis for its exclusion should be discussed.*
DPR: In the revised USEPA risk assessment (1/19/01), on p.11, it is stated that ". . .the mode of action for the carcinogenic potential in the Sprague-Dawley rat is not likely to be operative in humans." Because of all the evidence indicating an endocrine mechanism (of limited relevance in humans) it is further stated (on p.12) that "Therefore, a cancer risk assessment was not conducted for atrazine."

2. ***Mechanism of Carcinogenicity***

On page 102, second paragraph of the draft RCD, it is stated "Recent studies have indicated that, instead of causing cancer by a genotoxic mechanism, atrazine may induce aromatase, the enzyme that converts androgen hormones to estrogens. Such a mechanism would be anticipated to exhibit a threshold, thus making the linearized, low-dose cancer risk calculations inappropriate. Instead, the MOE calculations (above) for long term toxicity would be used to include cancer." The implication of such a statement is that the linearized low-dose cancer risk calculations included in the draft

*RCD are informative, but they should not be used for risk management or regulatory decision making. The draft RCD seems to be recommending that these decisions should be based solely on MOEs. This statement is in apparent contradiction to the statement on page 81 in the draft RCD which reads AHowever, the "**exact" (**to be added**)** mechanism of the induction of mammary tumors in the female SD rats by atrazine, remains unclear. There is some evidence for genotoxicity resulting from atrazine and/or unidentified derivatives of atrazineYTherefore, although there is substantial evidence for an endocrine mechanism for oncogenicity, further scientific experiments are needed to "make the case conclusive." to be replaced with: **"identify the exact mechanism(s)."** Thus the default non-threshold, low-dose extrapolation was **"also"** used in the risk assessment to address potential oncogenicity of atrazine in humans." This is in keeping with the statement on page three in the DPR "Summary of Toxicology Data for atrazine" which states that AInformation provided thus far does not establish that a threshold phenomenon exits for atrazine effects on reproductive hormonal changes or possible consequences thereof, including tumor development."*

The draft RCD postulates an endocrine mechanism of carcinogenicity by atrazine. An aromatase mechanism of action is emphasized, based on a single study with cultured carcinoma cells. Briefly, this theory assumes that atrazine induces the enzyme aromatase, thereby stimulating the conversion of androgens to estrogens, leading to higher circulating levels of cancer-inducing estrogens. Disruption of the hypothalamus/pituitary control of estrogen secretion is also mentioned at various places in the draft RCD. Nowhere in the draft RCD, however, is the proposed mechanism for atrazine clearly laid out. ***DPR: "DPR does not believe that the exact mechanism has yet been resolved. However, the preponderance of evidence is that an endocrine mechanism exists. Such mechanism(s) could include aromatase induction, an effect on the hypothalamic/pituitary axis and/or an effect on an endocrine hormone receptor. Although there is limited evidence for each of these three mechanisms, critical experimental evidence is lacking." will be added to the Risk Appraisal, p.99.*** *Significant, new findings on atrazine have been published in the past years which bear on its carcinogenicity and reproductive toxicity. Some were reviewed in a recent U.S. EPA document "Hazard and Dose-Response Assessment and Characterization: Atrazine" May 2000, posted on its Web site as a preliminary draft. It contains a coherent and well-written summary and discussion of atrazine´s proposed neuroendocrine mode of action. Unless the RCD can synthesize these findings into an endocrine mechanism of carcinogenicity by atrazine which is supported by all of the data, the mechanism must still be considered unresolved, as concluded by OEHHA in its 1999 PHG for atrazine. Accordingly, OEHHA evaluated the cancer risk from atrazine exposure by assuming a linear dose response.*
DPR: But see the comments on p.1 of this memo.

The following are excerpts from the draft RCD:

(a) Page 2, "There is evidence that the rat mammary tumors arose through an endocrine mechanism, which would be expected to have a (practical) threshold, suggesting that a NOEL/MOE may be the most appropriate method for estimating risk associated with atrazine exposure."

(b) Page 81, "It has been hypothesized that triazine herbicides, including atrazine, cause mammary tumors through an estrogen (or endocrine) mechanism (Ciba-Geigy, 1992d). Such a receptor-based mechanism, in the absence of genotoxicity, might justify the use of a threshold model for assessing risks to humans. However, the mechanism for the induction of mammary tumors in the female SD rat by atrazine, remains unclear. There is some evidence for genotoxicity resulting from atrazine and/or unidentified derivative(s) of atrazine (Tables 19-21)."

(c) Page 81, "Although there is substantial evidence for an endocrine mechanism for oncogenicity, further scientific experiments are needed to make the case conclusive. Thus, the default non-threshold, low-dose extrapolation was used in this risk assessment to address potential oncogenicity of atrazine in humans."

(d) Page 96, "Oncogenicity was assessed using a linear multi-stage model which assumes a non-threshold mechanism. It is possible that mammary tumors resulting from atrazine exposure in the female rat arose from an estrogenic (receptor-mediated) effect (Stevens et al., 1994; Tennant et al., 1994), which might be expected to show a threshold."

(e) Page 102, "Recent studies have indicated that, instead of causing cancer by a genotoxic mechanism, atrazine may induce aromatase, the enzyme which converts androgen hormones to estrogens. Such a mechanism would be anticipated to exhibit a threshold, thus making the linearized, low-dose cancer risk calculations inappropriate. Instead, the MOE calculations (above) for long-term toxicity would be used to include cancer."

The following are excerpts from OEHHA´s PHG:

(a) Page 1, "The exact mechanism of mammary tumors formation is not known. Atrazine is positive in a number of mutagenicity studies."

(b) Page 19, "It is hypothesized that atrazine may be acting by being metabolized to a DNA hypomethylating agent."

DPR: This is pure conjecture. Without a literature reference, the identity of the metabolite <u>and</u> an explanation of why its effects are restricted to the mammary gland of the female SD rat, this should not have been included in the PHG. It is suggested from recent studies that 2-OH or 4-OH metabolites of E2 oe E1 (formed by CYP1A1 and CYP1B1, respectively), subsequently oxidized to their quinones, are able to react with DNA. Such DNA damage, in E-2-sensitive tissues such as mammary gland, can be reduced by O-methylation of catechol estrogens and/or estrones (by catechol O-methyl transferase). It is therefore plausible to suggest that hypomethylation of estrogen/estrone metabolites is more important in causing breast cancer than is DNA hypomethylation. However, because the roile of atrazine in this process is unclear, it is currently considered premature to incorporate this into a risk assessment of atrazine.

(c) Page 31, "The exact mechanism of tumor induction by atrazine is not known. Recently, the registrant has submitted a myriad of studies that suggest that hormones play a role in the induction of mammary tumors in Sprague-Dawley rats. It has been hypothesized that atrazine administration accelerates the age related endocrine changes in Sprague-Dawley rats leading to earlier onset or increased incidence of mammary tumors. Atrazine is positive in a number of mutagenicity studies. It has been suggested that atrazine is metabolized at sites other than the liver to genotoxic compounds."

(d) Page 36, "Recent studies suggest that atrazine increases the ratio of estradiol metabolites 16-α-OHE to 2-α-OHE-1. 16-α-Hydroxyestrone can react directly with DNA, enhanced breast cell growth and increase oncogene and virus expression . . ."

(e) Page 36, "The role of ovarian hormone in the development and progression of mammary tumors is well documented (Bernstein and Press, 1998), but the actual mechanism of action is not known."

From the above, it is apparent that there are inconsistencies both within the draft RCD itself, and between the RCD and the PHG with regard to interpretation of the available data on the mechanism of carcinogenicity of atrazine. At the time the PHG was finalized, OEHHA concluded that the mechanism of action was unknown. In the light of new information published since the completion of the PHG, this conclusion still appears to be valid. ***DPR: The USEPA has reached a different conclusion, see Revised Risk Assessment of 1-19-2001. In addition, the studies showing that***

triazines induce aromatase were completed in 2000, although not used by USEPA in this risk assessment. *However, the draft RCD appears to reach a different conclusion, namely, that an "endocrine mechanism" is likely to be operative. Nowhere in the RCD is the proposed endocrine mechanism clearly laid out, however.* ***(DPR: see above)*** *Given the importance of this conclusion to the ensuing risk assessment approach taken in the RCD, it is critical that a summary and critical evaluation of the mechanistic data related to this proposed mechanism be added to the "Risk Assessment" section of the RCD.*

Furthermore, it is suggested on page 96 of the draft RCD that induction of aromatase by atrazine leading to increased estrogen biosynthesis is the mechanism of mammary tumor induction. Some of the studies submitted to DPR include measurements of estrogen levels in atrazine-treated animals. We suggest that the tumor incidence be plotted against estrogen levels to determine if the data support this mechanism. This type of analysis should be included in the RCD.
DPR: see Tables 8 and 11 and associated description of Thakur, 1991a,b and McConnell, 1995 in the RCD.

Interpretation of Data on Testicular Interstitial Tumors

With respect to the carcinogenicity data, the draft RCD notes:

(a) Page 2, "Oncogenic effects were observed in the form of mammary gland tumors in female SD rats and interstitial cell tumors in male rats. The latter effect was observed only at the HDT and was probably secondary to increased life-span."

(b) Page 30, "Neoplastic lesions which increased in treated groups included testicular interstitial cell tumors (adenomas), which were present in 1.5% of controls and 10.5% of males at 1000 ppm ($p<0.05$, Fisher's exact test). Because this is an age-related tumor (Capen, 1996), it may be a direct result of the significantly longer life-span of males at 1000 ppm, but not at lower doses."

(c) Page 81, "Benign testicular interstitial cell tumors were also increased in the highest dose group. The increase was statistically significant, but may have been secondary to the increased lifespan in that group

Interstitial cell tumors, also known as Leydig cell tumors (LCT) are the most common neoplasm of the testis of rats and mice, especially in aged animals. According to the data summarized on page 30 of the draft RCD, the incidences of LCTs were 1.5 percent and 10.5 percent in control and in atrazine-treated rats (highest dose tested

(HDT) group), respectively. The incidence (10.5 percent) of LCT seen in the treated rats is about two-fold higher than the high-end of the range (1 to 5 percent) in reported historical controls of the same strain. Therefore, aging alone is unlikely to account for the significantly increased incidence. ."
DPR: DPR does not see how this statement can be made, especially when followed by . . . . .

As summarized in the draft RCD (on page 28), "Survival in males was significantly increased; for example, after two years at 1000 ppm, only 26% of rats had died vs. 44% of controls (p<0.01)." A significant difference in the survival rate is not always associated with a significant change in the mean life span. For example, as OEHHA has noted on page 17 of the atrazine PHG document, the survival rate in high-dose female Sprague-Dawley (SD) rats was significantly lower than that of the control, but there was no significant difference in the mean life span (days) between these two groups. ***DPR: But, as noted in the RCD on p.28, "Dosed females had reduced lifespan; for example, after two years at 500 and 1000 ppm, 63% (p<0.01) and 57% (p<0.01) had died vs. 38% of control rats. This was apparently related to mammary tumor formation as the average lifespan in non-tumor-bearing animals was longer in 1000 ppm females than in controls." In order to clarify, "lifespan" will be replaced with "survival" in the RCD.*** *Without data on the mean life span of male rats, it is difficult to make a conclusion that the life span in atrazine-treated rats is prolonged. Therefore, we recommend that the RCD include a comparison in the mean life span between high-dose male SD rats and the control males in order to address this issue.*
DPR: An elevated incidence of interstitial cell tumors, which are common in old rats, was observed in the only group of males that survived significantly (p<0.01) longer than controls, those at the HDT. At 2 years, 74% survived at this dose vs. 56% for controls. Considering that the HDT, 1000 ppm, was probably in excess of the MTD for many of these rats (mean body weight was reduced by 19%, p<0.01, see p.28), DPR fails to see a scientific basis for dwelling on this issue.

Other Carcinogenicity Issues

1. The draft RCD states on page 28, "Neoplastic changes resulting from atrazine administration were restricted to an increased incidence of mammary tumors in the female SD rat; no increases in tumors were consistently found in the male SD rat or in Fischer rats or mice, of either sex." This statement may be misleading in that it limits the atrazine-induced neoplastic changes to mammary tumors. The data presented in the draft RCD do not support this statement. For example, the document states:

(a) On page 28, "In an acceptable FIFRA Guideline oncogenicity study of atrazine, ... an increased incidence of benign testicular interstitial cell tumors in males was also noted, at the highest dose tested (HDT) only."
DPR: see above

(b) On page 28, "In another non-FIFRA guideline study using the Fischer rat, there was no increase in mammary tumors, although there was an increased incidence of uterine tumors and leukemia/lymphoma at the HDT."
DPR: This was not replicated in an acceptable study with F344 rats.

(c) On page 80, "Atrazine-related neoplastic changes detected in a FIFRA-guideline oncogenicity study consisted of an increased incidence of malignant and/or benign mammary tumors in female Sprague-Dawley rats and an increased incidence of benign testicular interstitial cell tumors in male Sprague-Dawley rats (Mayhew, 1986)."

1. By the one-sided Fisher´s Exact test, the 200 ppm groups for mammary fibroadenomas ($p<0.081$) and combined tumors ($p<0.077$) are very close to statistical significance relative to controls (page 40, table 13B and text). Given the suggestion made in the draft RCD that compound-induced weight loss in the treated groups may have reduced tumor incidence in those animals relative to controls, it is plausible that the F-344 rats did develop tumors in response to atrazine and statistical significance was not attained simply due to the weight loss. We recommend that this possibility be more fully discussed in the RCD.
DPR: It is quite possible that mammary tumors in F344 rats were masked by weight loss, but DPR does not consider $p<0.08$ to be close to significant, especially when there are as many as 60 rats per dose group.

2. The draft RCD notes on page 34 that "At 9 months, progesterone blood level was unaffected by treatment in SD rats; estradiol was elevated by 37% and prolactin by 157% at 400 ppm (Table 8). The study confirmed the effect of tumor induction seen in the Mayhew, 1986 study. Data were generally consistent with the possibility that atrazine caused elevated estradiol levels in early adult life, which may have resulted in elevated incidence or earlier onset of these tumors." According to the data presented in Table 8 (page 35), compared to the control group, the blood level of estradiol was increased by 37 percent (31.2 " 28.1 compared to 22.8 " 20.6 in the control) while progesterone was decreased by 36 percent (7.4 " 4.1 compared to 11.6 " 1.0 in the control), but neither change was statistically significant. While DPR notes the increase (37 percent) in estradiol, the decrease (36 percent) in progesterone was interpreted as "unaffected." We recommend revising the draft RCD to present a consistent interpretation of these findings.

DPR: "…progesterone blood level was reduced by 36%…" will be incorporated on p.34 of RCD.

Seasonal Exposures to Atrazine

1. The draft RCD for atrazine does not include assessments of occupational, seasonal exposures to this chemical. No clear reasons were provided for this important omission. The brief statement made in the first paragraph on page 84 that Aapplications are made from the ground and these are not considered seasonal," is not scientifically justified and does not support the exclusion of seasonal exposures to atrazine from this risk assessment. OEHHA recommends that seasonal exposure be evaluated in the RCD.

DPR: The determination that there is no seasonal exposure was made by the WH&S Branch of DPR.

2. Occupational exposure to atrazine is represented in the draft RCD by Annual Average Daily Doses (AADD) for annual exposure assessment and by Lifetime Average Daily Doses (LADD) for lifetime cancer risk assessment. Potentially greater short-term exposures are not evaluated in the draft RCD. Note that atrazine can be applied by farmers over the course of three consecutive days and by commercial applicators for 15 consecutive days. OEHHA believes that these short-term exposures should be evaluated. We recommend estimating doses for these short-term exposures using upper-bound exposure estimates for these situations, rather than average exposure values such as those used for the AADD and LADD estimates. These short-term exposures should be evaluated by comparison to an appropriate sub-chronic endpoint. OEHHA recommends performing this seasonal evaluation and including the results of this evaluation in the draft RCD.

DPR: see above.

Reproductive Toxicity

1. *In this section (page 62), the draft RCD notes the existence of only one "acceptable FIFRA-guideline reproductive toxicity study." No mention is made of the considerable body of data on reproductive parameters, which are discussed in a later section on endocrine toxicity (pages 72 to 77). Addition of a cross-reference from the endocrine section to this section on reproductive toxicity is recommended.*

2. *The single study summarized in this section was a two-generation rat reproduction study (Mainiero et al., 1987) in which atrazine was administered in the diet at concentrations of 0, 10, 50, or 500 ppm (resulting in doses of approximately 0, 0.5, 2.5 or 25 mg/kg-day). Parental effects noted at the high concentration consisted of decreased body weight gain and food consumption, and increases in relative testes weights (considered secondary to the decreased body weights).*

Of this study, the draft RCD notes: "Decreased postnatal F_2 pup body weight was present in all treated male groups at birth (3-percent) and continued throughout the observation period, reaching statistical significance at day 21 (7-10 percent). U.S. EPA noted this effect at $\geq$ 50 ppm at 21 days. The study authors suggested that the weight decrease was due to pup consumption of the maternal diet. Although no data were submitted to support this suggestion, DPR considered that it probably had some validity: pup weight loss was not noted at 14 days, when exposure would have been predominantly via the dams' milk, but only at 21 days, when consumption of the diet and the dams' milk would have resulted in an 'overdose' for pups, on a mg/kg basis. Pup weight loss was therefore not considered toxicologically relevant as an adverse reproductive effect. ...The reproductive NOEL was $\geq$ 500 ppm (25 mg/kg/day), based on a lack of reproductive toxicity at this dose."

The interpretation presented in the draft RCD of pup growth data in a multigenerational reproductive toxicity study does not appear to be scientifically valid. In the absence of data on atrazine secretion in milk or on pup consumption of treated feed, the conclusion that pups received an "overdose" of atrazine is speculative. Addition of the test compound to feed or drinking water is the standard means of treatment in multigenerational reproductive toxicity studies. As pups approach weaning age, it is known and expected that they will consume some of their dam′s feed and water. Thus, the potential for direct exposure of pre-weaning pups to the test compound is generally understood, and is not considered a basis for discounting a finding of altered pup growth. We recommend that the RCD be revised to include an alternative interpretation of these data; the dose level of atrazine received by the pups is not an "overdose."

DPR: It is common for pesticides to cause effects post-weanling but not pre-weanling. This is clearly the case here because the pup body weight was not affected at 14 days, but it was at 21 days. It is Medical Toxicology policy <u>not</u> to consider such effects to be adverse <u>reproductive</u> effects.

In this particular case, pup weights were reduced at birth and "throughout the observation period," although the difference only became statistically significant at 21 days postnatal. There are a number of potential toxicological mechanisms that could have resulted in this pattern of impaired pup growth. For example, the finding of reduced birth weights suggests that there may have been a prenatal component to the observation. The initial deficit could then have been compounded by lactational exposure to atrazine, preventing any opportunity for catch up growth, and eventually resulting in a statistically significant deviation from the normal growth curve. Alternatively, atrazine treatment could simply have impaired milk production. Decreased milk production could, in turn, have depressed pup growth, whether or not any atrazine was actually secreted in the milk. Either scenario represents a toxicologically relevant effect, although the former could arguably be categorized as developmental toxicity, rather than "reproductive toxicity." Nonetheless, these data do not appear to have been taken into account in characterizing the risk for the developmental toxicity of atrazine. OEHHA recommends that the draft RCD examine the aforementioned alternatives for reduced pup growth based on other toxicological studies reviewed in the draft RCD: for example, endocrine, developmental and pharmacokinetics studies.

DPR: OEHHA speculates on atrazine´s accumulation in milk or effects on milk production. In Appendix VI (Dietary Exposure Assessment), pp. 6 and 7, studies are described that measured the residues in milk of dairy cows fed high levels of atrazine. It did <u>not</u> accumulate in the milk, 2 ppm in diet translating to 0.013 ppm in milk.

Endocrine effects are noted in the draft RCD as a possible mechanism for the induction of mammary tumors. However, there is no mention of the "endocrine" changes as manifestations of the reproductive toxicity of atrazine. We recommend including a discussion of the impact of atrazine-induced endocrine changes on reproductive toxicity in the RCD.

DPR: The TOC lists the sections of the RCD. It seems unnecessary to add cross-references between the various sections. This is covered in the Risk Assessment and Risk Appraisal, Sections IV and V.)

Developmental Toxicity

1. In this section (page 63), four standard developmental toxicity studies were evaluated in the draft RCD: three rat studies and one rabbit study. One study for each

species is considered acceptable under Federal Insecticide, Fungicide and Rodenticide Act guidelines. It seems questionable that "abortions" (loss of entire litters) in rabbits is considered in the draft RCD as a manifestation of maternal, rather than developmental, toxicity. However, given that there were other effects (as tabulated on page 65 of the draft RCD) at the lowest-observed-adverse-effect level (LOAEL) for maternal and developmental toxicity (75 mg/kg-day in both cases), this distinction makes no practical difference.

2. *Data on postnatal pup growth from the multigenerational reproductive toxicity study (Mainiero et al., 1987) should also be considered in the discussion of developmental toxicity.*

DPR: see above.

2. ***Endocrine Effects***

A number of studies discussed in the draft RCD (pages 73 to 77) clearly suggest a role for atrazine in reproductive toxicity, for example:

(a) On page 73, Tesac et al. (1992) stated, "A daily injection of atrazine or desethylatrazine to pregnant or lactating rats influenced the pituitary-gonadal axis in the offspring of both sexes."

(b) On page 73, Eldridge (1993 a, b) stated, "...it is not possible to conclude that humans will not be affected by triazines because of interspecies differences in estrus cycles."

(c) On page 74, Safe et al. (1995) stated, "Atrazine lengthens cycles and causes persistent estrus [in the SD rat]. Its site of action is thought to be between the hypothalamus and the pituitary."

(d) On page 75, Morseth (1996a) stated, "Prior to ovariectomy, atrazine caused a dose-dependent disturbance in estrus cycling, particularly a prolongation of diestrus and of estrus.... It was concluded that atrazine leads to a delay in ovulation (and associated prolonged estrus) by disturbing the surges of release of LH and prolactin. The LOEL and NOEL values for hormonal disruption were 40 and 5 mg/kg/day, respectively."

(e) On page 77, Cooper et al. (1996) stated, "The effects of atrazine on ovarian function in SD and Long-Evans hooded rats have been studied. . . . Atrazine disrupted estrus cyclicity in both strains. At the mid-dose, atrazine increased the number of

days in diestrus for both strains, without a change in the number of days in estrus. . There was a corresponding increase in blood progesterone level and a decrease in estradiol level, which when combined with prolonged diestrus is known as pseudopregnancy. At the HDT, SD rats showed a similar effect, correlated with a slight increase in the level of both hormones. However, LE-hooded rats displayed a reduction in the level of both hormones at this dose, a condition known as anestrus."

The draft RCD discusses these studies only in the context of considering their relevance to the carcinogenic effects of atrazine. No mention is made of the strong weight of evidence provided here for the reproductive toxicity of atrazine. Effects such as delayed sexual maturation, prolonged cycle length, and accelerated reproductive senescence are all manifestations of reproductive toxicity as defined by U.S. EPA in their "Guidelines for Reproductive Toxicity Risk Assessment" (1996). The agency defines reproductive toxicity as:

"The occurrence of biologically adverse effects on the reproductive systems of females or males that may result from exposure to environmental agents. The toxicity may be expressed as alterations to the female or male reproductive organs, the related endocrine system, or pregnancy outcomes. The manifestation of such toxicity may include, but not be limited to, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, gestation, parturition, lactation, developmental toxicity, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems."

DPR: The endocrine experiments conducted by Syngenta (formerly Novartis) and others followed the USEPA review of the 1986 chronic study (Mayhew) in which mammary tumors were found in the female SD rat. Endocrine experiments were designed to determine whether or not there was an endocrine mechanism for these tumors. The standard multi-generation reproduction study (using the same strain of rat, p.62 RCD) did not show any reproductive toxicity that might warrant such additional studies.

More specifically, U.S. EPA notes the following:

(a) "Significant evidence that estrous cycle (or menstrual cycle in primates) has been disrupted should be considered an adverse effect. Included should be evidence of abnormal cycle length or pattern, ovulation failure, or abnormal menstruation."

(b) "Significant effects on measures showing a decrease in the age of onset of reproductive senescence in females should be considered adverse. Cessation of normal cycling, which is measured by vaginal smear cytology, ovarian histopathology, or an endocrine profile that is consistent with this interpretation, should be included as an adverse effect."

(c). "Significant alterations in circulating levels of estrogen, progesterone, testosterone, prolactin, LH, or FSH may be indicative of existing pituitary or gonadal injury. When significant alterations from control levels are observed in those hormones, the changes should be considered cause for concern because they are likely to affect, occur in concert with, or result from alterations in gametogenesis, gamete maturation, mating ability, or fertility. Such effects, if compatible with other available information, may be considered adverse and may be used to establish a NOAEL, LOAEL, or benchmark dose."

U.S. EPA's guidelines were published according to a procedure which involved considerable intra- and inter-agency review, as well as consideration of public comments and review by U.S. EPA's Science Advisory Board. As such, they represent the best currently available scientific principles for use in assessing risk for reproductive harm. The draft RCD should follow these guidelines/principles to the extent possible in conducting the risk assessment and provide the rationale for deviating from these guidelines/principles.
DPR: As presented in this RCD, endocrine effects occurred only at higher doses than those that are cardiotoxic. Therefore, this consideration is irrelevant with regard to this risk assessment

(e) ***Susceptible Subpopulations***

1. *The draft RCD for atrazine did not identify any groups that may be especially susceptible to this chemical. However, data presented in the draft RCD (pages 73 to 77) suggest that such groups exist and would include infants and children, especially those one to six years old. Reasons in support of our conclusion are listed below:*

(a) Potential acute and chronic dietary exposures to atrazine from all commodities with U.S. EPA tolerances and from drinking water are the highest for infants and children among exposures calculated for 21 population subgroups (see tables 31 and 34 in the draft RCD).

(b) Endocrine effects caused by atrazine also may be more harmful for infants and children than for adults. These effects are summarized in the draft RCD on page 99 "atrazine inhibited the binding of [^{3}H]-5a-dihydro-testosterone to receptors in the hypothalamus, pituitary and prostate of male rats, in vitro, and in vivo; numerous reports showed premature reproductive senescence, along with other effects which could have arisen through endocrine mechanism(s), in female SD rats; triazine herbicides induced the enzyme aromatase in human placental carcinoma cells, in vitro, and an analogous effect could account for the reduced testosterone and increased estradiol levels which have been reported in the blood of male Wistar rats after dosing; administration of atrazine to the rat for 15 days resulted in increased levels of thyroid hormones, T_3 and T_4 (thyroxine) in the blood. It is difficult to know the role that this information might have in risk assessment because the mechanisms for these effects are not fully understood."

(c) Infants and children may also be at relatively greater risk from cumulative exposures to other triazines that have similar mechanism(s) of action (endocrine effects) (OEHHA, 1999).

1. *While available developmental toxicity studies failed to show fetal or embryonic toxicity at doses of atrazine less than those affecting dams, two atrazine metabolites, desisopropylatrazine (DIPA) and diaminochlorotriazine (DACT), caused clear developmental effects in rats at doses lower than those affecting dams. Developmental effects produced by DIPA included an increased incidence ($p<0.01$) of fused sternebrae (one and two) at 25 and 100 mg/kg-day. NOAELs for developmental and maternal toxicity (decreased body weight) were 5 mg/kg-day and 25 mg/kg-day, respectively. A NOAEL of 2.5 mg/kg-day in a developmental rat study with DACT was based on incompletely ossified or unossified bones, including the hyoid, interparietal and parietal bones. The maternal NOAEL of 25 mg/kg-day was based on reduced body weight and food consumption. The significance of these findings for the potential of atrazine to cause developmental effects should be addressed in the draft RCD.*

2. *In general, endocrine effects are not regarded in the draft RCD as useful for risk assessment purposes because "the mechanisms for these effects are not fully understood." However, the RCD also states that "there is considerable evidence to support an endocrine mechanism of mammary carcinoma induction" and reviews studies on page 77 which suggest a role for endocrine disruption in delaying sexual maturity in male and female rats. Despite these data, the RCD disregards an extra ten-fold uncertainty factor under FQPA for developmental effects. OEHHA*

recommends that the draft RCD reevaluate the use of endocrine effects for assessing the potential health impacts of exposure to atrazine.

3. *We recommend that the draft RCD include a more detailed discussion of whether the MOE calculations sufficiently account for a potentially greater susceptibility of children. OEHHA recommends application of an additional uncertainty factor to take into account the potential greater sensitivity of infants and children when evaluating atrazine toxicity for exposures that can be quantified (e.g., drinking water, food residues) (Stoker et al., 2000).*

DPR: Higher potential exposure is not the same as "greater susceptibility" of children.

Pharmacokinetics

The draft RCD on pages 17 to 19 provides information on the metabolism of atrazine after oral exposure in humans and rats and a discussion of interstrain/interspecies comparisons of atrazine metabolism based on in vitro assays in hepatocyte cultures (pages 18 and 19). OEHHA recommends that the draft RCD discuss the role of atrazine metabolites in the overall toxicological response arising from exposure to the parent compound. Furthermore, this section should also address the distribution of atrazine and its metabolites into milk because of atrazine´s potential developmental effects.

DPR: The toxicity of the chlorometabolites has been described extensively e.g. pp. 21, 23-27, 46-50, 58, 60-61, 66, 68, 76-77. Moreover, these have been included in the dietary exposure analysis, in Appendix VI. The issue of possible milk accumulation has been addressed above. DPR does not consider that 2 ppm (diet) leading to 0.013 ppm (milk) is an example of bioaccumulation. The toxicity of hydroxyatrazine, a degradate that is apparently not formed in humans and which is thought to act differently, has also been described in detail in the RCD.

Tolerance Assessment

1. *No information is provided in the draft RCD on the types of atrazine residues considered for tolerance assessment (pages 100 to 101). We recommend that a more specific description of the residues (e.g., parent chemical only or parent chemical and its metabolites) be included since atrazine´s metabolites have toxicity similar to that of the parent compound.*

DPR: The atrazine tolerances include (3) chlorotriazine metabolites. The purpose of the tolerance assessment is to determine whether or not residues at tolerance have an adequate MOE. The precise make-up of the residues of atrazine and/or metabolites comprising the tolerance level of residue is irrelevant.

2. *We recommend providing a table with the estimates of the maximum residue contributions (calculated by using the tolerance level and a 100 percent crop treated assumption), the anticipated residue concentrations, and their representations as percentages of the acceptable daily intake, reference dose or NOAEL for chronic exposure.*

DPR: The use of the TMRC (by USEPA) has been discontinued since 1996 (FQPA). It has been discredited as being overly conservative because it is impossible, in practice, to imagine the same person being subjected to the maximum permissible pesticide residue for every RAC, simultaneously.

3. *The tolerance assessment section of the draft RCD does not take into account the numerous endocrine effects of atrazine, namely, prostate inflammation in male offspring, delay in puberty in rat studies, central nervous system as mode of action for atrazine (neuroendocrine alterations in the hypothalamus), and uncertainty in the toxicological data base regarding CNS alterations. U.S. EPA´s Scientific Advisory Panel (SAP) in their final report (SAP Report No. 2000-05) concludes that "neuroendocrine model of carcinogenic action may be relevant to infants and children exposure, but the effects may have a long latency and may not become apparent until puberty or even later." Neither does the RCD consider cumulative exposures to other triazine compounds. OEHHA recommends application of one additional uncertainty factor of ten for all aforementioned variables to take into account a potential greater sensitivity of infants and children when evaluating atrazine tolerances for exposures that can be quantified (e.g., drinking water, and food residues).*

DPR: Only acute toxicity considerations are examined in the tolerance assessment for a pesticide, as explained on pp. 100-101 of the RCD. The NOEL used for tolerance assessment was 5 mg/kg/day, for both maternal and developmental toxicity, in a rabbit developmental toxicity study with a LOEL of 75 mg/kg/day. The <u>lowest</u> MOE was >2000. It is not considered appropriate to introduce additional margins of exposure requirements when there is no evidence that the developing rabbit is more susceptible to the toxic effects of atrazine than the adult rabbit.

Risk Characterization

The "Risk Characterization" section of the draft RCD for atrazine (pages 91 to 93) refers only to uncertainties in the current methodology and general assumptions used in risk assessment. We recommend that this section be expanded to include a discussion of the uncertainties specifically related to atrazine. These include the quality of the existing database, quality and limitations of the exposure data, data gaps (if any), and uncertainties related to toxicological responses, such as immunotoxicity, neurotoxicity, and endocrine effects for which risk assessment methodologies have not been developed. The section on "Risk Characterization" should also include a discussion of sensitive populations, including children and infants.
DPR: The section referred to above (pp. 91-93) merely states the MOEs and Risks determined for Occupational and Dietary exposure. The uncertainties in these calculations are discussed in the Risk Appraisal Section (pp.95-99). As stated above, there is no evidence that "children and infants" are more "sensitive" only that they may have greater theoretical dietary exposure, according to the TAS calculations (Table 31).

Specific Comments

Page 19, top paragraph: The 24 hour bioconversion rates for hepatocytes from the three species vary over a range of 2.9-fold, yet it is stated that "bioconversion of atrazine by human hepatocytes is an order of magnitude slower than other species." Please clarify this apparent inconsistency.
DPR: ". . . an order of magnitude" has been replaced with "..slightly.."
Page 22, top paragraph: The abbreviation for extramedullary hematopoiesis (EMH) should be defined here rather than later on page 24.
DPR: corrected.
The Barrater and Reborn 1995a and 1995b references are not included in the "Reference section."
DPR: These should be "Biradar and Rayburn, 1995a,b" on p. 104; these will be changed on pp.54 and 59.
Page 66, second paragraph: For the DIPA study, the maternal NOAEL should read 5 mg/kg-day rather than 25 mg/kg-day, as per the "Toxicology Summary" (page 39).
DPR: reductions in maternal body weight were only 3% (day 9) and 4% (day 13), although both were statistically significant, $p<0.05$. These are not considered toxicologically significant.

Page 72, third paragraph: "The suppression of the LH peak would stimulate the release of estrogens in the (intact) SD rat (which would stimulate the growth of mammary tumors)." We recommend that this statement be referenced.

DPR: It is considered inappropriate to give literature citations in a "Summary" such as this paragraph.

Page 81, third paragraph: "Dietary restriction of female SD rats dosed with the genotoxic carcinogens N-methyl-N-nitrosourea or 7,12-dimethylbenz[a] anthracene resulted in a reduced incidence of mammary tumors compared with free-feeding rats. The additional cancers resulting from these carcinogens were abolished at 30 percent and 40 percent dietary restriction. It therefore seems probable that the mechanism by which atrazine results in mammary tumors is different from those of these compounds." It is unclear how this conclusion follows from the studies discussed. Please clarify.

DPR: As stated earlier in this paragraph, atrazine results in an increased incidence of mammary carcinomas with increasing dose, even though body weight and food intake are reduced dramatically. This phenomenon is unlike these (genotoxic) carcinogens, where reducing the calories in the diet reduces the tumors (caused by MNU or DMBA).)

Page 81, fourth paragraph: The statement that decreased blood estradiol, elevated progesterone and prolonged diestrus are opposite to the conditions favoring mammary tumor induction should be referenced.

DPR: a reference to Section III.J will be added.

Page 95, third paragraph: "However, increased EMH could have been secondary to an increased incidence of mammary carcinomas." This statement was made previously on page 29, second paragraph. As discussed there, a correlation between EMH and adenocarcinoma was observed at interim sacrifice but not at final sacrifice. We recommend that citations be provided of published studies to support the hypothesis that EMH is sometimes caused by mammary tumors.

DPR: As stated on p.29 (RCD), the study author (Mayhew) suggested that the hyperplasia and EMH could have resulted from ulcerated and/or necrotic mammary tumors. This statement is not referenced by Mayhew but seems plausible.

Page 95, third paragraph: The 100 percent rate of EMH in control F-344 rats is offered as a justification for discounting EMH as an endpoint for chronic atrazine toxicity in SD rats. However, as illustrated in Table 4A of the draft RCD, control female SD rats suffered EMH of the spleen at a rate of only 18 percent by two years compared to 22 to 43 percent in the treated groups. OEHHA recommends that control SD rats, rather than control F-344 rats be used as the appropriate control group for evaluating atrazine-induced EMH in SD rats.

DPR: They were, see Table 4!
Page 96, second paragraph: "This is unlike other known (genotoxic or estrogenic) mammary gland carcinogens, which continue to increase the incidence of mammary tumors in ovariectomized rats." We recommend that a reference be provided.
DPR: to be added, "Geschickler, C/F. & Byrnes, E.W., 1942. Factors influencing the development and time of appearance of mammary cancer in the rat in response to estrogen. Arch. Pathol. 33:334-356.
Li, J.J. & Li, S.A., 1995. The effects of hormones on tumor induction. Ch.11, Sec.III in Chemical Induction of Cancer. Ed. J.C. Arcos, Bukhauser, Boston, USA."
Page 96, second paragraph: The draft RCD states that "Each authority has determined that atrazine is unlikely to be a human carcinogen." However, the International Agency for Research on Cancer (IARC) categorizes atrazine as having "inadequate evidence of carcinogenicity." IARC finds that the data do not allow a determination of whether or not atrazine is carcinogenic in humans. We recommend that the statement "unlikely to be a human carcinogen" in the RCD be expanded and clarified.
DPR: ". . or has inadequate evidence of carcinogenicity" to be added on p.96. IARC risk assessments are of uncertain validity because they generally rely on only published reports.
Page 96, third paragraph: "Furthermore, genotoxic nitroso compounds are usually highly reactive and induce tumors close to the site of their production or first contact with an organism..." We recommend that this statement be referenced.
DPR: A description of the following study will be added to the RCD on p.96: "In Sasaki et al., 2000[1] the effects of nine N-nitroso compounds were reviewed. All were carcinogenic at multiple sites in both sexes of both rats and mice (and several other animal species also, in some cases). The most common sites for cancer were stomach, colon, lung, liver, kidney, bladder. None of them caused mammary tumors of any sort in any species. Using the COMET assay in the mouse in vivo, all of these N-nitroso compounds caused DNA damage in stomach, colon, liver and lung. Eight of them also caused DNA damage in the bladder and a different eight also caused DNA damage in the kidney. None of these N-nitroso compounds resulted in DNA damage in mammary gland tissue. Thus, there was excellent concordance between the sites of genetic damage in a short-term test (COMET) and the sites of cancer in chronic studies."

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[1] Y.F. Sasaki *et al.*, 2000 The Comet assay with multiple mouse organs: comparison of Comet assay results and carcinogenicity with 208 chemical selected from the IARC monographs and U.S. NTP carcinogenicity database. ***Crit. Rev. Toxicol. 30(6):*** 629-799.