Section 4

EVALUATION OF THE MAMMALIAN TOXICOLOGY AND TOXICOKINETIC/METABOLISM

Comment on the extent of the toxicology database

At various positions throughout the document, it is stated, under subheadings for the several atrazine metabolites, that "no studies were provided". This is not to be taken to indicate that the toxicology data package was deficient. In fact, the data package on atrazine is very extensive whilst the data package on its metabolites was significantly more than is normally provided for pesticides.

Addition of extra text in response to comments on the first draft

Novartis (formerly Ciba-Geigy; organisation and name changed during the course of this review) provided a number of further studies and some further technical comment, in response to their review of the first draft of this report. A few of these studies/reports have been assessed but in the main, the extra information has been included as extra comment or text in the appropriate location in this report, and identified as such. The decision not to evaluate studies in detail was taken if they were only interim reports of longer studies or if they did not add any substantial new information.

EVALUATION OF THE MAMMALIAN TOXICOLOGY AND TOXICOKINETIC/METABOLISM

1. INTRODUCTION

1.1 Regulatory history of health considerations in Australia

Atrazine is a triazine herbicide used pre-emergence and early post-emergence for selective control of broad-leaf and grassy weeds in various food crops (such as corn and sorghum), forestry plantations and in non-crop situations. Atrazine has been in use in Australia for more than 20 years.

The toxicology of atrazine was first considered by the National Health & Medical Research Council (NH&MRC) of Australia for the Department of Health & Family Services (hereafter called the Department) in 1985. The toxicology of atrazine has been evaluated and reviewed by the Department on a number of occasions since then. This has occurred for a number of reasons:

- the identification, by the IBT Taskforce Program, of atrazine as having IBT-generated data in its toxicological data package which required either verification or replacement;
- as part of the Technical Grade Active Constituent (TGAC) Clearance Scheme (1985-1992), the Department reviewed the toxicology profile of some 400 agricultural chemicals (including atrazine);
- a number of supplementary data submissions have been made over time, with a special focus on the carcinogenicity potential of atrazine.

In addition, there has been a number of applications for clearance of atrazine from new sources of manufacture (ie. new TGACs) and/or new end-use products (EUP) containing atrazine as the active ingredient.

The regulatory history of key activities associated with atrazine in Australia is summarised in the Table below.

1.1.1 Health & other standards

In Australia, health standards for agricultural and veterinary chemicals, such as the poison schedule, first aid and safety directions and an acceptable daily intake (ADI), are recommended or set by the Department. Poisons schedules are established by the National Drugs and Poisons Schedule Committee (NDPSC) of AHMAC (Australian Health Ministers Advisory Council), formerly known as the Drugs and Poisons Schedule Standing Committee (DPSSC) of the NH&MRC. In the case of ADIs and maximum residue limits (MRLs), these were formerly established by the Pesticide and Agricultural Chemicals Standing Committee (PACSC) of the NHMRC; in 1992 the Department became directly responsible for establishing MRLs, a function

subsequently transferred to the National Registration Authority for Agricultural & Veterinary Chemicals (the NRA) in June 1994.

History of public health considerations of atrazine in Australia

DATE	REGULATORY ACTIVITY
1983- 1984	MRLs established for corn, grain and fodder, sorghum, broom millet & established lucerne, sweetcorn, experimental MRL in cereal grains;
Nov 1984	PACSC noted atrazine toxicology data package contains some IBT studies. Under watching brief until replacement studies generated
Dec 1985	Toxicology data (Ciba-Geigy) evaluated by Department. PACSC noted replacement chronic mouse (commenced Nov. 1992) and rat (Oct. 1984) studies were underway. NOEL of 0.6 mg/kg/day, based on a 2-year rat study used to establish an ADI of 0.0003 mg/kg/day - a 2000-fold safety factor used, pending submission of replacement chronic mouse study.
June 1986	Supplementary data (Ciba-Geigy) reviewed. PACSC: no change to NOEL/ADI.
Aug 1986	DPSC confirm NOEL and exempt-from-scheduling status, scheduling to be reassessed on receipt of replacement chronic studies.
Nov 1986	2-year rat study reviewed. High incidence of mammary tumours in controls with a dose-related trend for increased incidence in atrazine-treated rats. PACSC agree atrazine of low oncogenic potential but requested final report of ongoing chronic studies in rodents ASAP. Request for MRL for water. PACSC- no justification for establishing MRL for water, as current label warned against contamination of dams, waterways or drains, so GAP should not result in residues.
Feb 1987	PACSC requested company to comment on report of three mouse carcinogenicity studies when only one submitted to date. MRLs for lupins established.
May 1987	DPSC reject IBT mouse chronic study and published paper submitted by Ciba-Geigy.

History of public health considerations of atrazine in Australia cont'd

DATE	REGULATORY ACTIVITY
Aug 1988	Additional toxicology data submitted (Koor Intertrade). No change to NOEL. Ciba-Geigy reminded that replacement IBT studies due May 1986.
Nov 1988	Rat (SD strain) chronic study (Ciba-Geigy) evaluated. Company asked for further clarification and to provide IARC study in Fischer rats.
Aug 1989	TGAC clearance application (Amalgamated Chemicals) evaluated. PACSC consider application for MRL for potable water pending policy consideration of Public Health Committee of NHMRC.
March 1990	SCOT reviewed available cancer epidemiology data and agreed no conclusive data but that the matter should be kept under review.
July 1990	SCOT recommended a review of triazines.
Nov 1990	Toxicology evaluation of TGAC data (Ciba-Geigy & Amalgamated Chemicals). DPSC agreed that rodent studies showed no carcinogenic potential and available epidemiological data showed no association between atrazine exposure and cancer; confirmed exempt status for scheduling and agreed to clearance of TGAC. NOEL of 0.5 mg/kg/day established in 2-year rat study with an estimated ADI of 0.005 mg/kg/day.
May 1991	SCOT, whilst noting tumour findings in SD rats, agreed no epidemiology data to independently assess hazard to humans.
Nov 1991	New combination EUP application (Macspred) for use in plantations evaluated.
Dec 1992	New source TGAC (Mineral & Chemical Traders) approved. No change to ADI or schedule.
Aug 1993	New source TGAC (Makhteshim-Agan) approved. No change to ADI or poison schedule.

History of public health considerations of atrazine in Australia cont'd

DATE	REGULATORY ACTIVITY
Jan 1994	ACPH considered atrazine use and water contamination issues, noting public concern raised in Tasmania following use in establishment of eucalypt plantation and contamination of stream water. ACPH recommended development of forestry guidelines to reduce possibility of water contamination with pesticides and agreed to review the ADI and the drinking water guideline value.
May 1994	ACPH reviewed toxicology and considered rat mammary tumours as not being appropriate to assess the risk to humans. ACPH4 confirmed the NOEL of 0.5 mg/kg/day and ADI of 0.005 mg/kg/day and the water quality health guideline value of 0.02 mg/L.
Oct 1994	New combination EUP (Chemspray) approved for home garden use.
Nov 1994	Extensive package of supplementary toxicology studies evaluated. No change to NOEL/ADI. Schedule moved from Exempt to S5.
Aug 1995	Evaluation of toxicology data for new combination EUP (Sandoz) approved for control of broad-leaf weeds in sugar cane. No change to ADI or poison schedule.
May 1995	Nominated onto the NRA's ECRP Priority Review Candidate List
1996	Cycle 1 ECRP review

NOEL/ ADI

The current ADI for atrazine is 0.005 mg/kg/day based on a NOEL of 0.5 mg/kg/day established in a 2-year chronic study in rat. Atrazine is currently in Schedule 5 of the SUSDP.

MRLs

Atrazine has residue limits (maximum residue limits, MRLs) set for a variety of crops and non-food uses. The MRLs for atrazine, as detailed in the MRL Standard (30 June 1994) are outlined in the Table below.

Table: Australian maximum residue limits for atrazine

CODEX Classification	*FOOD*	*MRL (mg/kg)*
FC0001	Citrus fruits	*0.1
MO0105	Edible offal (mammalian)	*0.1
FB0269	Grapes	*0.1
VD0545	Lupin (dry)	*0.02
GC0645	Maize	*0.1
MM0095	Meat [mammalian]	*0.01
ML0106	Milks	*0.01
FI0353	Pineapple	*0.1
VR0589	Potato	*0.01
SO0495	Rape seed	T*0.01
GC0651	Sorghum	*0.1
GS0659	Sugar cane	*0.1
VO0447	Sweet corn (corn-on-the-cob)	*0.1

* denotes that the maximum residue limit (MRL) has been set at or about the limit of analytical determination.

Drinking Water Guidelines

A Guideline Value of 0.5 µg/L and a Health Value of 20 µg/L have been set for drinking water (Guideline value - above this level, action should be taken to identify source of atrazine and take action to prevent further contamination; Health Value - based on 10% of the ADI). Details of the Australian drinking water guidelines for atrazine and a comparison with international values are shown in Attachment 1.

Existing chemicals review program

Atrazine is one of 80 agricultural and veterinary chemicals identified as candidates for priority review under the ECRP. It was selected on public health grounds on the basis of concern that, as a relatively persistent chemical in the environment, it could have endocrine-disruptor and carcinogenic potential.

Following data call-in processes, a number of additional data submissions on the toxicology of atrazine have been received from industry, users, and the public. These data, together with all previously submitted data have been evaluated and are detailed in the report below.

Attachment 2 summarises toxicology data submission details (sponsor companies and dates) relating to toxicological and public health aspects of atrazine.

1.2 International status

The US EPA announced a special review of the triazine herbicides in November 1994, apparently arising from concerns from speculated links to breast cancer incidence. In the USA where atrazine was first registered in 1958 or 1959, it is estimated that 90-120 million lbs of triazines are used each year, with atrazine accounting for up to 80 million lbs of that (mainly in corn). The concern is that atrazine is contaminating drinking water because of its relative stability and mobility in soil. However, there has been a "massive" response to the US EPA from farmers and farmers federations who claim that atrazine is safe, cheap and effective. Ciba-Geigy suggested that economic losses in agriculture could range from US$1.2 to US$2.8 billion. DuPont Agricultural products has agreed to completely phase-out the related triazine, cyanazine, by the end of 1999.

It has been reported that atrazine has been banned in Germany (March 1991), and Denmark (1994; withdrawn from registration by Ciba in 1993). In Sweden, registration was cancelled in 1989 and in Italy, authorisation has been suspended (temporarily in 1990; extended in 1991), pending EU evaluation. In Finland and Norway, registration has been withdrawn by Ciba.

UK evaluations of atrazine are available, prepared by the Pesticides Safety Directorate of the Ministry of Agriculture, Food and Fisheries (MAFF); these assessments, dated May 1992 and July 1993, were initiated following the identification of concerns about the fate and behaviour of the triazine herbicides in soil and water. An ADI of 4 µg/kg bw/day was set, on the basis of NOELs in chronic oral rat studies and an admissible operator exposure level of 50 µg/kg bw/day was set on the basis of a NOEL in a subacute dog study. The proposed environmental quality standard (EQS) in water was 2 µg/L based on the annual combined average of atrazine and simazine, with a maximum allowable concentration (MAC) of 10 µg/L for atrazine and simazine combined. UK Water Supply (Water Quality) Regulations 1989 require levels of individual pesticides in drinking water at the tap to be less than 0.1 µg/L. It was recommended that the use of atrazine be permitted on cropped land but not on non-crop land (industrial or amenity situations, fallow, wasteland, footpaths, roads, tennis courts etc.), that it not be aerially sprayed, and that application on crops not exceed a set maximum rate per year. Home garden use was considered acceptable.

Atrazine was reviewed by the International Agency for Research on Cancer in 1991 (IARC, 1991). It was classified in Group 2B (possibly carcinogenic to humans).

2. TOXICOKINETICS AND METABOLISM

Atrazine is a relatively old herbicide, approved at a time when regulatory requirements for toxicokinetic studies were less stringent and when experimental methodology for the conduct of such studies was more limited than now eg. some techniques employed in earlier studies contributed to direct *in vitro* degradation of atrazine, leading to incorrect assumptions about metabolic transformations occurring *in vivo*. This, coupled with the fact that multiple metabolites are generated from atrazine in plants and animals (see Attachment 15, Major degradation steps of atrazine in plants and animals, and Attachment 16 Metabolic pathway of atrazine in the goat), has meant that many of the available company studies on the absorption, distribution, metabolism and elimination of atrazine have been somewhat piecemeal and confusing. A relatively recent study (Paul, Dunsire & Hedley, 1993) has gone a long way towards comprehensively assessing atrazine toxicokinetics in rats.

2.1 Atrazine metabolism and pharmacokinetics in *in vivo* studies in rodents.

In Sprague-Dawley rats, atrazine undergoes close to complete absorption from the GI tract after oral administration and is rapidly eliminated, predominantly in the urine. Bile is a minor elimination route. Apart from erythrocytes, tissue residues were low, and there was no evidence of accumulation, except for erythrocytes, due to s-triazine binding to haemoglobin (Hb). This binding is specific for rodent Hb due to its structure, and is not found in other species (Hamboeck *et al*, 1981). The binding of s-triazine metabolites to rodent haemoglobins appears irrelevant to other species. Atrazine undergoes almost complete metabolism; the major path is stepwise N-dealkylation via desisopropyl atrazine or desethylatrazine to the major metabolite, diaminochlorotriazine. The triazine ring remains intact (Paul, Dunsire & Hedley, 1993).

Studies by the oral route in other rat strains confirm the ostensibly complete oral absorption of atrazine from the GI tract, the predominance of the urinary excretion route, the presence of a significant number of urinary metabolites, the presence of highest residue levels in erythrocytes and the lack of formation of radioactive CO_2 from uniformly ring-labelled ^{14}C-atrazine (ie. no evidence of breakdown of the triazine ring).

The metabolism of atrazine in rats has been described in some detail (Orr, 1987). It is readily dealkylated to give either of the monodealkylated metabolites (desethylatrazine, desisopropylatrazine) which in turn can be further dealkylated, conjugated with reduced glutathione, or excreted directly. The didealkylated-s-triazine (diaminochlorotriazine; DACT), the major metabolite, is readily excreted but also may be subject to GSH conjugation. Glutathione conjugates of atrazine, desethylatrazine and desisopropylatrazine may suffer loss of any remaining alkyl substituents to give the conjugate of

DACT or may enter into the mercapturic acid pathway directly. At some point during the metabolism of these sulfur-containing conjugates, they are acted upon by a carbon-sulfur lyase to give the 2-mercapto-s-triazines. These can be excreted or methylated to give 2-methylthio-s-triazines which can be excreted directly or further oxidised to give the corresponding S-oxides; these are the electrophiles putatively involved in the covalent binding of s-triazine residues to rodent haemoglobin. A minor alternative pathway involves the oxidation of a primary carbon on the side chain of atrazine or either of the monodealkylated metabolites to a carboxylate, to give metabolites which are excreted without further modification.

Thus, the main metabolic pathway is the oxidative removal of alkyl side chains, with 2-chloro-4,6-diamino-s-triazine being the major metabolite. The 2-carbon-chlorine bond is stable to enzymic hydrolysis but is subject to conjugation via GSH transferase. Sulfur-containing metabolites are acted on to give 2-sulfhydryl-s-triazines which in are subject to methylation followed by oxidation to the corresponding S-oxides. Oxidation of the primary positions of the alkyl side chains is a minor alternative metabolic route.

2.2 Atrazine: comparative metabolism and pharmacokinetics in animals

The overall pathway for metabolism of atrazine (and other closely related chlorotriazine herbicides) is consistent across mammalian species, although quantitative differences exist due to species variations in the kinetics of individual steps. The liver is the primary site of xenobiotic metabolism, with pathway intermediates being isolated from this tissue. Metabolites isolated from tissues, excreta, milk and eggs are primarily pathway end-products. With the exception of the liver and kidney, all tissue concentrations of metabolites were lower than blood levels. All observations indicate that atrazine is rapidly metabolised in the liver, then excreted from the body with no significant accumulation and without metabolism in tissues (Thede, 1989).

In ruminants it appears that dealkylation reactions proceed more slowly than in rats, thus favouring formation of conjugates of atrazine than conjugates of the dealkylated metabolites, particularly diaminochlorotriazine (Roger, Caballa & Knaak, 1973). This possibly may explain the reported greater sensitivity of ruminants to adverse effects of atrazine than rats and other toxicology test species (Loosli, 1994).

In short-term oral dosing studies in goats (Madrid & Nichols, 1987a; Huhtanen, 1987a) and chickens (Madrid & Nichols, 1987b; Huhtanen, 1987b), the radioactive atrazine metabolite, diaminochlorotriazine (DACT) underwent a significant extent of excretion (>77% of the dose in goats within 24 h of the last dose; >81% of the dose in chickens within 24 h after the last dose). In the goat study, milk contained 2% of the total dose administered. In hen eggs, yolk levels of radioactivity ranged from undetectable to 0.363 ppm after 5 days of 0.55 mg/day of DACT feeding.

2.2.1 Hydroxyatrazine: not a metabolite in animals (?) but a plant metabolite

In a study in Sprague-Dawley rats (Bakke *et al*, 1972), 2-hydroxyatrazine and a number of other 2-hydroxylated metabolites were reported in urine (comprising, in total about 47% of urinary radioactivity). It was subsequently confirmed that the hydroxylation of the C-2 chlorine arose from the use of acidic extraction and chromatography conditions and that, using organic extraction and normal phase chromatography, there was no hydroxylation occurring at the 2-chloro position on the triazine ring of atrazine. Similarly, hydroxy metabolites identified in a peroral Sprague-Dawley rat study by Miles & Orr (1987) were artefacts of the formic acid elution used in the preliminary clean-up step acidic isolation procedure and they are actually excreted into the urine as the corresponding 2-chloro-s-triazines ie. metabolic hydrolysis of the carbon-chlorine bond does not occur (Orr, 1987). A published study using human skin microsome fractions has reported the formation of 2-hydroxy derivatives of atrazine (Ademola *et al*, 1993).

In corn treated with atrazine, hydroxyatrazine metabolites predominate (Capps, 1989).

Studies have been conducted in goats fed with radioactive hydroxyatrazine (Monford, 1991; Pickles, 1991). Over 4 days, up to 79% of the dose was accounted for in urine, faeces and GI contents, with 67% of the dose recovered in the urine and 15% in the faeces; milk contained less than 0.4%, indicating that feeding of atrazine-treated plant material to milking animals should be unlikely to leave significant residues in milk.

2.2.2 Nitrosoatrazine

In vivo formation of nitrosoatrazine did not occur in rats or goats after feeding atrazine or hydroxyatrazine simultaneously with high concentrations of sodium nitrite (Marco *et al*, 1976; Geissbuehler, 1975; Sumner & Cassidy, 1975; see main text for full citation).

2.3 Pharmacokinetics and metabolism in Rhesus monkeys and humans

In Rhesus monkeys, the principal route of elimination of an intravenous dose was the urine, with close to quantitative recovery in excreta over 72 h (Hui *et al*, 1995a). Two major metabolites found in urine were desethylatrazine and diaminochlorotriazine; no atrazine or desisopropylatrazine was detected. Plasma contained atrazine, desethylatrazine, diaminochlorotriazine and desisopropylatrazine. The expected glutathione pathway metabolite, the mercapturic acid of atrazine (atrazine mercapturate), was detected primarily in urine collected to 24 h (Simoneaux *et al*, 1996).

Six adult volunteers ingested a single 0.1 mg/kg oral dose of atrazine and blood and urine samples collected for 168 h (Davidson, 1988). Atrazine and desisopropylatrazine were detected in blood at very low levels. Desethylatrazine and diaminochlorotriazine were identified in blood for up to 24 h. Desethylatrazine appeared rapidly, peaking near 2 h and then rapidly declined with a half-life of 2.8 h; this probably resulted from liver P-450 oxidative dealkylation of atrazine. Desethylatrazine was renally eliminated and also further dealkylated to diaminochlorotriazine, which peaked in blood at about 5 h. Diaminochlorotriazine also appeared in urine, and possibly also undergoes further metabolism. Both metabolites disappeared from the blood with first order kinetics. Atrazine was undetected in urine. Desethylatrazine, desisopropylatrazine and diaminochlorotriazine accounted for 5.4%, 1.4% and 7.7% of the dose, respectively. Other metabolites were not been detected or quantified. Since significant amounts of atrazine were not found in blood or urine, this left 85% of the dose unaccounted for. Possibly atrazine was incompletely absorbed; however, in rodents the extent of absorption is >70%, even when administered in the diet. Another possibility is complete ring cleavage and metabolism to CO_2 and N_2; however, evidence from studies in rodents, goats, chickens and plants is that the triazine ring is biologically stable. Other possibilities include extensive biliary excretion or else, there are many other metabolites excreted into the urine which were not extracted and identified.

After percutaneous absorption of atrazine in male volunteers, desethylatrazine and diaminochlorotriazine were found in urine. The expected glutathione pathway metabolite, the mercapturic acid of atrazine (atrazine mercapturate), was detected at trace levels. No atrazine or desisopropylatrazine was detected. Plasma contained atrazine, desethylatrazine, desisopropylatrazine and diaminochlorotriazine (Simoneaux, 1996).

2.4 Dermal absorption studies

In *in vitro* and *in vivo* dermal absorption studies in rats and humans, atrazine in a suspension formulation (Gesaprim 500FW) and in a water-dispersible granule formulation (Gesaprim 90WDG) was poorly absorbed through both rat and human epidermis *in vitro*. The quantity of atrazine absorbed from the 500FW formulation by human skin was independent of the applied dose and remained constant at about 24 µg equiv/cm^2. Over 48 h, only 0.06%, 2.57% and 5.96% of the high-, mid- and low doses (1:1, 1:100 and 1:200 water dilutions of the 500 g/L suspension) were absorbed through human skin. The human epidermis was less penetrable than the rat epidermis as the absorption rates were 7- and 2-fold lower at the high and mid/low dose than those in the rat epidermis (Jack, 1994). Comparison of the extent of excretion (urine and faeces) after dermal application of Aatrex 4L formulation to animals and humans suggests this may also be the case *in vivo* (Chengelis, 1994; Hui *et al*, 1995).

In an *in vivo* study in men, the extent of percutaneous absorption of 5.63% and 1.18% at topically applied doses of 6.67 and 79 µg/cm2 was compared with rat

data of 24.4% and 26.2% for 10 and 100 µg/cm^2, respectively (Hui *et al*, 1995b). On this basis, it was claimed that the extent of percutaneous absorption in man was much less than in rats. However, in another rat dermal absorption study (26.9% and 21.6% 'absorbed' after 10 h at 10 and 100 µg/cm^2), most of the radioactivity considered to be absorbed was found in the dissolved skin after removal of surface radioactivity by detergent washing, with less than 1% of the dose excreted (Murphy & Simoneaux, 1987). From this comparison it is not appropriate to unequivocally conclude that the extent of percutaneous absorption in man is much less than in rats, although rats appear to retain much more in the skin (not removable by surface washing) than do humans, and absorption can continue from this depot.

The percutaneous absorption of radioactive atrazine in human skin was examined utilizing a flow-through *in vitro* diffusion system. About 16.4% of the applied dose was absorbed by the skin at 20 h, with <5% in the receptor fluid and 12% in skin supernates. Desisopropylatrazine and DACT were found in receptor fluid and skin supernates, and desethylatrazine in skin supernates. Since desisopropylatrazine represented about 50% of the total metabolites formed, this was a key step in the metabolism of atrazine. Further metabolism may proceed by cleavage of the N-deethyl group to give totally dealkylated atrazine. In a skin microsomal fraction supplemented with an NADPH-generating system, atrazine was metabolized to desisopropyl- and desethylatrazine; in addition, 2-hydroxy derivatives of atrazine were reported to be formed.

2.4.1 *In vitro* studies on atrazine metabolism

In a series of *in vitro* metabolism studies with atrazine, the following findings were reported:

- using rat liver subcellular fractions, N-dealklation was associated with the microsomes and the isopropyl group was more readily cleaved than was the ethyl substituent. Conjugation with reduced glutathione was associated with the cytosol and was a slower reaction than N-dealkylation. There was no evidence for the direct formation of 2-hydroxy-s-triazines *in vitro* (Dauterman & Muecke, 1974).

- Because of their established or presumed toxicological significance, nitroso derivatives of atrazine were studied, even though the formation of such compounds in soil, water, plants or animals has never been observed/reported. In liver fractions from male rats, nitrosoatrazine was exclusively metabolised by the GSH pathway while nitroso-hydroxyatrazine was very efficiently denitrosated. Radioactive nitrosoatrazine and nitrosohydroxyatrazine were rapidly eliminated from organs and tissues (with the exception of nitrosamine in erythrocytes, a rodent Hb specific feature for chlorotriazines) (Muecke, 1993).

- Following incubations with radiolabelled atrazine, Fischer rat, goat and human hepatocytes produced similar patterns of metabolites whereas the pattern arising from incubation with hepatocytes from Sprague Dawley (SD) rats was significantly different. The major compounds isolated from Fischer rat, goat and human hepatocyte incubations were unmetabolised atrazine and the two monodealkylated derivatives, desethylatrazine and desisopropylatrazine. In contrast, SD rat hepatocytes further metabolised these derivatives to the didealkylated metabolite, diaminochlorotriazine and a collection of unidentified polar metabolites (Simoneaux & Thede, 1988).

- The primary products of guinea pig, goat and human isolated hepatocyte incubations were the two monodealkylated derivatives, desethylatrazine and desisopropylatrazine, with minor evidence of further metabolism to didealkylated metabolite, diaminochlorotriazine. The different species appear to share common pathways, with different kinetics in each step of those pathways eg. rats appear to preferentially deisopropylate atrazine whereas CD-1 mice and humans preferentially deethylate it. Guinea pig, goat and Rhesus monkey hepatocyte incubations produced roughly equal amounts of the two monodealkylated metabolites. Diaminochlorotriazine accounted for 50-70% of total radiolabel in 24 h incubations with hepatocytes from CD-1 mice, rats (both strains) and Rhesus monkeys, but only 3-10% in incubations with guinea pig, goat and human hepatocyte cultures. Species and strain differences were also apparent in Phase II metabolism - SD rat, Fischer rat, CD-1 mouse and human hepatocyte incubations displayed very different arrays of Phase II metabolites (Thede, 1988). [The results of this study appear to somewhat at odds with some of the findings of the previous study (Simoneaux & Thede, 1988) insofar as that study suggested that major compounds isolated from Fischer rat, goat and human hepatocyte incubations were unmetabolised atrazine and the two monodealkylated derivatives, desethylatrazine and desisopropylatrazine whilst SD rat hepatocytes further metabolised these derivatives to the didealkylated metabolite, diaminochlorotriazine. Yet this study indicated that not only hepatocytes from SD rats but also from CD-1 mice, Fischer rats, and Rhesus monkeys rapidly metabolised atrazine to the didealkylated chloro-metabolite of atrazine, diaminochlorotriazine.]

- However, both studies (Simoneaux & Thede, 1988 and Thede, 1988) indicated that in human hepatocytes, the primary metabolites were the two monodealkylated derivatives, desethylatrazine and desisopropylatrazine, with minor evidence of further metabolism to the didealkylated metabolite, diaminochlorotriazine, in contrast to SD rats in which atrazine is rapidly metabolised to the didealkylated chloro-metabolite, diaminochlorotriazine. Thus, there could be some possible doubt as to the appropriateness of SD rats, in particular, as models for the toxicity of atrazine to humans. In *in vivo* studies in humans, the available data suggests an apparent significant discrepancy in the amount of DACT found; a study by Davidson (1988) recovered only 77% of an oral 0.1 mg/kg dose of atrazine in the urine as DACT, whereas a study by Catenacci et al (1993) suggested that in exposed workers (dermal and inhalation exposure), 80% was recovered in urine as

DACT. (This latter report has not been evaluated in detail, only the abstract has been reviewed).

3. ACUTE TOXICITY

3.1 Atrazine

A large number of acute toxicity studies have been performed on atrazine, its metabolites and several product formulations (end-use products, or EUPs). It may be concluded that, in laboratory test species, atrazine generally has low to very low acute oral, intraperitoneal and subcutaneous toxicity, low dermal toxicity, low inhalational toxicity, possibly may be a mild eye irritant, but is not a skin irritant. Conflicting results were obtained in animal studies designed to investigate skin irritation potential, although atrazine does not appear to be a dermal sensitizer in humans.

Atrazine is of low acute oral, subcutaneous and dermal acute toxicity with the lowest acute oral LD50 in mice of 1750 mg/kg (highest reported value: >10000 mg/kg) and in rats of 1869 mg/kg (highest value >8500 mg/kg); subcutaneous LD50's in mice and rats were >5000 mg/kg; and acute dermal toxicity LD50 values in rats were determined as being over the range >2000 to >3100 mg/kg and in rabbits, >4500 mg/kg.

Atrazine was generally of low acute intraperitoneal toxicity in rodents, with the LD50 in mice being 626 mg/kg whilst in rats, LD50 values in different studies ranged between 235 to >5000 mg/kg!

2

Acute inhalation was moderate to low, with an LC50 value of an atrazine mist aerosol in rats (4 h, nose only) being >710 mg/m^3 whilst in three other 4-hour exposure studies (two nose-only, the other a whole-body exposure), the LC50 was >5000 mg/m^3 .

In 4 separate studies, atrazine was either not an eye irritant or only a slight eye irritant in rabbits. Atrazine was not a skin irritant in rats, rabbits or humans.

Three skin sensitisation studies in guinea pigs gave conflicting results, with atrazine determined as a non-sensitiser, a sensitiser and a strong sensitiser. It was not clear why such a discrepancy was observed since these studies were conducted between 1985-1994 and were all conducted according to established test guidelines and GLP.

A study in humans, using a 0.5% w/v suspension of an 80W formulation in water, determined it not to be a sensitiser. Additionally, patch tests conducted on fifty human volunteers did not reveal any evidence of contact sensitivity with atrazine (atrazine 80W-F1-2858; composition not given) (Shelanski & Gittes, 1965). Correspondence from medical officers at Ciba-Geigy's St Gabriel and McIntosh manufacturing plants in the United States certified that

no cases of skin irritation or other atrazine-related illness have been seen at the plants (Cronan, 1988; Charters, 1989).

3.2 Atrazine metabolites

The atrazine metabolites tested viz. desethylatrazine, desisopropylatrazine, diaminochloro-triazine and hydroxyatrazine, all had low acute oral toxicity. The oral LD50s in rats was >5000 mg/kg for diaminochlorotriazine and for the plant metabolite, hydroxyatrazine but the two monodealkylated atrazine metabolites had lower LD50s: desethylatrazine (1890 mg/kg for males, 668 mg/kg for females) and desisopropylatrazine (2290 mg/kg for males, 810 mg/kg for females). [In the same study, the LD50 for technical atrazine was 3517 mg/kg (both sexes)].

Diaminochlorotriazine was a mild-moderate eye irritant and a slight (intact skin) or mild (abraded skin) skin irritant in rats.

3.3 Atrazine formulations

The tested formulations of atrazine were of low oral, dermal and inhalational toxicity.

The oral LD50 in rats for 'Gesaprim 500FW', a 500 g/L SC preparation, was >3000 mg/kg.

Marksman Herbicide', a mixture containing 250 g/L atrazine and 130 g/L dicamba, had an oral LD50 of approx. 5900 mg/kg, a dermal LD50 of >2000 mg/kg, and an inhalation LC50 of >3380 mg/m^3 (rats). It was a slight skin and eye irritant (rabbits) but not a skin sensitiser (guinea pigs).

4. SHORT-TERM REPEAT-DOSE STUDIES

4.1 Atrazine

In immature Tif:RAIf(SPF) rats, atrazine (at up to 400 mg/kg bw/d for 14 days) produced substantial retardation in body and organ weight gain (spleen, thymus and, additionally, in females, brain, liver and ovary). Over a two-week recovery period, weights had recovered to almost normal. The lack of historical data for young, rapidly-maturing rats makes some observed changes in blood chemistry, haematology and histopathology more difficult to interpret than in adults, but the fact that the effects were largely reversible suggests that atrazine delayed maturation but did not produce long-lasting effects, and there was no evidence of permanent effects on the immune system, haematopoietic tissues or endocrine organs. This non-standard study did not demonstrate a NOEL, although effects were minimal at 25 mg/kg bw/d [non-significant, very

marginal lower bodywt than controls; ALT increased about 24% cf. controls; slightly reduced absolute organ weights (spleen and thymus in males, liver, thymus and ovary in females); no ovulation in 1/10 females] (Fitzgerald, 1988). In a study in which atrazine (oral gavage at doses of 0, 100, 200, 400 mg/kg bw/d for at least 2 weeks; high dose reduced to 300 mg/kg on day 4) was given to female rats, there was one death at the 400 mg/kg dose. Bodyweight and thymus weights were reduced in all groups. Animals appeared thin and hunched at the 2 highest doses. Atrazine reduced mean oestrogen levels at 200 mg/kg and above, luteinizing hormone was lower at 300 mg/kg, while other hormones were unaffected. It was concluded that such effects could occur at lower doses in chronic studies, thus influencing mammary tumour development in a strain of rats already predisposed to developing them (Morseth, 1990).

A 4-week Wistar rat dietary study, (0, 100, 500, 2000 and 4000 ppm atrazine; estimated intake 5, 25, 100 and 200 mg/kg bw/d) showed decreased food consumption at all doses, reduced weight gain at 25 mg/kg bw/d and above, and increased relative testes weights at 100 and 200 mg/kg bw/d. Assuming the reduced food intake was a reflection of palatability of the diet, a NOEL for this study could be set at 100 ppm (est. 5 mg/kg bw/d), based on these effects.

In a 21-day dermal study in rabbits (0, 10, 100 and 1000 mg/kg bw/d atrazine), the NOEL was determined to be 10 mg/kg/d (females), based on small effects on bodyweight gain and a possible increase in plasma cholesterol at the next highest dose of 10 mg/kg/d. The NOEL in males could be set at 100 mg/kg/d, based on bodyweight and food consumption effects, a small decrease in mean erythroid parameters and WBC counts, a small decrease in mean total serum protein, a decrease in mean serum chloride, and an increase in spleen weight (absolute and relative), all observed at the next highest dose of 100 mg/kg/d (Huber *et al*, 1989).

4.2 Desethylatrazine

No short-term repeat-dose studies provided.

4.3 Desisopropylatrazine

No short-term repeat-dose studies provided.

4.4 Diaminochlorotriazine

Female Sprague Dawley rats were treated with diaminochlorotriazine (DACT) by gavage (0, 100, 200 and 400 mg/kg bw/d) for at least 2 weeks. There was one death during the first few days in the high-dose group, with a further 7 after reducing the high dose to 300 mg/kg/d on day 4. Bodyweight and thymus weights were reduced in all groups. Animals appeared thin and hunched at the 2 highest doses. Absolute spleen weights were decreased at 300 mg/kg. Leutinizing hormone was decreased in all DACT groups, oestrogen and progesterone were lower at 200 mg/kg/d and above, and prolactin was decreased at 100 and 200, but not at 300 mg/kg/d (Morseth, 1990).

In a pilot 4-week dietary study with DACT in beagle dogs, a suitable upper dose for testing was considered to be less than 1500 ppm. The NOEL was considered to be 500 ppm (14.1 mg/kg/d in males, 13.9 mg/kg/d in females), on the basis of the faecal, bodyweight, food consumption and cardiac effects at 1000 ppm (21 mg/kg bw/d in males, 27.2 mg/kg/d in females) and above (Swallow *et al*, 1989).

4.5 Atrazine formulations

A dermal toxicity study in New Zealand White rabbits with 'Marksman Herbicide' (containing 250 g/L atrazine and 130 g/L dicamba) utilised topical application to the skin under occlusive bandages for 6 hours/day on 15 or 16 occasions over 21 days, at doses of 0, 40, 200 and 1000 mg/kg bw/d. No significant treatment-related gross pathological findings were detected apart from those of skin. At the high dose, mild hyperkeratosis and acanthosis was seen. No other significant histological lesions were found. It was concluded that Marksman Herbicide causes slight to moderate dermal irritation, but no systemic toxicity when repeat dermal doses are given at up to 1000 mg/kg bw/d (Morrow, 1986).

5 SUBCHRONIC STUDIES

5.1 Atrazine

A 90-day Sprague-Dawley rat dietary study (0, 200, 1000 and 5000 ppm) showed mortality, reduced bodyweight and food consumption, haematological changes, arrested spermato-genesis at higher doses. Disregarding the very small effect of atrazine on bodyweight gain during week 1, the NOEL in this study may be taken as 200 ppm (estimated 20 mg/kg bw/d), on the basis of reduced food intake, body weights, calcium deposition in the renal pelvis and splenic haematopoiesis at the next higher dose of 1000 ppm (Tisdel & Harrison, 1977a). Given the concern about the possible endocrine disruptor

effects of atrazine, the finding of atrophic degenerate testes with no spermatogenesis at the high dose of 5000 ppm was noted.

A 3-month dietary study in Wistar rats at 0, 20, 100 and 1000 ppm showed reduced food consumption and weight gain at the high dose only. On the basis of these findings, the NOEL was 100 ppm (est. 5 mg/kg bw/d) (Amalgamated Chemicals, 1984).

Sprague-Dawley derived rats (Tif: RAIf (SPF) hybrids of RII/1 x RII/2) were given atrazine in the diet (0, 10, 50 and 500 ppm) for 13 weeks. The NOEL for atrazine in this study was 10 ppm (0.60 mg/kg bw/d) for males, 50 ppm (3.35 mg/kg bw/d for females), on the basis of depressed bodyweight gain at the next highest dose of 50 ppm (3.3 mg/kg bw/d) in males, and depressed bodyweight gain and food intake, increased water intake, increased relative liver weight, and splenic haemosiderin deposition, at the next highest dose of 500 ppm (35.3 mg/kg bw/d) in females. Over a one-month recovery period, a partial recovery of bodyweights took place at the high dose, food intake recovered (with a small overcompensation in high-dose females), increased water intakes returned to normal and the increased relative weight of liver in high-dose females returned to normal; in high-dose females there was little evidence of recovery in the increased incidence of splenic haemosiderosis (Bachmann, 1994).

A 90-day dog dietary study (0, 200, 632 and 2000 ppm) showed anaemia, decreased bodyweights and food consumption at higher doses. The initial histopathology report from the Warf Institute (Madison, Wisconsin) indicated arrested or decreased spermatogenesis at 632 and 2000 ppm; however, detailed re-investigation of the slides by another pathologist from Hazleton Labs America (Madison, Wisconsin) concluded that “no compound-related changes were observed in the testes examined histopathologically”. On the basis of effects on bodyweight and food intake seen at the lowest dose used in this 90-day dog study (200 ppm), a NOEL was not established. Discounting the food intake and bodyweight effects at the low dose, a NOEL of 200 ppm (approx. 5 mg/kg bw/d) could be set, based on reduced testicular weights (other signs of testicular toxicity are in question in this study) and anaemia at the next highest dose tested of 632 ppm (est. 16 mg/kg bw/d) (Tisdel & Harrison, 1977b).

5.2 Desethylatrazine

Desethylatrazine was administered in the diet to Sprague-Dawley derived rats (approx. 6-weeks old) at 0, 500 and 1000 ppm for 13 weeks. There were relatively limited adverse effects at both doses, viz. reduced bodyweight gain and food intake, so that animals were up to 17% and 23% lighter than controls in the respective groups. Mild anaemia was seen at 1000 ppm. A NOEL was not established in this study (Drake, 1971a).

Desethylatrazine was administered in the diet to rats (Tif: RAIf (SPF) hybrids of RII/1 x RII/2) at 0, 10, 50 and 500 ppm for 13 weeks. Only minor adverse

effects were seen, restricted to the highest dose. The NOEL for desethylatrazine in this study was 50 ppm (calculated as 3.2 mg/kg bw/d in males, 3.35 mg/kg bw/d in females), based on reduced bodyweight and food intake (both sexes), minimal changes in haematology and AP activity (females), and a small increase in relative liver weight (females) at the next dose of 500 ppm [35.2 (males) and 38.7 (females) mg/kg bw/d] (Gerspach, 1991).

Desethylatrazine was administered in the diet to beagle dogs at 0, 15, 100 and 1000 ppm for 13 weeks. The findings suggested that the target organs for desethylatrazine in dogs were the heart and kidney. Electrocardiographic findings of fibrillation in 1 of 4 high-dose females and haemorrhagic inflammation in the right atrial wall of the heart of 1 of 4 high-dose males indicated possible cardiac effects, whilst mild renal tubular epithelial hyperplasia/basophilia was reported in 3 males and 2 females at the high dose. On the basis of these effects at 1000 ppm (28.9-32.2 mg/kg bw/d), the NOEL for this study was 100 ppm [3.7 (males) and 3.9 (females) mg/kg bw/d] (Rudski *et al*, 1992).

5.3 Desisopropylatrazine

Desisopropylatrazine was administered in the diet to Charles River albino rats at 0, 500 and 1000 ppm for 90 days. Desisopropylatrazine, at 1000 ppm in the diet of rats led to reduced weight gain in males and a slight increase in relative weight of the liver in high-dose females. On the basis of a small effect on bodyweight gain at the lowest dose tested of 500 ppm, a NOEL for desisopropylatrazine in this study was not established (Smith, 1971).

Desisopropylatrazine was administered in the diet to 5 to 6-week old rats (Tif: RAIf (SPF) hybrids of RII/1 x RII/2) at 0, 10, 50 and 500 ppm for 13 weeks. At 500 ppm it resulted in activation of the thyroid gland and hypertrophy of TSH-producing cells in the pituitary gland of males, and a degree of extramedullary haematopoiesis in the spleen and liver of females, together with a slight increase in relative weight of the liver in high-dose females. The NOEL for desisopropylatrazine in this study was 50 ppm (3.2 mg/kg bw/d in males, 3.3 mg/kg bw/d in females), based on these findings at the high dose [34.9 (m) and 37.5 (f) mg/kg bw/d] (Schneider, 1992).

Desisopropylatrazine was administered in the diet to beagle dogs at 0, 15, 100, 500 and 1000 ppm for at least 14 weeks. Effects related to the dosing of desisopropylatrazine in dogs were seen at dietary concentrations $\geq$ 500 ppm and were limited to reductions in bodyweight parameters, food consumption and possible heart weight decrease. On the basis of these effects, the NOEL for this study was 100 ppm (3.8 mg/kg bw/d). Anaemia was seen to develop in 2 of 8 dosed dogs (littermates, although this was reasoned to be unrelated to treatment. In view of the small number of animals per group, it is not possible to unequivocally conclude whether or not this is the case (Thompson, Batastini & Arthur, 1992).

5.4 Diaminochlorotriazine

Diaminochlorotriazine was administered in the diet to Sprague-Dawley (SD) derived rats (approx. 6-weeks old) at 0, 500 and 1000 ppm for 13 weeks. Treatment resulted in some adverse effects at both doses tested viz. reduced bodyweight gain and mild anaemia. Increased weights of thyroids (high dose males and dosed females) and lungs (both doses) were noted, although no pathological findings were reported for any of these organs. A NOEL was not established in this study (Drake, 1971).

Diaminochlorotriazine was administered in the diet to Crl:CD (SD) BR rats (approx. 6-weeks old) at 0, 10, 100, 250 and 500 ppm for 90 days (Pettersen *et al*, 1991). Oestrus cycle data were reported in a study addendum (Pettersen *et al*, 1991). DACT resulted in reduced bodyweight gain in females at 250 ppm and both sexes at 500 ppm. At 100 ppm and above, the compound affected oestrus cycling; a statistical analysis of oestrous cycle data collected during the study indicated that this metabolite increased both the length of the oestrous cycle and the incidence of persistent oestrus in rats at doses of 100 ppm or over. Analysis of serum oestradiol, progesterone, prolactin and corticosterone did not show any treatment-related effects but there were only a limited number of animals sampled. The NOEL for DACT in this study was 10 ppm (0.7 mg/kg bw/d), based on the oestrous-cycle effects at 100 ppm (7.6 mg/kg bw/d).

5.5 Hydroxyatrazine

A 13-week rat feeding study with hydroxyatrazine (0, 10, 100, 300 & 600 ppm) showed a small reduction in bodyweight gain (accompanied by some reduction in food consumption in males) at the high dose of 600 ppm. The main target organ of the metabolite appeared to be the kidney, with treatment-related decreases in urine specific activity and increases in urine volume at the high dose. This was associated with gross and microscopic histopathological kidney changes in both sexes (toxic nephrosis), minimal to mild at 300 ppm, very apparent at 600 ppm. The NOEL for these effects was 100 ppm (6.3 mg/kg bw/d in males, 7.35 mg/kg bw/d in females) (Rudzki, McCormick & Arthur, 1989).

A 13-week beagle dog feeding study with hydroxyatrazine (0, 15, 150, 1500 and 6000 ppm) showed a treatment-related reduction in bodyweight gain, accompanied by some initial reduction in food consumption at doses of $\geq$ 1500 ppm. The main target organ was the kidney, with treatment-related decreases in urine specific activity and increases in urine volume. This was associated with gross and microscopic histopathological kidney changes in both sexes (chronic nephropathy). The NOEL for these effects was 150 ppm (5.8-6.2 mg/kg bw/d) for both male and female dogs (Chau, McCormick & Arthur, 1990).

6 CHRONIC STUDIES

6.1 Atrazine

In an early published study (Innes *et al*. 1969), two strains of mice [(C57BL/6 x C3H/Anf)F1 and C57BL6 x AKR)F1] were given atrazine (source and purity unstated) in 0.5% gelatine by gavage from 7 days of age at an initial dose of 21.5 mg/kg bw/d (not adjusted with increasing bodyweight) up until 4 weeks of age, then the compound was administered in the diet at 82 ppm until necropsy at approximately 18 months. Atrazine and simazine were included in a table of compounds which did not cause a significant increase in tumours after oral administration, at a dose which was claimed to be a maximally-tolerated dose (MTD). Otherwise, no further information was given about any findings after the administration of these two compounds and the report is of limited value in assessing atrazine toxicity.

A 21-22-month CD-1 mouse dietary oncogenicity study with atrazine (0, 10, 300 and 1000 ppm; est. 0, 1.5, 45 and 150 mg/kg bw/d) showed possible slight increases in alveolar cell tumours. However, there was no obvious dose-related increase and the upper incidences were within historical control levels, it was considered that these observations were unrelated to treatment with atrazine. The NOEL may be taken as 300 ppm (estimated as 45 mg/kg bw/d), based on increased mortality (female only), reduced body weight (both sexes), and macroscopic kidney pathology (granular/irregular surface, or pale/white appearance) at the next dose of 1000 ppm (Sumner, 1981). A validation Report of this IBT study indicated that it was performed in accordance with its protocol, and that deficiencies in study were generally of a minor nature and would not have affected the final conclusions.

Atrazine did not affect the incidences of neoplasms in CD-1 mice at levels of 0, 10, 300, 1500 or 3000 ppm in the diet for 91 weeks (0, 1.2, 38.4, 194 and 450 (male) and 0, 1.6, 47.9, 246.9 and 482.7 (female) mg/kg bw/d. Survival of high-dose females was reduced, mean bodyweight and bodyweight gain was reduced at 38.4/47.9 mg/kg bw/d and above, mean food consumption, water intake, haemoglobin, RBC, and haematocrit were reduced, and the incidence of atrial thrombi was increased at 194-246.9 and 450-482.7 mg/kg bw/d. The NOEL was at least 1.2-1.6 mg/kg bw/d, based on small decreases in bw/bw gain at the next higher dose of 38.4/47.9 mg/kg (Hazelette & Green, 1987).

Albino rats were fed at 0, 1, 10 and 100 ppm of atrazine 50W (a fine, wettable, light beige powder "reportedly" containing 48.25% active ingredient; all doses were calculated in terms of this analysis) in diet until 65 weeks when the 1 ppm group was switched to 1000 ppm. The study was terminated at 104 weeks. A NOEL of 100 ppm (approx. 4 to 6 mg/kg bw/d) was determined for the effects of atrazine in depressing body weight and food consumption. However, this was a relatively minor effect and its significance is clouded by the fact that the 1000 ppm dose was not started until week 65 of the test. The study was of limited value because of the low survival rates and the fact that a limited

number of "representative animals" were taken for a limited range of assays (Keller, 1961)

In a two-year IBT study in Charles River strain albino rats, atrazine was administered in the diet at 0, 10, 100 and 1000 ppm (Spindler & Sumner DD, 1981). Mixing of samples and errors in pathology records meant that re-evaluation of the microscopic pathology could not be completed and the study could not be validated. The deficiencies in the study do not allow for the establishment of an overall NOEL and the study is not useful in assessing the chronic toxicity or oncogenicity of atrazine.

Sprague-Dawley CD rats fed technical atrazine at 0, 10, 70, 500 and 1000 ppm in the diet for 2 years showed reduced food consumption, bodyweight gain, anaemia, decreased blood glucose and triglycerides at 500 and 1000 ppm. Survival of high-dose females was lower than controls. A number of pathological effects, some of which were of doubtful significance, were noted at 1000 ppm; these included retinal degeneration, increases in kidney calculi and microcalculi in males, centrilobular degenerative changes in the livers of female rats, and hyperplasia in a number of tissues. Possible neoplastic changes were restricted to the testes of male rats and the mammary glands of females. The incidence of the testicular interstitial cell tumours was as follows: 1/65 (control), 3/65 (10 ppm), 2/67 (70 ppm), 2/67 (100 ppm) and 7/67 (1000 ppm), i.e. there was no clear dose-response relationship and the increased incidence in the 1000 ppm group was not marked, particularly in view of the increased survival to term (67% *vs* 44%) at this dose. Fibroadenomas and adenocarcinomas were found in the mammary glands of female rats; percentages of female rats with such tumours (all types, single or multiple) were as follows: control, 53.0%; 10 ppm, 60.9%; 70 ppm, 69.1%; 500 ppm, 72.3%; 1000 ppm, 87.5% and the total number of tumours/rats examined was control, 55/66; 10 ppm, 68/64; 70 ppm, 91/68; 100 ppm, 130/65; 1000 ppm, 138/64. Mammary tumours were significantly increased at 500 and 1000 ppm and trend analysis indicated 10 ppm as a NOEL (approx. 0.4 to 0.6 mg/kg bw/d). In male rats a NOEL may be set at 70 ppm (approx. 2.4-3.3 mg/kg bw/d), on the basis of decreased body weight, behavioural effects, palpable tissue masses (sides) and skeletal muscle (rectus femoris) degeneration at the next highest dose of 500 ppm (Mayhew, 1986).

In a published study (Pinter *et al*. 1990) conducted by the Hungarian National Institute of Hygiene using Fischer 344/LATI rats, there was an increase in benign mammary tumours in males (although with a possible slight increase in latency cf. those tumours in control animals), an increase in malignant uterine tumours in females, and an increase in haematopoietic system tumours. It is possible that atrazine treatment may have affected hormonal balance since the mammary gland and uterine tumours may be hormone-dependent tumours. There was no increase in mammary tumours in females, while the dosed males actually had a slightly increased longevity cf. control animals. The increase appears to be largely attributable to the significantly longer lifespan of the high-dose animals. However, neither tumour to age adjustment nor comparison to background control data of the laboratory were performed in this study

(historical control data was not available). In a later Hazleton study, no increase in mammary tumours was noted in male Fischer 344 rats at the HD (400 ppm); the increase in the Pinter study was only seen at 750 ppm, not at 375 ppm.

Sprague-Dawley rats, taken from the F1 generation of a 2-generation reproduction study (no. 852063), were dosed with atrazine at 0, 10, 50 or 500 ppm for 8, 35, 52 or 104 weeks; these rats had been previously exposed to the same dose levels *in utero*. Considering non-neoplastic changes, treatment-related changes at 500 ppm included reductions in mean body weight and food consumption (both sexes); reduction in mean erythroid parameters (females) and increases in mean serum cholesterol (females). The latter finding was also seen at 50 ppm. On the basis of these LOELs, the NOEL in males was 50 ppm (2.3 mg/kg bw/d) and 10 ppm (0.7 mg/kg bw/d) in females. Mammary tumours were not significantly increased in treated female rats. [A previous study in the same strain (Mayhew, 1986) showed significant increases in mammary tumours at 500 and 1000 ppm (72% and 88% incidence respectively); this difference between the two studies cannot be accounted for on the basis of differences in control incidences, which were almost identical viz. 55% & 53% incidence]. Whilst there was an apparent increase in pituitary tumours in high-dose females, the incidence in control, low- and mid-dose animals was unusually low and that the incidence at the high-dose was well within the normal range of incidence for this strain and laboratory. Of the mammary tumour-bearing animals with pituitary neoplasms, 75% of the pituitaries stained positive for prolactin. Thus, results from this complex protocol indicate some hormonal influence of the pituitary on the mammary gland but the exact significance is equivocal (Rudzki, McCormick & Arthur, 1991; Ackerman, 1991).

[Of the following 4 studies in female rats, two (one SD and one Fischer 344 study) were designed to investigate any correlation of serum hormone levels and oestrous cycle data with the onset of mammary tumours.]

Two female SD rat studies (both using two dosage levels of 70 and 400 ppm in the diet) showed a negative trend in survival at the high dose (400 ppm) of atrazine (due to earlier onset of deaths in one study and an increased incidence in the other). There was also an earlier onset of palpable masses in both studies at the high dose. Mean bodyweight and bodyweight gains were reduced in both the low-dose (70 ppm) and high-dose groups in one study and the high-dose group only, in the other; this was associated with an initial slight reduction in food consumption. One study showed an increased incidence of fluid-filled uteri and the non-neoplastic lesion, mammary galactocele, in both the high-dose and low-dose groups (Osherhoff, 1990a; Thakur, 1991a). However, neither of these effects were noted in the other study (Thakur, 1992a). The 2 studies showed that there was an earlier onset of mammary and pituitary tumours in the high-dose groups from both studies, without an overall increase in their life-time incidence. It was not possible to set a NOEL for one study (Osherhoff, 1990a; Thakur, 1991a) because of bodyweight effects and fluid-filled uteri at the LD. In the other study, however (Thakur, 1992a), the NOEL

was 70 ppm (3.0-3.5 mg/kg bw/d), based on reduced bodyweight, slightly increased mortality rate and earlier onset of mammary tumours at the high dose (approx. 21 mg/kg bw/d). In both studies the NOEL for the earlier onset of mammary tumours was 70 ppm (3.0-3.7 mg/kg bw/d). There was no effect of atrazine on the incidence of pituitary tumours in either study.

From hormone assays (oestradiol and progesterone) and oestrous cycle data, it was suggested that atrazine above a certain threshold may accelerate the ageing of the neuroendocrine system, leading to an earlier onset of mammary (and pituitary) tumours.

Two carcinogenicity studies were conducted in Fischer F344 rats, the first (Osheroff, 1990b; Thakur, 1991b; Eldridge *et al*, 1993) designed to investigate the effect of chronic atrazine dosing on the oestrous cycle and on selected hormone levels (oestradiol, progesterone). Both studies showed that the only toxicological effect of atrazine was a slight reduction in bodyweight/bodyweight gain, accompanied by an initial reduction in food consumption. In this strain there was no effect on survival, palpable masses or on any tumour incidence, including pituitary and mammary tumours. In the second study (Thakur, 1992b), an increase in high-dose males (400 ppm) of the non-neoplastic kidney lesion, transitional cell hyperplasia, was noted. Based on bodyweight reductions, the NOELs for both studies were 70 ppm (3.4-4.2 mg/kg bw/d).

Investigation of oestrous cycling and assays of hormone levels in the first study (Osheroff, 1990b; Thakur, 1991b; Eldridge *et al*, 1993) did not find any effects of atrazine on the percentage of days female Fischer rats spent in oestrus. Normal age-related changes in control and treated animals included a shift in oestrous cycle patterns towards an enhancement of the percent of total days spent in proestrus at the expense of days in oestrus. Whereas the time Sprague-Dawley controls spent in oestrus tended to increase with age, that in Fischer rats tended to decrease, especially after 15 months. Serum progesterone levels rose continuously throughout the study whereas oestradiol levels increased over approx. the first year then declined. Oestradiol and progesterone concentrations were not significantly affected by atrazine dosing.

A study was conducted to determine the effect of atrazine dosing for 12 months on the mammary and pituitary glands of female SD rats, the oestrous cycle, and plasma levels of oestradiol, luteinising hormone, progesterone and prolactin. Atrazine was administered to female Crl:CD (SD) BR rats (approx. 6 weeks old) at dietary levels of 0, 15, 30, 50, 70 and 400 ppm for up to 12 months. There did not appear to be any information on blood hormone levels; it is assumed that these will be reported in a study addendum. There were no statistically significant differences in the incidence of mammary gland adenomas fibroadenomas or adenocarcinomas at feeding levels up to 400 ppm, when analysed individually, although when combined, there was a significant increase at 400 ppm. A significant positive trend for onset time existed when adenomas and fibroadenomas were combined or when all three tumour types were combined. No trend was evident if 400 ppm results were excluded. The

NOEL for mammary tumour incidence and onset time was 70 ppm (4.1 mg/kg bw/d) (Pettersen & Turnier, 1995). Findings of this study were consistent with previous studies which indicated that, although the number of SD female rats with tumours increased at 12 months, this increase resulted from an earlier onset, without an overall increase in mammary tumour incidence following lifetime (2-year) exposure.

Following the completion of this ECRP review, Novartis submitted an interim report of a further 2-year SD rat dietary study (0, 25, 50, 70 and 400 ppm); at one year, no mammary carcinomas or fibroadenomas were observed in ovariectomized rats (control or treated) whereas there was a low incidence in intact rats, with an increase, cf. controls and other treatment goups, at the 400 ppm dose [Morseth SL (1996) Chronic 12/24 month study in rats with atrazine technical: 12-month interim report. Corning Hazleton Inc, Vienna VA, USA. Report no. CHV 2386-108. Date: 24 Oct. 1996]

A 105-week dog dietary study with Atrazine 80W (0, 15, 150 and 1500 ppm calculated as the active ingredient) provided a NOEL of 150 ppm (est. 3.8 mg/kg bw/d) in terms of decreased food consumption and bodyweight and increased adrenal weights (and possibly ovaries and uteri) at the next dose of 1500 ppm. At this high dose there were no apparent effects on mortality, on the limited haematology, clinical chemistry and urinalysis parameters determined, or on macroscopic and microscopic pathology (Woodard *et al*, 1964). This study is of limited value because of the small number of animals used (3/sex/group).

Beagle dogs (4-6/group) received 0, 15, 150 and 1000 ppm (est. 0, 0.38, 3.8, 25 mg/kg bw/d dietary atrazine technical for at least 52 weeks. At 25 mg/kg bw/d, one female died, bodyweights, bodyweight gains and food consumption were reduced, relative male liver weights were slightly increased, platelet counts were increased, and serum protein and albumin was slightly decreased. There was marked cardiac toxicity in high dose animals only (both sexes), with irregular heartbeat, increased heart rate, ECG alterations, atrial fibrillation, moderate to severe gross or microscopic cardiac lesions consisting of dilatation of left and right atria, and myocardial degeneration of the atria. All other parameters were normal. The NOEL was 150 ppm (3.75 mg/kg bw/d) (O'Connor, McCormick & Green, 1987).

The company considered that the decreased P-II amplitude in 150 ppm females was not treatment related because: the decrease occurred in only one animal on one day and was reversed the next; the change was of small magnitude (0.1 mV) and was significant only due to a decrease in the SEM rather than the mean, and ECGs were measured only in 0.1 mV increments; P waves in dogs vary spontaneously with foreleg position change, and as a result of respiratory arrhythmia; P wave amplitudes distributions are usually skewed, making parametric statistical comparisons unreliable; and spontaneous variations of 0.1 mV are common. The company considered that the atrial dilatation in one 150 ppm male dog was not related to treatment because: atrial size was estimated qualitatively, after formalin fixation; several control animals exhibited mild

dilatation, but were considered within the normal range; there was no evidence of microscopic liver or heart lesions in the same animal, and ECGs and all other parameters were normal; and a review of the data by a scientist experienced in canine electrocardiography concurred with a diagnosis of polyarteritis (Wetzel, 1989).

6.2 Desethylatrazine

No chronic studies were provided.

6.3 Desisopropylatrazine

No chronic studies were provided.

6.4 Diaminochlorotriazine

Beagle dogs (8-10/group) received dietary diaminochlorotriazine, an atrazine metabolite, for 13 or 52 weeks. Dose levels were 0, 5 or 100 ppm for 13 or 52 weeks (low- and mid-dose groups). High-dose animals were given 1500 ppm for 6 weeks, reducing to 750 ppm from week 7 because of toxic signs; females remained on this dose until termination (weeks 13 or 52) or through week 13 with a 39-week recovery period (2 animals), whilst males remained on 750 ppm through week 8 then put on control diet (0 ppm) for weeks 9-13 because their condition at the lower dose did not improve. Two males underwent scheduled sacrifice at week 13 and the remainder were re-established on 750 ppm until necropsy at 52 weeks. Effects in high-dose animals only were 5 male and 2 female deaths, impaired cardiac function, bodyweight loss, anaemia, decreased serum albumin, calcium and cholesterol, increased mean spleen, liver and kidney weights, gross and microscopic cardiac lesions, fluid accumulation, and liver, testes, bone marrow and thymus pathology. The NOEL was 100 ppm (3.2-3.9 mg/kg bw/d in males, 2.7-3.8 mg/kg bw/d in females) (Thompson, Batastini & Arthur, 1990).

Comment: Gross and microscopic changes were reported in the right atria of dogs orally dosed at $\geq$ 1500 ppm in a 4-week pilot study, indicating an early onset of the cardiac inflammation produced by DACT (Swallow, Hazelette & Arthur, 1989).

6.5 Hydroxyatrazine

Hydroxyatrazine was administered in the diet to Crl:CD(SD)BR Sprague-Dawley derived rats (70/sex/group, with an extra 10/sex or the control and high-dose groups) for up to two years; doses were 0, 10, 25, 200 and 400 ppm. Ten/sex (plus an extra 10/sex for control and high dose) were scheduled for sacrifice at one year. The kidneys and lower urinary tract were the target

organs for hydroxyatrazine in rats. The NOEL for hydroxyatrazine in this study was 25 ppm in males (0.962 mg/kg bw/d) and 10 ppm in females (0.475 mg/kg bw/d), based on kidney toxicity and pathology at the next highest dose (200 ppm in males, 25 ppm in females). No decrease of mammary tumour onset time was observed in this study. A maximum-tolerated dose was clearly exceeded at 400 ppm, based on decreased survival, reduced bodyweight gains and the extent of renal damage (Chow & Emeigh Hart, 1995).

7. REPRODUCTION STUDIES

7.1 Atrazine

Weanling Charles River albino rats (10 males and 20 females/dose group) were fed atrazine 80W (an 80% wettable powder) in the diet at levels of 0, 50 and 100 ppm (calculated on the active ingredient). After 74 days of dietary administration, parent animals were mated (2:1) and the study continued over 3 generations. Test and control rats were comparable in terms of survival, mean body weights, general appearance and behaviour, and reproductive performance. Data for litters/group, total still-births, live young/litter, birth weight, percentage alive at weaning, mean weanling weight and, where measured in the F3b offspring, organ weights (liver, kidney and heart) and microscopic pathology, indicated that 100 ppm was an appropriate overall NOEL. No teratogenic effects were noted. There was an absence of statistical analyses but otherwise the study appeared to be reasonably well reported (Hollinsworth, Woodard & Woodard, 1966).

Atrazine at dietary concentrations up to 500 ppm did not cause any impairment in the reproductive performance of Charles River (CRCD, VAF/PLUS) rats in a two-generation study. The NOEL was 50 ppm [between. 2.73 (male) to 3.45 (female) mg/kg bw/d], on the basis of reduced food consumption, bodyweights, and bodyweight gains in parental animals, at the next higher dose of 500 ppm (Mainiero *et al*, 1987).

Two mixtures of pesticides and a fertilizer (ammonium nitrate), representative of established groundwater contamination in California and Iowa, were both tested (at 1x, 10x and 100x the median concentration found in the groundwater surveys) in a continuous breeding study in COBS Crl:CD-1 (ICR) VAF/Plus outbred albino Swiss mice. The 1x mixtures were made up as follows (ng/mL concentration given in brackets, unless otherwise stated):-

- IOWA mix: alachlor (0.9); atrazine (0.5); cyanazine (0.4); metolachlor (0.4); metribuzin (0.6); ammonium nitrate (10 µg/mL); propylene glycol (512 µg/mL; used as solubilizer)

- CAL mix: aldicarb (9.0; 1;1:1 ratio of aldicarb, sulfoxide and sulfone); atrazine (0.5); dibromochloropropane (0.01); 1,2-dichloropropane (4.5);

EDB (0.9); simazine (0.3); ammonium nitrate (10 µg/mL); propylene glycol (512 µg/mL; used as solubilizer)

Animals were 11weeks of age at the start of the continuous breeding phase of the study which consisted of controls (40 breeding pairs) and three dose groups (20 pairs/group). Following 7 days pre-mating exposure to dosed drinking water, animals were housed as breeding pairs for 98 days, with continuous exposure. Randomly-selected F1 pups were weaned and exposed to dosed drinking water, with breeding for 7 days at age 74 $\pm$ 10 days, then housed singly until delivery of litters. There were no significant effects on any parameter measured in the reproductive toxicity studies, even at doses of IOWA and CAL mixtures at 100x mean groundwater contamination levels. It was specifically noted that epididymal sperm concentration, percent motile sperm, percent abnormal sperm, and testicular spermatid head count were not affected, nor was there any testicular or epididymal pathology. Oestrous cyclicity, as measured by vaginal cytology in mice treated with IOWA water, was not affected (Heidel *et al*. 1993).

7.2 Atrazine metabolites

No reproduction studies on any of the metabolites of atrazine were available for review.

8. DEVELOPMENTAL STUDIES

8.1 Atrazine

Atrazine was not teratogenic in rats (strain not stated) when dosed on days 6-15 of gestation at 100, 500 or 1000 mg/kg bw/d. A NOEL of 100 mg/kg for embryotoxic effects can be set, based on decreased mean live fetal body weight, increased early and late resorptions, and retarded ossification at the next higher dose of 500 mg/kg. The developmental deficits are attributed to maternal toxicity, with reduced body weight gain (mid- and high doses), reduced food consumption (all doses), and prominent clinical signs of toxicity (mid- and high dose and possibly the low dose group, but study report too brief to absolutely confirm this) (Fritz, 1971).

Studies in rats (strain not stated) were conducted with (1) dietary dosing at 0, 50, 100, 200, 300, 400, 500 or 1000 ppm throughout gestation; (2) subcutaneous injection at 0, 50, 100, 200, 800, 1000 or 2000 mg/kg on days 3, 6 *and* 9; and (3) subcutaneous injection at 1000 or 2000 mg/kg on days 3, 6 *or* 9. No teratogenic effects were reported but embryotoxicity (decreased litter size, increased resorptions) was observed after SC administration (NOEL was 200 mg/kg). This embryotoxic effect was greatest when atrazine was administered on day 6. The only parameters assessed were pups/litter, weaning weight and resorptions. No changes were observed following oral

administration of atrazine up to 1000 ppm in the diet. Following sc administration, the NOEL was 200 mg/kg for decreased litter size and increased resorptions. At 2000 mg/kg subcutaneously no live pups were born, although uterine resorption sites were readily apparent. This embryotoxic effect was greatest when atrazine was administered on day 6 of gestation (Peters & Cook, 1973).

Atrazine was not teratogenic in Charles River Crl:COBS CD(SD) BR rats at gavage doses of 0, 10, 70, 700 mg/kg bw/d, given on days 6-15 of gestation. Delayed ossification, decreased foetal size and weight correlated with decreased maternal bodyweights and food consumption, clinical signs. A NOEL of 10 mg/kg bw/d for fetotoxicity is appropriate, based on developmental delays in ossification. Maternotoxicity was apparent at the two higher doses at which fetotoxicity was observed (Infurna & Arthur, 1984; Infurna *et al*. 1988).

Atrazine was not teratogenic in Charles River [Crl:COBS CD(SD)BR] rats at gavage doses of 0, 5, 25 or 100 mg/kg bw/d given on gestation days 6-15. Reduced food consumption, bodyweights, bodyweight gains and increased salivation indicated maternotoxicity in high dose females only. Minor skeletal variations were increased in the highest dose group. Based on these findings, the overall NOEL was 25 mg/kg bw/d (Giknis, 1989a).

Atrazine technical was not teratogenic in NZ White rabbits at 0, 1, 5 and 75 mg/kg bw/d by gavage on days 7-19 of pregnancy. Signs of maternotoxicity at 5 mg/kg included decreased food consumption during the dosing period (only significantly different from controls on days 17 and 19), decreased body weight gain during the dosing period, and, at the high dose, bloody vulvae, changes in stool characteristics (absent or small and soft), decreases in food consumption, decreased body weight and body weight gain during the period of compound administration. Embryotoxicity and fetotoxicity was evident at the high dose and included increases in resorptions and percent post-implantation losses, decreases in foetal viability, decreases in fetal bodyweight, and delayed ossification. Based on these findings, the NOEL for fetotoxicity and embryotoxicity was 5 mg/kg bw/d and for maternotoxicity, 1 mg/kg bw (Arthur & Katz, 1984; Infurna *et al*.,1988).

Two mixtures of pesticides and a fertilizer (ammonium nitrate), representative of established groundwater contamination in California and Iowa, were both tested (at 1x, 10x and 100x the median concentration found in the groundwater surveys) in a rat teratology study. The 1x mixtures were made up as follows (ng/mL concentration given in brackets, unless otherwise stated): IOWA mix: alachlor (0.9); atrazine (0.5); cyanazine (0.4); metalochlor (0.4); metribuzin (0.6); ammonium nitrate (10 µg/mL); propylene glycol (512 µg/mL; used as solubilizer): CAL mix: aldicarb (9.0; 1;1:1 ratio of aldicarb, sulfoxide and sulfone); atrazine (0.5); dibromochloropropane (0.01); 1,2-dichloropropane (4.5); EDB (0.9); simazine (0.3); ammonium nitrate (10 µg/mL); propylene glycol (512 µg/mL; used as solubilizer). Sprague Dawley Crl:CD BR VAF/Plus outbred Charles River CD rat dams (23-24 dams per group) were

exposed to normal water, water plus vehicle, or dosed water on gestation days 6 to 20. There were no signs of maternal toxicity, apart from a significant increase in maternal water consumption in the 100x CAL group during gestation, although a trend to increasing water intake was seen pre-dosing (days 0-6). The only other statistically significant finding was an increase in late fetal deaths at the 100x concentration in the IOWA study; the percent of litters with late fetal deaths was 4.8% (or 0.3% late fetal deaths/litter) cf. 0% in all other groups, an observation not considered to reflect an effect related to dosing. Under the conditions of these well conducted and reported studies, there were no significant effects on any parameter measured in the developmental toxicity studies, even at doses of IOWA and CAL mixtures at 100x mean groundwater contamination levels (Heidel, 1993).

Two groups of 6 ewes were fed atrazine at 15 and 30 mg/kg bw/d mixed in alfalfa meal, by stomach tube, throughout gestation plus 30 days postpartum. All high-dose animals died during gestation between day 36 and 60; one had failed to conceive, three had had embryonic deaths, and two had normal fetuses. At 15 mg/kg, all ewes delivered full-term, normal, live lambs which nursed without clinical signs of poisoning (Binns & Johnston, 1970; abstract only).

8.2 Desethylatrazine

Desethylatrazine was administered to virgin female rats (strain not stated) (24 mated animals/group) by gavage at doses of 0, 30, 100 and 200 mg/kg bw/d (10 mL/kg) on days 6-15 post-coitum. The maternal NOEL may be taken as 5 mg/kg bw/d, based on the small reductions in food consumption and body weight gain during the first half of the treatment period at the next highest dose of 25 mg/kg, and a small increase in embryonic resorptions. The fetal NOEL was 25 mg/kg bw/d, based on an increase in the incidence of fused sternebrae-1 and -2, and poor ossification of the proximal phalanx of posterior digit-5 at the next highest dose of 100 mg/kg bw/d. There was no evidence of teratogenicity (Fritz, 1972).

Desethylatrazine was administered to approximately 2-month-old virgin female Tif: RAIf (SPF) rats (hybrids of RII/1 x RII/2) (24 mated animals/group) by oral gavage at doses of 0, 5, 25, and 100 mg/kg bw/d (10 mL/kg) on days 6-15 post-coitum. The maternal NOEL was 5 mg/kg bw/d, based on the small reductions in food consumption and body weight gain during the first half of the treatment period at the next highest dose of 25 mg/kg. The fetal NOEL was 25 mg/kg bw/d, based on an increase in the incidence of fused sternebrae-1 and -2, and poor ossification of the proximal phalanx of posterior digit-5 at the next highest dose of 100 mg/kg bw/d. There was no evidence of teratogenicity (Marty, 1992).

8.3 Desisopropylatrazine

Desisopropyatrazine was administered to approx. 2-month-old virgin female Tif: RAI f (SPF) rats (hybrids of RII/1 x RII/2) (24 mated animals/group) by gavage at doses of 0, 5, 25, and 100 mg/kg bw/d (10 mL/kg) on days 6-15 post-coitum. The maternal NOEL was 5 mg/kg bw/d, based on the small reductions in food consumption and body weight gain during the first half of the treatment period at the next highest dose of 25 mg/kg. The fetal NOEL was 5 mg/kg bw/d, based on an increase in the incidence of the anomaly, fused sternebrae-1 and -2 at the next highest dose of 25 mg/kg bw/d. There was no evidence of embryotoxicity or teratogenicity (Marty, 1992b).

8.4 Diaminochlorotriazine

Diaminochlorotriazine was not teratogenic in Charles River Crl: COBS CD (SD) BR rats (26 sperm-positive animals/group) at gavage doses of 0, 2.5, 25, 75 and 150 mg/kg bw/d on days 6-15 of gestation. Embryotoxicity was evidenced by an increase in resorptions and post-implantation loss at 150 mg/kg bw/d and fetotoxicity by decreased mean fetal weights of both sexes at 75 and 150 mg/kg bw/d, and an increase in skeletal variations. The maternal and fetal NOEL was 2.5 mg/kg bw/d, based on the transient slight reductions in food consumption and body weight gain, and an increase in the incidence of skeletal variations, at the next highest dose of 25 mg/kg bw/d (Hummel *et al*, 1989).

8.5 Hydroxyatrazine

Hydroxyatrazine was administered by gavage to Crl: COBS CD (SD) BR rats (26/group) at doses of 0, 5, 25, 125 mg/kg bw/d on gestation days 6 to 15. Food consumption and bodyweight gain were reduced in high dose dams only and foetal weights were slightly reduced in this group. The effect on fetal weight was less than 5% and was probably a result of the larger litter size in the high-dose group. The incidences of incompletely ossified hyoid and interparietal bones and non-ossified forepaw metacarpals and proximal phalanges were increased in the high dose group, and may be secondary to maternotoxicity. There were no compound effects on any other foetal or reproductive parameters. Thus there was no evidence of embryotoxicity or teratogenicity in this study and the overall NOEL for maternal and fetal effects was 25 mg/kg bw/d (Giknis, 1989b).

9. GENOTOXICITY STUDIES

Atrazine technical and several of its metabolites have been comprehensively tested for genotoxicity in bacteria, *Drosophila*, and mammalian cells *in vitro* and *in vivo*, with predominantly negative results. For atrazine and each of its metabolites, studies are presented in the following order; gene mutation assays; chromosomal effects assays; other genotoxic effects; and cell transformation assays.

9.1 Atrazine

In a series of *in vitro* gene mutation assays in *Salmonella typhimurium*, there was no evidence that atrazine caused gene mutation, with or without metabolic activation; 6 separate studies were cited (Simmon & Poole, 1977; Arni & Mueller, 1978; Ciba-Geigy Japan, 1979; Ciba-Geigy Switzerland, 1986; Butler & Hoagland, 1989). Similarly, in *in vitro* rec-assays with *Bacillus subtilis* H17 and M45 and reverse mutation assays with *Escherichia Coli*, with or without metabolic activation, there was no evidence of mutagenicity (Ciba-Geigy Japan, 1979). In gene conversion assays in *Aspergillus nidulans* and *Saccharomyces cerevisae*, no mutagenic effects of atrazine were seen, either with or without metabolic activation (de Bertoldi *et al*, 1980).

In a host-mediated assays, male Swiss Webster mice received either a single dose or 5 consecutive daily doses of atrazine by oral intubation. Simultaneously, *S. typhimurium* strains TA 1535 (which detects base-pair substitution mutations) and TA 1538 (which detects frameshift mutations) were injected subcutaneously. After 4 h, total viable and mutant cells were enumerated by plating serial dilutions of peritoneal exudate. Doses of atrazine tested were 500, 1000 and 2000 mg/kg in the acute study and 275, 5500 and 1100 mg/kg in the repeat-dose study with TA 1535. Doses were 550, 1100 and 2200 mg/kg in the acute study, and 275, 5500 and 1100 mg/kg in the repeat-dose study with TA 1538. Atrazine was negative for host-mediated gene mutation activity (Simmon & Poole, 1977).

A series of chromosomal effects assays were conducted with atrazine.

In a nucleus anomaly test in somatic interphase nuclei in Chinese hamsters (6/sex/group) given two daily oral doses of 0, 282, 564 and 1128 mg/kg atrazine and killed 24 h later; there was no increase in cells displaying nuclear anomalies (Hool, Langauer & Mueller, 1981a).

In a mammalian cytogenetics test in NMRI-derived male mice (15/dose group; 12 controls), germinal epithelium was examined for chromosomal aberrations following peroral dosing with atrazine at 0, 44 and 1332 mg/kg bw/d orally for 5 consecutive days. Mice were killed 5 days after the first dose and 3 h after an i.p. injection of colcemide (10 mg/kg). The testes were processed and one hundred metaphase plates from 8 animals/group were examined for chromatid aberrations, chromosomal aberrations, chromatid gaps and chromosomal pulverisation. No specific chromosome abnormalities were seen in the spermatogonia (Hool & Mueller, 1981b). The same negative results were obtained in a repeat study (as above but with dosing on days 0, 2, 3, 5 and 9, with no specific chromosome abnormalities seen in spermatocytes (Hool & Mueller, 1981b).

In a dominant lethal test, mature NMRI-derived male Tif.MAGf (SPF) mice (20/group) were given single oral doses of 0, 444 and 1332 mg/kg of technical

atrazine followed by mating with females (2-3 months old) at weekly intervals for 6 weeks; females were autopsied on day 14 of pregnancy. There were no reported clinical signs in males and no effects on pregnancy rate, or the numbers of implantations, live embryos or embryonic deaths ((Hool & Mueller, 1981b). In a second study young adult NMRI-derived male Tif:MAGf (SPF) mice (30/group) were given single oral gavage doses of 0, 500, 1000, 2000 and 2400 mg/kg of technical atrazine, followed by mating with two virgin females at the following intervals; days 1-4, days 4-8, days 8-12, and weeks 3, 4, 5, 6, 7 and 8. Females were autopsied on day 13-15 of pregnancy. No significant effects on mating frequency, pregnancy rate, or the numbers of implantations, live embryos or embryonic deaths were seen. The positive control, cyclophosphamide, gave the expected results (Hertner, 1993). Thus, atrazine did not induce dominant lethal mutations in male mice at single oral doses as high as 2400 mg/kg bw.

Atrazine was administered to groups of male and female mice (Tif: MAGF, SPF) by oral gavage at 2250 mg/kg bw and sacrificed at 16, 24 and 48 h, or was administered at doses of 562.5, 1125 and 2250 mg/kg bw, with sacrifice after 24 h (8/sex at each dose and time point). The percentages of micronuclei were not increased significantly in any of the treated groups (Ceresa, Langauer & Arni, 1988).

In toxicity studies, F344/N rats and B6C3F1 mice were given pesticide/fertilizer mixtures in their drinking water; two mixtures of pesticides and a fertilizer (ammonium nitrate), representative of established groundwater contamination in California and Iowa, were tested at 0.1x, 1x, 10x and 100x the median concentration found in the groundwater surveys. *In vivo* genetic toxicology assays were conducted on peripheral blood erythrocytes from the female mice at the 13-week interim evaluation of the toxicity study (induction of micronuclei) and on splenocytes from male rats and female mice at the 13-week interim evaluation (induction of micronuclei and SCEs). Both the 1x mixtures contained atrazine at 0.5 ng/mL. Results of tests for induction of micronuclei in peripheral blood erythrocytes of female mice treated with CAL water were negative. With the IOWA mix, significant increases were seen at 10x and 100x concentrations but they were within the normal range of micronuclei in historical control animals. SCE frequencies in splenocytes of male rats and female mice were marginally increased in mice and rats receiving the CAL mixture but neither species exhibited increased frequencies of micronucleated splenocytes. None of the above changes were considered to be of biological significance (National Toxicology Program, 1993).

A series of studies conducted to investigate other possible genotoxic effects of atrazine viz. DNA damage as measured by unscheduled DNA synthesis or directly by the alkaline elution technique, were reviewed.

In two separate *in vivo* studies on freshly isolated hepatocytes prepared from male Tif:RAIf(SPF) rats, atrazine did not cause any unscheduled DNA synthesis when exposed for 5 h at concentrations up to 1670 µg/mL (Puri & Mueller, 1984a; Herner, 1992). Similarly, no UDS was observed in cultured

human fibroblasts exposed for 5 h at concentrations up to 150 µg/mL (Puri & Mueller, 1984b).

Sprague-Dawley rats received a single 875 mg/kg bw dose of atrazine and were killed 12, 24, 36, 48 and 72 h. DNA damage was assessed in liver, kidney, stomach and lung by the technique of alkaline elution, which indirectly measures the number of single strand breaks.

DNA fragmentation was also evaluated after administration of 5 or 15 daily doses of 350 mg/kg bw, with sacrifice after 12 h. DNA lesions were detected in stomach and kidney, and at a lower level in liver, but not in lung. DNA breaks/lesions were present 24 h after a single dose, reaching a maximum 12 h later in liver and kidney, and 72 h later in stomach mucosa. With repeated dosing, the DNA elution rate was significantly increased after 5 days treatment in kidney and stomach, and after 15 days in liver (Pino *et al*, 1988).

No cell transformation assays with atrazine were available for assessment.

A review conducted under the Environmental Research Programme of the Commission of the European communities, indicated that atrazine was negative in a large range of *in vitro* tests but was positive in a forward mutation test with *A. nidulans* ($8AG^R$ locus; activation with potato microsomes) and *S. coelicolor* ($Strp^R$ locus; potato microsomes), in a mitotic cross-over test in *A. nidulans* (tpa A1; potato microsomes) and in a UDS test in EVE cells (3 mM; potato microsomes). Results were negative for genotoxicity in many *in vivo* studies but positive results were cited in a host-mediated assay for forward mutations in *E. coli* (100 mg/kg bw po, in 10% ethanol) and in *S. pombe* (1000 mg/kg po), for chromosome breakage in mouse bone marrow cells (2000 mg/kg bw in olive oil), and for dominant lethals in mouse spermatids (1500 mg/kg po in olive oil) (Adler, 1980).

An extensive review of genotoxicity studies on atrazine has been published (Brusick, 1994). The reviewer concluded that not all of the conflicting genotoxicity studies for atrazine could be reconciled, on the basis of differences in study methods, atrazine purity, or route of administration, however the overall conclusion was that in most test systems atrazine was not genotoxic. Use of the weight-of-evidence approach resulted in a conclusion that atrazine does not pose a mutagenic hazard.

9.2 Desethylatrazine

Desethyltrazine was negative in a standard an Ames test using *S. typhimurium* TA100, 97, 98, 1535, 1537 and 1538, when tested in the absence of S9 at concentrations up to 10 µmol/plate (Butler & Hoagland, 1989). In a more extensive experiment (Deparade, 1989), with and without a metabolic activation system with *S. typhimurium* TA100, 98, 1535, 1537 and *E. coli* WP2uvrA, there was no evidence that this metabolite had any gene mutation activity at concentrations up to 5000 µg/0.1 mL.

In a micronucleus test, desethylatrazine was administered to male and female Tif: MAGF, SPF mice by gavage at (1) 480 mg/kg, with sacrifice at 16, 24 and 48 h and (2) 120, 240 and 480 mg/kg, with sacrifice at 24 h (8/sex at each dose and time point). Polychromatic erythrocytes from bone marrow were analysed for micronuclei (5/sex/group). Desethylatrazine showed no evidence of clastogenic or aneugenic effects under the test conditions (Ogorek, 1991a).

Cultured of freshly-isolated rat hepatocytes [male Tif:RAIf(SPF)]were exposed to desethylatrazine for 16-18 h at of 9.25, 27.7, 83.3, 250, 500 and 1000 µg/mL (duplicate experiment; 4 cultures/group). No significant increase in unscheduled DNA synthesis (assessed by incorporation of ^{3}H-thymidine) caused by desethylatrazine was observed at any of the concentrations used (Geleick, 1991).

No cell transformation assays were reported.

9.3 Desisopropylatrazine

In a replicated test (Deparade, 1990), with and without a metabolic activation system using *S. typhimurium* TA100, 98, 1535, 1537 and *E. coli* WP2uvrA, there was no evidence that this metabolite had any gene mutation activity at concentrations up to 5000 µg/0.1mL

In a micronucleus test, desisopropylatrazine was administered to male and female Tif: MAGF, SPF mice by gavage at (1) 480 mg/kg, with sacrifice at 16, 24 and 48 h and (2) 120, 240 and 480 mg/kg, with sacrifice at 24 h (8/sex at each dose and time point). Polychromatic erythrocytes from bone marrow were analysed for micronuclei (5/sex/group). Desethylatrazine showed no evidence of clastogenic or aneuploidy-inducing effects under the test conditions (Ogorek, 1991b).

Cultured of freshly-isolated rat hepatocytes [male Tif:RAIf(SPF)]were exposed to desisopropylatrazine for 16-18 h at 7.4, 22.2, 66.6, 200, 400 and 800 µg/mL (duplicate experiment; 4 cultures/group). No significant increase in unscheduled DNA synthesis (assessed by incorporation of ^{3}H-thymidine) caused by desethylatrazine was observed at any of the concentrations used (Geleick, 1991).

No cell transformation assays were reported.

9.4 Diaminochlorotriazine

In a replicated test (Deparade, 1987), with and without a metabolic activation system and using *S. typhimurium* TA100, 98, 1535, 1537 and *E. coli* WP2uvrA, there was no evidence that this metabolite had any gene mutation

activity at concentrations up to 3000 µg/0.1 mL. The highest concentration tested (5000 µg/mL) precipitated in the soft agar.

In a micronucleus test, diaminochlorotriazine (DACT) was administered to male and female Tif: MAGF, SPF mice by gavage at (1) 5000 mg/kg bw, with sacrifice at 16, 24 and 48 h and (2) 1250, 2500 and 5000 mg/kg bw, with sacrifice at 48 h (8/sex at each dose and time point). Polychromatic erythrocytes from bone marrow were analysed for micronuclei (5/sex/group). DACT showed no evidence of clastogenic or aneuploidy-inducing effects under the test conditions (Strasser, 1988).

Diaminochlorotriazine was negative in an unscheduled DNA repair test in human fibroblasts *in vitro*, over a concentration range of 5.56 to 600 µg/mL (duplicate tests, with 4 cultures per test group); visible compound precipitation was seen at 300 and 600 µg/mL (Meyer, 1987).

Diaminochlorotriazine was tested for DNA-damaging effects *in vitro* on primary hepatocytes from male Tif: RAIf(SPF) rats. Concentrations from 0.10 up to 400 µg/mL were used (triplicate experiments; 4 cultures/group). Unscheduled DNA synthesis (UDS) was assessed by ^{3}H-thymidine incorporation and autoradiography. Viability tests on the cells showed that very high levels of cell death occurred at concentrations of >350 µg/mL. There was no evidence of a biologically significant increase in UDS in the cultures treated with diaminochlorotriazine (Hertner & Puri, 1988).

No cell transformation assays were reported.

9.5 Hydroxyatrazine

Hydroxyatrazine was negative in an Ames test using *S. typhimurium* TA100, 97, 98, 1535, 1537 and 1538, when tested in the absence of S9 at concentrations up to 10 µmol/plate (Butler & Hoagland, 1989). In another study (Arni & Mueller, 1981), hydroxyatrazine at concentrations up to 102 µg/plate, with or without metabolic activation (S9 from Aroclor-induced rat liver) was negative. In a more extensive study (Deparade, 1988), with and without a metabolic activation system from Aroclor-induced rat liver, and using *S. typhimurium* TA98, 100, 1535 and 1537, there was no evidence that this metabolite had any gene mutation activity at concentrations up to 5000 µg/0.1 mL (duplicate assays).

In a micronucleus test, hydroxyatrazine was administered to male and female Tif: MAGF, SPF mice by gavage at (1) 5000 mg/kg, with sacrifice at 16, 24 and 48 h and (2) 1250, 2500 and 5000 mg/kg, with sacrifice at 24 h (8/sex at each dose and time point). Polychromatic erythrocytes from bone marrow were analysed for micronuclei (5/sex/group). Hydroxyatrazine showed no evidence of clastogenic or aneuploidy-inducing effects under the test conditions (Ceresa, 1988).

Hydroxyatrazine was negative in an unscheduled DNA repair test in human fibroblasts *in vitro*, over a concentration range of 13.89 to 150 µg/mL (duplicate tests, with 4 cultures per test group); visible compound precipitation was seen from 125 and 1500 µg/mL (Meyer, 1988).

Hydroxyatrazine was tested for DNA-damaging effects *in vitro* on primary hepatocytes from male Tif: RAIf(SPF) rats. Concentrations from 3.125 to 1500 µg/mL were used (triplicate experiments; 4 cultures/group); visible compound precipitation was seen at all doses in the first test and from 12.5 µg/mL in the second test ie. not at 3.125 and 6.25 µg/mL. Unscheduled DNA synthesis (UDS) was assessed by ^{3}H-thymidine incorporation and autoradiography. Cytotoxicity tests up to 4000 µg/mL showed that 1500 µg/mL was the highest usable concentration. There was no evidence of a biologically significant increase in UDS in the cultures treated with diaminochlorotriazine (Hertner, 1988).

No cell transformation assays were reported.

10. SPECIAL STUDIES

In a short-term hepatocarcinogenicity assay, male F344 rats (6 weeks old) were dosed with diethylnitrosamine (DEN) i.p. at 200 mg/kg bw and two weeks later treated with atrazine incorporated in the diet for 6 weeks, before undergoing necropsy; at week 3 all rats underwent partial (2/3rds) hepatectomy. Hepatocarcinogenic potential was assessed by comparing the number and area of glutathione-S-transferase (placental form)-positive foci in the livers cf. controls given DEN alone. Atrazine at 500 ppm in the diet of 15 rats was negative (Hasegawa & Ito, 1992).

The potential carcinogenicity of mixtures of pesticides was investigated in a short-term test for hepatocarcinogenic potential (as detailed above). Pesticide mixtures were alachlor plus atrazine, and glyphosate, alachlor, atrazine and "inerts". All mixtures proved positive. When tested individually, alachlor was positive, glyphosate was borderline, and atrazine was negative. There was no information on the concentration of chemicals in the diet or on the number of animals/test (Cabral *et al*, unspecified date).

Atrazine and simazine were administered by gavage to Sprague-Dawley and Fischer rats at 0, 100 and 300 mg/kg bw/d for 2 weeks. While the reduction in oestradiol in SD rats contrasts with the increases noted in long-term SD rat studies, the increase in oestrous cycle length, an2d days spent in oestrus suggests that the triazines (and atrazine much more than simazine) may hasten ageing of the endocrine system in SD rats (Eldridge *et al*, 1994).

Wetzel *et al* (1994) provided studies to support the hypothesis that atrazine acts to accelerate reproductive ageing of the neuroendocrine system in female Sprague Dawley rats, resulting in an earlier onset of mammary tumours

stemming from prolonged oestrogen exposure; the authors argue that a threshold can be established for this effect of atrazine in this strain of rats.

Studies were conducted on the interaction of atrazine, simazine and diaminochlorotriazine (DACT), a common metabolite, with rat uterine oestrogen receptors (ER). None of the compounds at concentrations up to 100 mM competed with radioactive oestradiol binding (5 nM) to ER in extracted uterine tissue under equilibrium conditions (at 4°C). The triazines were about 10^5 less potent than oestradiol itself in causing 50% reduction in labelled oestradiol binding to ER (at 25°C). In '*in vivo*' studies in ovariectomised rats, PO doses of 300 mg/kg bw/d of atrazine, simazine and DACT for 2 days reduced uterine ER binding capacity about 33%, 39% and 24%, respectively ie. rat uterine tissue had a diminished ability to take up or retain oestrogen; it was not possible from the results to know whether this reduction was due to a triazine occupation of receptor sites or whether the ER population was diminished in the tissue of treated animals. The authors concluded that responses to triazine treatment would be best explained by effects on events other than, or in addition to, effects on ER binding of oestrogen (Tenant *et al*, 1994a; 1994b)

A brief review of the carcinogenicity of substituted, symmetrical triazines (s-triazines), was provided. The reviewer drew attention to the fact that the mammary tumour response observed in various female Sprague-Dawley rat studies (those conducted according to current standards) has not been consistent. Considering the large variation noted in the spontaneous occurrence of mammary tumours in SD rats, it was considered that the inconsistent response was not surprising. The results of the recent studies showing an earlier onset of mammary tumours (without an increase in total tumour incidence) has led to the hypothesis that certain triazines can produce an endocrine-mediated imbalance which results in precocious reproductive ageing, with the possible earlier onset or increased incidence of mammary tumours (Stevens *et al*, 1994).

In a paper titled 'Medical Hypothesis: Xenoestrogens as Preventable Causes of Cancer', Mary Wolff and co-workers (Davis *et al*, 1993) proposed that a number of chemicals (eg. chlorinated organic compounds, polycyclic aromatic hydrocarbons, triazine herbicides) acted as xenoestrogens to increase the incidence of breast cancer; this cited another paper by Ghinea *et al* (1988) as providing evidence of the oestrogenicity of atrazine, stating that 'hormone release' [was] increased by atrazine'; however, the results of the cited study, using cultured human thyroid cancer cells treated '*in vitro*' with oestradiol and dehydro-epiandrosterone in the presence of other hormones (thyroid hormones and insulin) and some pesticides including atrazine, did not seem to indicate any oestrogenic action of atrazine; if anything, it suggested a weak antagonistic or competitive effect of atrazine on oestrogen.

In direct tests of oestrogenic bioactivity, neither atrazine, simazine nor the common metabolite, diaminochlorotriazine (DACT) at PO doses of 20, 100 and 300 mg/kg/d over 3 days significantly increase the uterine weight of

ovariectomised female SD rats; the highest dose (approx. 10% of the LD50) caused body weight loss viz. about 14% for atrazine, 11% for simazine and 17% for DACT cf. about 1% loss for controls, over the three days. In a similar experiment but with SC injections of oestradiol (2 µg/kg) on days 2 and 3, 300 mg/kg of the chlorotriazines reduced uterine weight in comparison to animals given oestrogen alone ie. appeared to antagonise the uterotrophic effects of the replacement oestrogen; the average reduction was 27% below the control uteri. DACT was also significantly active at 100 mg/kg. Body weight losses were similar to those in the previous experiment (Tennant *et al*, 1994a).

None of the chloro-s-triazines at 300 mg/kg bw/d po stimulated incorporation of [^{3}H]thymidine into uterine DNA of immature SD female rats; however, doses of 50 mg/kg/d or more, in a dose-related manner, significantly reduced thymidine incorporation into uterine DNA extracted from immature rats given single injections of 0.15 µg oestradiol (Tennant *et al*, 1994).

Expression of progesterone receptor binding is an even more specific test of oestrogen action. Oral doses of 300 mg/kg (but not 50 mg/kg) of the three triazines significantly reduced expression of progesterone receptor binding in cytosol fractions prepared from uteri of ovariectomised rats injected sc with 1 µg oestradiol (using labelled synthetic progesterone ligand, 17alpha,21-dimethyl-19-norpregna-4,9-diene-3,20-dione [17beta-methyl-^{3}H]). Furthermore, uterine progesterone receptor levels were not stimulated in rats given po doses up to 300 mg/kg of these triazines without oestradiol injections (Tennant *et al*, 1994).

Overall, results from the three studies summarised in the previous paragraphs suggest that these three triazines possess no intrinsic oestrogenic activity but are capable of weak inhibition of oestrogen-stimulated responses in the rat uterus; this inhibition may play a role in the changes in reproductive endocrine function previously observed in female SD rats.
In a preliminary study to a 4-week study to examine the effects of atrazine on the LH surge in female Sprague Dawley rats, -oestradiol was administered sc to ovariectomised female SD Crl: Cd BR rats at an age of about 8 weeks. Atrazine (300 mg/kg) was administered by oral gavage for 3 days, beginning the day after surgery. At designated intervals plasma was analysed for LH and prolactin (PRL) by RAI. In control animals the LH surge was apparent at 1500 h, with a peak at 1800 h (biological time, 9 h after the light cycle began) and did not return to baseline by the final collection period (2200 h). Atrazine attenuated the LH surge at 1500 h, the time range in which the LH peak is expected to occur in young intact animals. The data also suggest that the LH surge may have been delayed in the atrazine-treated animals cf. controls (in a similar manner to the age-related delay seen in middle-aged animals when compared to young animals). PRL levels rose over the course of the day; the failure of PRL levels to drop as expected late in the day in either control or atrazine-treated animals is likely to be related to the stress of repeated bleeding of the animals in this experiment (5 time points each). Atrazine did not affect the level of this hormone. In conclusion, atrazine (300 mg/kg bw/d for 3 days po) decreased the LH surge in female SD rats (Morseth, 1996a, 1996b).

A study was designed to elucidate the hormonally-mediated mechanisms underlying the earlier onset of mammary tumours in female SD rats (Morseth 1996c). Atrazine was administered to female Sprague Dawley Crl:CD BR rats at 0, 2.5, 5, 40 and 200 mg/kg bw/d by gavage. Animals were ovariectomised after 28-31 days of dosing and 10 days prior to sacrifice. Oestradiol was administered via a subcutaneous capsule implanted 3 days before blood sampling ie. 7-days after the ovariectomies. Atrazine at 40 and 200 mg/kg decreased the LH surge and prolactin (PRL) levels and affected oestrous-cycle patterns by causing persistent dioestrus as well as episodes of prolonged oestrus. A dose of 5 mg/kg/d did not disrupt oestrous cycle patterns or affect plasma PRL, but inconclusive results were obtained for LH levels; no LH suppression was noted in repeat-bleed animals whereas LH levels appeared to be decreased in the non-repeat bleed animals. A dose of 2.5 mg/kg/d was a clear NOEL for atrazine effects on the LH surge, PRL, and oestrous cycles. The study demonstrated that young female SD rats dosed with high levels of atrazine began to display one of the early events of reproductive senescence, namely, reduced surges of pituitary LH. This neuroendocrine failure leads to oestrous cycle disruption which, in SD females, produces an endocrine environment which is favourable for mammary tumour growth later in life.

Results of an interim study submitted by Novartis after the completion of this ECRP review indicated an earlier onset of persistent oestrus in female SD rats at 400 ppm atrazine in the diet, with no LH surge at this dose; doses of 50 ppm atrazine or lower had no effect on oestrous cycling or the LH surge (Morseth, 1996; see main text for full reference citation).

The potential oestrogenic activity of atrazine and simazine was investigated in the immature female rat uterus and in the oestrogen-responsive MCF-7 human breast cancer cell line and the PL3 *S. Cerevisiae* yeast strain which is dependent on the presence of oestrogenic substances to grow on selective media (Connor *et al*, 1996). These studies are summarised as follows:

Oral treatment of immature 21-day-old female Sprague Dawley rats with 50, 150 or 300 mg/kg bw/d of atrazine or simazine for 3 days did not significantly induce rat uterine weight or cytosolic progesterone receptor levels (animals sacrificed 20 h after the last dose). Uterine peroxidase activity was decreased by both compounds at the two higher doses. 17 -Oestradiol (E2) injected intraperitoneally (10 µg/kg/d) caused a 6.2-, 8.9- and 9.7-fold increase in rat uterine weight, cytosolic progesterone levels and uterine peroxidase activity, respectively. In rats co-treated with ip E2 plus po atrazine or simazine, some doses slightly inhibited E2-induced cytosolic progesterone receptor binding and uterine peroxidase activity but there was no dose-response relationship (and no inhibition at the high dose of atrazine). The high dose of simazine produced a small inhibition of the E2-induced increase in uterine wet weight.

In MCF-7 cells, atrazine and simazine did not affect E2-induced cell proliferation or nuclear progesterone receptor levels (measured in two different types of assays). Data suggest that for these two responses neither chloro-s-

triazine exhibits oestrogen agonist or antagonist activity. Luciferase activity in MCF-7 cells transiently infected with the Gal4-oestrogen receptor chimeric construct and a Ga14-regulated luciferase reporter gene exhibited dose-dependent increases following E2 treatment and several other endocrine receptor agonists (including the weak bisphenol A and nonylphenol). However, neither atrazine nor simazine (tested up to 10 µM) had any significant effect on luciferase activity. Furthermore, in cells co-treated with E2 plus atrazine or simazine, the triazines did not inhibit the E2-induced responses.

Engineered PL3 cells contain the URA3 gene which encodes for orotidine-5'-monophosphate decarboxylase, an enzyme involved in uracil synthesis; the regulatory region of this gene contains three tandem oestrogen responsive elements and OMP decarboxylase can be induced in the presence of functional oestrogen receptors in the presence of oestrogenic substances. These cells were transformed with a vector containing human oestrogen receptor cDNA (YEp10-HEGO). Neither atrazine or simazine (at concentrations as high as 10 µM) promoted the growth of PL3 cells on minimal media lacking uracil, whereas growth was observed on similar media supplemented with 1 nM E2. Evidence was cited to suggest that their lack of activity was not due to their ability to penetrate the cells. (The weak industrial oestrogens, bisphenol A and *p*-nonylphenol were also capable of producing transformant growth.)

Overall, these results indicate that atrazine and simazine do not exhibit 17 -oestradiol-mediated oestrogenic agonist activity in 7 different measured oestrogen-regulated responses. In co-treatment studies, the two chloro-s-triazines did not inhibit E2 responses in *in vitro* cellular assays, but weakly inhibited some 17 -oestradiol-induced responses in the rat uterus *in vivo*. It is possible that this weak anti-oestrogenic activity may be to an indirect interaction with endocrine-receptor-mediated signal transduction pathways eg. TCDD and related compounds exhibit a broad spectrum of ant-oestrogenic activities which interact between the ER and aryl hydrocarbon (Ah) receptor-induced endocrine-response pathways.

Atrazine and desethylatrazine inhibited testosterone metabolism in male Fischer rat anterior pituitary and hypothalamus *in vitro* (0.92 µM, or approx. 0.2 mg/mL) and *in vivo* (120 mg/kg bw/d orally for 7 days) (Babic-Gojmerac *et al*, 1989).

In 26-week toxicity studies, F344/N rats and B6C3F1 mice were given pesticide/fertilizer mixtures in their drinking water; two mixtures of pesticides and a fertilizer (ammonium nitrate), representative of established groundwater contamination in California and Iowa, were tested at 0.1x, 1x, 10x and 100x the median concentration found in the groundwater surveys. The 1x mixtures were made up as detailed elsewhere in the summary. Investigations included clinical pathology, neurobehavioural and neuropathological studies, organ weight analysis, histopathology (including examination of the reproductive system included sperm morphology and vaginal cytology) and assessment of

sperm motility, numbers of motile and non-motile sperm, sperm density, testicular spermatid head count (to quantify spermatogenesis).

In mice, 1/20 control females (IOWA mix), 1/20 females (IOWA mix at 100x) and 1/20 male mice (CAL mix at 100x) died early but no clear adverse effects were seen in clinical signs, body weight, water consumption, clinical pathology, neurobehavioural tests, reproductive system, organ weight, or histopathological evaluations. All rats survived and there were no effects on bodywt gains, water consumption, clinical signs, neurobehavioural signs, clinical pathology, or histopathology (including a detailed examination of the reproductive system). With the IOWA mix, there possibly was a marginal increase in absolute and relative liver weights with increasing dose; the increase, albeit statistically significant (at 1x, 10x and 100x doses) may not be biologically significant, particularly when compared with the higher relative liver weights in the control animals in the CAL mix cf. the IOWA mix controls (NTP, 1993)

Immunotoxicity of an atrazine formulation, AAtrex, was examined in C57Bl/6 female mice following sublethal exposure to equivalent ½-1/64 LD50 doses of the herbicide. Body weight was not affected and there were no dose-related changes in organ weight, spleen cell number or cell viability. There were no significant changes in the frequency of L3T4-positive and Lyt-2-positive T-cells. Functional *in vitro* assays of mitogen activation showed no marked effects of atrazine exposure on lymphocyte stimulation by lipopolysaccharide (LPS), phytohemagglutinin (PHA) or Con-A. Interleukin-2 production by splenic cells was not affected and no dose-related effect could be concluded from a transient suppression of a primary humoral IgM response to sheep erythrocytes or from a transient inhibition of a specific T-cell response to alloantigens in mixed lymphocyte reaction. Exposure to equiv. ½-1/16 LD50 doses augmented phagocytic activity of peritoneal macrophages, without any dose-related effect. Normal humoral and cellular responses were restored by 14-40 days after exposure. Overall, transient and reversible immunosuppression of humoral-mediated and cell-mediated responses and activated macrophage phagocytic activity could not be attributed to a direct chemical-related effect of sublethal exposure to atrazine (Fournier *et al*, 1992).

The effect of atrazine on mouse haemopoietic progenitors (CFU-S and GM-CFC) and on peripheral blood (leukocytes and reticulocytes) after a single ip injection of 58.65 mg/kg bw was studied. The peripheral blood leukocyte level was not modified but reticulocytes dropped severely, with prompt recovery. Haemopoietic progenitors were severely affected but they recovered and reached normal levels in a few days. These results demonstrated a haemotoxic effect of atrazine after a large acute dose (Mencoboni *et al*, 1992).

A published paper noted that, under synthetic reaction conditions, atrazine could undergo nitrosation. N-Nitrosoatrazine in water underwent photodecomposition, with the half-life near the surface of water in sunlight calculated as being less than 10 min. It was also rapidly decomposed under fluorescent light. The study did not encompass any environmental monitoring

to indicate whether it was formed in the environment and, if so, whether it was stable in soil or in water (Wolff *et al*, 1976).

Lymphocyte cultures from 42 pesticide applicators and 16 controls were examined for chromosomal aberrations during mid-winter and again during the peak summer period of intense spraying activity. Cultures from exposed individuals were reported to show a marked increase in the frequency of chromatid lesions, especially noticeable among workers exposed primarily to herbicides. Whilst there appeared to be a very significant increase in chromatid breaks in exposed individuals, the number of chromatid breaks in controls (unexposed individuals) were also much higher than the mid-winter value in exposed workers; on this data, it could be argued that herbicide exposure might offer compensatory protection against chromosome damage. Also only a limited number of cells were scored per individual. It was not possible to make any firm conclusion about the chromosome damaging properties of atrazine because the workers examined were exposed to over 11 herbicides and 3 fungicides, in addition to a range of insecticides (Yoder, Watson & Benson, 1973).

11. HUMAN STUDIES

11.1 Epidemiological studies

The incidences of soft-tissue sarcoma (statistically significant), Hodgkins disease (HB) and non-Hodgkins lymphoma (NHL) in white male Kansas residents aged 21 or older, from 1986 to 1982, were studied in relation to farm herbicide use, as determined by phone interviews with patients and controls, or their next of kin, and herbicide suppliers. NHL was found to be associated with farm herbicide use, and the relative risk of NHL increased significantly with duration or frequency of herbicide use. However, the study overestimated risks of NHL by comparing exposed farmers with non-farmers, rather than unexposed farmers. Study data shows an increased risk of NHL in non-exposed farmers. If exposed farmers are compared with unexposed farmers the risks of NHL are statistically the same. Furthermore, the relative risk associated with exclusive use of triazines is reduced, and is no longer statistically significant. The data may suggest confounding factors associated with farming (Hoar *et al*, 1986).

Between 1980-1985 in Alessandria Province, Italy, all women histologically confirmed to have primary malignant epithelial tumours of the ovary were compared with randomly selected (plus or minus five years, no bilateral oophorectomy) referents. There were 65 cases (27 decedents) and 126 referents in the study. Subjects, or decedents' relatives were interviewed to determine occupational history and herbicide exposure. 'Exposed' individuals were defined as those who were involved in the preparation or use of triazine herbicides or who worked in corn cultivation with reported use of herbicides (triazines were reportedly used in all herbicide treated corn cultivation). Reproductive risk factors for ovarian cancer such as age, number of live births, use of oral contraceptives, miscarriages, abortions etc. were recorded. Risk factors for ovarian tumours were increased by use of oral contraceptives and short menstrual cycles, whereas parity had a protective role. Relative risks were 2.7 for 'definitely exposed' subjects, and 1.8 for 'possibly exposed' subjects. Risk factors increased with increased years of exposure. The risk factor for unexposed agricultural workers was one ie. no different than from unexposed non-agricultural people. Triazine exposure was not quantified. Although a number of reproductive factors for ovarian tumours were controlled, other known non-reproductive factors such as obesity, smoking and alcohol were uncontrolled, and could affect the results.

A critique of the above paper (Minder, 1990) concluded, on the basis of statistical aspects, classification of the exposed women, and possible confounders and biases, that the case for an association between ovarian cancer and triazines was weak, and for a causal link, even weaker. A second, unpublished critique (apparently solicited by Ciba-Geigy) was prepared by JV Watson, a Consultant Oncologist with the MRC, Cambridge, UK ('Report on Triazine Herbicides and Ovarian Epithelial Neoplasms'). Whilst not containing

many specific points which would raise major concerns about the study, it highlighted the problems and pitfalls in the conduct of such epidemiological studies, and concluded that the paper raised more questions than it answered.

In a published study of agricultural risk factors for leukaemia amongst white men in Iowa and Minnesota (case-control interview study of 578 white men with leukaemia and 1245 controls), the authors concluded that there were slight but significantly elevated risks for farmers cf. non-farmers for all leukaemia (odds ratio 1.2) and for chronic lymphocytic leukaemia (OR 1.4). However, when considering exposures to individual chemicals, there was no evidence of a linkage between atrazine use and leukaemia in white males (Brown *et al*, 1990).

In a published case-control interview study (Hoar-Zahm *et al*, 1993), the role of atrazine in the development of non-Hodgkin's Lymphoma (NHL) was investigated in three case-referent studies conducted in 4 mid-western states (Nebraska, Iowa, Minnesota and Kansas), using a total of 993 white men with NHL and 2918 population-based referents. While the odds ratio for NHL with atrazine use was 1.4 (95% CI 1.1-1.8) in the combined studies, when adjusting for the use of 2,4-D and organophosphorus (OP) insecticides, the atrazine-NHL association was much reduced (to less than unity in all but one state). The data provide little evidence that atrazine use is associated with NHL in white males.

Mortality rates were studied in 1472 workers at a triazine manufacturing plant in Louisiana (6 months or more of herbicide production-related work) and compared with general population mortality rates. Overall mortality of workers was much lower than the general population (13 observed *vs* 28 expected total). Cancer rates were normal (3 observed *vs* 3.7 expected cancer deaths). Deaths due to non-Hodgkins lymphoma initially appeared higher than normal in workers (observed/expected = 2/0.2) however one case of nasopharyngeal cancer was originally misreported. The study did not establish whether any of the workers had physical contact with herbicides during manufacturing. The low mortality (13 deaths in total) severely limited the capacity of the study to discern cause-specific mortalities (Delzell *et al*, 1989).

A significantly larger epidemiological follow-up study of workers at two atrazine production plants in the United States showed an increase of observed-over-expected levels of non-Hodgkin's lymphoma (NHL) and soft tissue sarcoma (statistically significant). The actual numbers of cases was very low, however, and some of the workers concerned had only been employed for short periods. Therefore, a causal relationship to atrazine exposure is difficult to establish for either condition although it is not possible to completely rule out (Delzell *et al*, 1996; Sathiakumar *et al*, 1992, 1995; Delzell & Suthiakumar, 1992, 1995a, 1995b, 1996).

Correspondence included in the submission from medical officers at the Ciba-Geigy Corporation's plants at St Gabriel and McIntosh, USA, certified that no cases of skin irritation or other illness due to atrazine had been seen in these Ciba-Geigy plants (Cronan, 1988; Charter, 1989). In an epidemiological study

conducted by Ciba-Geigy at their Schweizerhalle plant, 154 pairs of employees were compared to determine if there was any increase in health disorders amongst those working with atrazine. The study did not find any changes in clinical parameters measured. With regard to all diseases that occurred since 1975, gastritis ("occasional curable gastritis or gastroenteritis") was diagnosed in a higher number of cases, but independent of the duration of exposure. It was concluded that it was unlikely that there could be any causal relationship between exposure and this effect (Gass & Stalder,1990/1993).

11.2 Exposure studies

Male Wistar rats received commercial atrazine in drinking water for 1 or 3 weeks. The principal rat urinary metabolite at the two time points was desisopropylatrazine.

In railway workers engaged in weeding operations on railway lines, the main urinary metabolites were the mono-dealkylated metabolite desisopropylatrazine and the di-dealkylated metabolite, diaminochlorotriazine. The sum of their concentrations in urine correlated with atrazine dust concentrations measured in the breathing zone. However, inhaled atrazine may not be the major source and percutaneous absorption could be a more important route (Ikonen, Kangas & Savilainen, 1988).

Dermal exposure to atrazine was very low for boom spray operators, higher for spray gun operators and the highest for mixer-loaders; during mixing/loading, maximum exposure was on forearms. Potential respiratory intake was very much lower than potential dermal exposure for mixers/loaders and spray-gun operators, and, although not significantly higher for boom spray operators, was greater than their potential for dermal exposure to atrazine (reference unknown; study reported in an existing evaluation on Departmental file).

Atrazine air concentrations and worker skin deposits (whole body) ranged from 0.07-0.53 mg/m^3 and 4.11-10.66 mg/h, respectively, during its manufacture and packaging. Unchanged atrazine in urine, which was correlated with exposure, was only a minor fraction of the total absorbed. Total atrazine absorption, assuming 20% of the inhaled dose and 10% of the dermal dose was absorbed, was estimated to be 3-8 mg per shift (Catenacci *et al*, 1990).

In six manufacturing workers, total atrazine exposure varied from 10 to 700 µmol per workshift (cutaneous and respiratory exposure). Urinary metabolites included bi-dealkylated metabolites (80%), desisopropylated metabolites (10%), desethylated (8%) and atrazine (2%). Fifty percent (50%) of the absorbed dose was eliminated in the first 8 h, completely in just over 24 h (Catenacci *et al*, 1993).

A summary paper (Loosli, 1994) provided a brief overview of the toxicology of atrazine, with advice on biological monitoring and first aid instructions. Although ruminants apparently have a low tolerance to triazine herbicides,

these compounds have low acute toxicity in laboratory mammals, and are not considered to be teratogenic or mutagenic. No signs or symptoms of poisoning have been seen in humans due to atrazine. Thus, there is no intervention on the basis of the atrazine component of formulations, and an antidote is neither known nor needed. Quantitative exposure determination relies on metabolites in urine. The diaminochlorotriazine metabolite can be measured by gas chromatography. All s-triazines can be detected by chemically transforming metabolites into cyanuric acid ie. trihydroxytriazine; however, this can be generated from other sources and low urinary levels have to be interpreted with caution. Ciba-Geigy Safety Data Sheets recommend an exposure limit of 4 mg cyanuric acid per litre of urine for industrial workers who handle triazines every day, corresponding to a triazine uptake of 10 mg/person/day. Field workers do not need to be monitored.

Since atrazine breaks down more slowly than most other current generation herbicides and is detectable in surface waters for a longer period of time after application, assessments of human exposures to atrazine through drinking water have been carried out for the populations of the states of Iowa, Illinois, and Iowa (Richards *et al*, 1995). The assessments indicated that atrazine in drinking water does not represent a significant human health threat, based on current knowledge of atrazine toxicity. Exposures to atrazine above the lifetime health advisory level of 3.0 ppb did not exceed 0.25% of the population in any of the three states and between 94-99% of the assessed population had exposure concentrations less than 1 pbb. Some of the highest groundwater concentrations observed in the study were known to be of point-source origin, arising from accidents or improper pesticide handling practices.

In the USA, the EPA has established the following Health Advisory Levels (HALs) for atrazine:-

Exposure Duration	Popul'n Segment	HAL (ppb)	Safety Factor
1 day	child	100	100
10 day	child	100	100
7 year	child	50	100
7 year	adult	200	100
70 year	adult	3	1000

The additional 10-fold safety factor was used in calculating the lifetime HAL because the US EPA ranks atrazine as a class C (possible) carcinogen. For calculation of the lifetime (70 year) HAL, it is assumed that only 20% of atrazine exposure comes from drinking water and includes an additional 5-fold factor to account for this. However, in the USA over 30 years of use, atrazine has not been detected in edible portions of plants or livestock nor has it been detected in market-basket surveys; results suggest that at least 95% of non-occupational exposure to atrazine occurs through drinking water. Thus, the 20% assumption provides an additional safety factor which approaches 5-fold.

The maximum contaminant level (MCL) is a legally-enforceable drinking water standard; for atrazine it is equal to the lifetime HAL of 3 ppb ie. 0.003 mg/kg or 3 µg/kg (Richards *et al*, 1995).

The 1992 Australian Market Basket Survey (AMBS) (National Food Authority, Australian Govt Publishing Service) conducted assays for atrazine and simazine in meat and cereal foods. No residues of either herbicide were detected. Because of the use pattern of these herbicides (just before or after crop emergence) it was considered unlikely that residues would be detected in food.

A number of applicator and field-worker exposure studies have been provided (Rosenheck, Phillips & Selman, 1993; Hofherr, 1995; Honeycutt, Bennet & DeGeare, 1996). For handgun applicators and a homeowner simulation using a push cyclone spreader, potential dermal exposure was much greater than potential inhalation exposure (Rosenheck, Phillips & Selman, 1993). At the time this review was completed, exposure monitoring data from the Honeycutt, Bennet & DeGeare (1996) study had not been compiled or submitted.

12. DISCUSSION

12.1 General toxicity

Studies submitted confirmed the low acute toxicity of the technical grade material but indicated a possibly somewhat higher toxicity of some of the metabolites, especially in females. Thus in different acute studies, desethyl- and desisopropylatraine were similar to, to up to twice as toxic as atrazine, whilst diaminochlorotriazine (DACT) and hydroxyatrazine were similar to, or possibly marginally less acutely toxic than atrazine. However, DACT was more toxic than atrazine in a comparison of several short-term repeat-dose studies and a chronic study, in terms of mortalities and clinical signs. Although a guinea-pig sensitisation test showed atrazine to be a strong skin-sensitising agent, a human skin sensitivity (patch) test did not give a positive reaction in any of 50 subjects.

In an assessment of repeat-dose feeding studies with **atrazine**, the consistent toxic effects noted across species (mice, rats, dogs) were relatively non-specific and included reduced bodyweight gain reduced food consumption and some liver enlargement at high doses (consistent with the reported effects of other triazines).

Mild anaemia was sufficiently common to indicate that inhibition of haematopoiesis was an effect of atrazine treatment at higher doses used in toxicology studies, a reversible effect also noted after large acute doses (Mencobini *et al*, 1992). Anaemia, altered haematological parameters and/or haematopoietic system toxicity were noted in short-term repeat-dose studies (rabbits), subchronic studies (rats, dogs) and chronic studies (mice, rats, dogs).

Other less-consistent non-neoplastic toxicological findings with dietary atrazine included:

- Possible testicular effects ie. small testes at high doses in 90-day weanling rat (5000 ppm) and beagle dog (2000 ppm) studies (with contradictory reports as to whether there was testicular pathology in the dog study at 632 ppm and above), and small and/or cyanotic testes reported in one (Spindler & Sumner, 1981) of a number of chronic rat studies.

- Possible cardiovascular effects, with atrial thrombi observed at high doses (1500 and 3000 ppm) in a chronic mouse study, and atrial fibrillation, ECG changes and gross and microscopic cardiac lesions in a 1-year dog study (1000 ppm).

- Possible kidney toxicity, with macroscopic kidney pathology (granular/irregular surface and/or white/pale appearance) in a chronic mouse study, and pelvic calculi/microcalculi in one (Mayhew, 1986) of a number of chronic rat studies.

- Somewhat reduced survival of females at the high doses of 1000 ppm and 3000 ppm in two chronic mouse studies and in one (Mayhew, 1986) of a number of chronic rat studies; in the other chronic rat studies, mortalities were either not affected or there was a small increase in survival in males.

A number of other observations [eg. single reports of reduced thymus weights (rat 14-day study), retinal degeneration (rat chronic study; Mayhew, 1986), increased pituitary weight (rat 1-year study), muscle tremors (1-year dog study)] were not seen in more than one study. Hormonal effects of atrazine (measured in SD and F344 female rats) are discussed below (under 'Genotoxicity and Carcinogenicity')

In subchronic feeding studies with **desethylatrazine**, there were similar findings to atrazine viz. reduced bodyweight gain and food consumption (rats and dogs), mild anaemia (rats and dogs), small increases in liver weight (rats), cardiac effects of atrial fibrillation or right atrial pathology (dogs), and mild renal tubular epithelial hyperplasia /basophilia (dogs).

In subchronic feeding studies with **desisopropylatrazine**, there were similar findings to atrazine viz. reduced bodyweight gain and food consumption (rats and dogs), possible mild anaemia (dogs) or some evidence of it (rats), with extramedullary haematopoiesis in spleen and liver, liver weight increases (rats), and increases in heart weight (dogs), although without accompanying pathology. Additionally, thyroid activation and hypertrophy of TSH-producing cells in the pituitary were reported in rats (subchronic study).

In repeat-dose feeding studies with **diaminochlorotriazine** (short-term repeat-dose, subchronic, and chronic studies have been conducted) there were similar clinical signs to atrazine, with reduced bodyweight gains and food intake (rats

and dogs), mild anaemia (rats), and cardiovascular effects (dogs; sino-atrial arrest at 2500 ppm and atrial haemorrhagic lesions at 1500 ppm); several other pathological effects in dogs (viz. in liver, thymus and testes) were ascribed as being secondary to the cardiovascular effects. Additionally, thyroid weights were increased (rats; subchronic study) although without accompanying pathology (cf. desisopropylatrazine which caused thyroid activation). Similar to atrazine, DACT increases the length of the oestrous cycle in SD rats and the incidence of persistent oestrus, with a reduction in circulating oestrogen levels at high doses (cf. atrazine).

In repeat-dose feeding studies with **hydroxyatrazine** (subchronic and chronic studies provided), consistent findings included reduced bodyweight gain and food consumption, anaemia (rats), findings comparable to the effects of atrazine. Effects on kidneys were noted across studies and species tested (rats and dogs), with increased kidney weights, urinalysis effects and gross and microscopic pathology (rats and dogs) and chronic progressive nephropathy (rat chronic study), with several other effects in rats (testicular degeneration, some cardiomyopathy) ascribed as secondary to the CPN.

12.2 Carcinogenicity & endocrine-system effects

A series of two-year chronic feeding studies in rats (Sprague-Dawley and Fischer-344) have been carried out to determine the extent (if any) of the carcinogenic action of technical grade atrazine on the pituitary and mammary glands and its effect on hormone levels and oestrous cycles in females.

Thirteen (13) different oncogenicity studies on atrazine (reported in 17 separate reports) were reviewed, together with one utilising hydroxyatrazine; the outcomes are briefly summarised and collated as follows:-

Atrazine

(1) ***Mouse dietary study*** (Innes *et al*, 1969). Atrazine without oncogenic activity in two strains of mice (18/sex/strain) at 82 ppm in the diet for 18 months. Limited details and study of limited value.

(2) ***Mouse dietary study*** (Sumner, 1981). CD-1 mice (60/sex/gp) given atrazine at 0, 10, 300 and 1000 ppm for 21-22 months; possible small increase in alveolar cell tumours at the low dose and mid dose but not high dose - also well within historical control incidence (validated IBT study).

(3) ***Mouse dietary study*** (Hazelette & Green, 1987). CD-1 mice (60/sex/gp) given atrazine at 0, 10, 300, 1500 and 3000 ppm for at least 91 weeks; reduced survival in high-dose females but no neoplastic effects.

(4) ***Rat dietary study*** (Keller, 1961). Albino rats (strain?) (30/sex/gp) given atrazine at 0, 1, 10 and 100 ppm for 104 weeks, with increase of 1 ppm to 1000 ppm at week 65; no neoplastic effects reported but study of limited value because of low survival rates.

(5) ***SD Rat dietary study*** (Spindler & Sumner, 1981). Charles River albino rats (60/sex/gp) given atrazine at 0, 10, 100 and 1000 ppm for 2 yrs; no indication of increased mammary tumours but, although supportive, this IBT study was not considered adequate to assess the carcinogenic potential of atrazine.

(6) ***SD Rat dietary study*** (Mayhew, 1986). SD rats (70-90/sex/gp) given atrazine at 0, 10, 70, 500 and 1000 ppm for 2 yrs; increased survival in high-dose males, decreased in females. Trend analysis indicated a NOEL for increased mammary tumour incidence in females of 10 ppm (approx. 0.4-0.6 mg/kg bw/d).

(7) ***Fischer rat dietary study*** (Pinter *et al*, 1990). Fischer-derived rats (50-56 sex/gp) given atrazine at 0, 375 and 750 ppm for 126 weeks; survival was very low, although there was enhanced survival in dosed males and possibly females. No increase in mammary tumours in females. Small increases in benign mammary tumours in 750 ppm males (but increasing latency with increasing dose!), malignant uterine tumours in females, and haemopoietic system. Study probably of limited value.

(8) ***SD Rat dietary study*** (Rudzki, McCormick & Arthur, 1991; Ackerman, 1991). SD Rats (50/sex/gp, from the F1 generation of a 2-generation reprodution study, exposed *in utero* to the same dose levels) were given 0, 10, 50 and 500 ppm atrazine for 104 weeks; mammary tumour incidences not increased in dosed females. Apparent increase in pituitary tumours in females at the high dose but within historical controls and incidences in other groups were unexpectedly low.

(9) ***Female SD rat dietary study*** (Osheroff, 1990a; Thakur, 1991a). Female SD rats (70/gp) were given atrazine at 0, 70 or 400 ppm for up to 2 years; at 400 ppm there was a somewhat earlier incidence of mammary and pituitary tumours, without any increase in their overall lifetime incidence.

(10) ***Female SD rat dietary study*** (Thakur, 1992a). Female SD rats (60/gp) were given atrazine at 0, 70 or 400 ppm for at least 2 years; no effect at either dose on the onset or incidence of pituitary tumours. Earlier onset, but not increased incidence of mammary tumours in females at 400 ppm (and lower incidence than controls at 70 ppm).

(11) ***Female Fischer rat dietary study*** (Osheroff, 1990b;Thakur, 1991b; Eldridge *et al*, 1993). Female F344 rats (70/gp) were given atrazine at 0, 10, 70, 200 or 400 ppm for up to 104 weeks; no effect at any dose on the onset or incidence of pituitary, mammary, ovarian or uterine tumours.

(12) ***Fischer rat dietary study*** (Thakur, 1992b). Fischer F344 rats (60/sex/gp) were given atrazine at 0, 10, 70, 200 or 400 ppm for at least 2 years; no effect at any dose on the onset or incidence of tumours.

(13) ***Rat dietary study*** (Pettersen & Turner, 1995). Female SD albino rats (55/gp) given atrazine at 0, 15, 30, 50, 70 and 400 ppm for 12 months; combined incidence of mammary gland adenocarcinomas, fibroadenomas and adenomas showed a small increase at 400 ppm, with a NOEL for incidence and onset time of 70 ppm (4.1 mg/kg bw/d).

Hydroxyatrazine

(14) ***SD rat dietary study*** (Chow & Emeigh Hart, 1995). SD rats (70+10/gp) were given **hydroxyatrazine** at 0, 10, 25, 200 or 400 ppm for up to 104 weeks; no effect at any dose on the time of onset of mammary tumours, although reduced incidence at the high dose, probably as a consequence of earlier deaths.

Discussion

In Sprague-Dawley rats (2 studies) no increase in the overall incidence of pituitary or mammary tumours was seen with atrazine but there was a somewhat earlier onset of mammary fibroadenoma/carcinoma in the 400 ppm group (20 mg/kg bw/d) (see summary Table at Attachment 8). Similarly, there appeared to be an earlier onset of pituitary tumours (Table in Section 6. and Attachment 8). Oestrous cycle and oestrogen results showing an increased number of days in oestrus or under oestrogen dominance suggest that this earlier onset of mammary tumours may relate to an accelerated ageing of the neuro-endocrine system. (In Sprague-Dawley rats, the time spent in oestrus tended to increase with age - see Attachment 5).

Unlike atrazine-treated Sprague-Dawley rats which tended to spend more days in oestrus or under oestrogen dominance than did age-matched untreated controls, Fischer rats did not exhibit any effects on the length of the oestrous cycle, oestradiol or progesterone levels with atrazine dosing. The results of the lifetime studies in female Fischer-344 rats indicate that the only toxicological effect of atrazine was reduced bodyweight gain (with a NOEL of 70 ppm, or approximately 3.5 mg/kg bw/d). There was no evidence for a carcinogenic effect of atrazine. It also may be noted that the proportion of time Fischer rats spent in oestrus tended to decline with age, in contrast to SD rats.

The mammary tumour response observed in various female Sprague-Dawley rat studies (those conducted according to current standards) has not been completely consistent. Thus, the first study (Spindler & Sumner, 1981) revealed a non dose-related increase in the incidence of fibroadenomas (at 10 and 1000 but not 100 ppm). In a second study (Mayhew, 1986) there was a dose-responsive increase in adenocarcinomas but no effect on fibroadenomas. In a subsequent study (female offspring culled from the F2 generation of a 2-generation reproduction study), there was no increase in mammary tumours (Rudzki *et al*., 1990). The recent studies provided with the most recent submission from Ciba-Geigy (Wetzel *et al*., 1993) showed an earlier onset of mammary tumours, without an increase in total tumour incidence. Considering the large variation noted in the spontaneous occurrence of mammary tumours in SD rats (Haseman *et al*., 1986), it may be that the inconsistent response in the various studies is not surprising. Overall, the findings have led to the hypothesis that certain triazines can produce an endocrine-mediated imbalance which results in precocious reproductive ageing in SD rats, with the possible earlier onset or increased incidence of mammary tumours.

In a published study (Pinter *et al*, 1990) using Fischer 344/LATI rats, there was an increase in benign mammary tumours in HD males (with a small increase in latency cf. tumours in control animals) but not in females. The increase in

male mammary tumours may be attributable, at least in part, to the significantly longer lifespan of 750 ppm males than controls. However, neither tumour to age adjustment nor comparison to background control data of the laboratory were performed in this study. In a later Hazleton study, no increase in mammary tumours was noted in male Fischer 344 rats at the HD (400 ppm); the increase in the Pinter study was only seen at 750 ppm, not at 375 ppm. An increase in malignant uterine tumours in females and an increase in haematopoietic system tumours also was noted. It is possible that atrazine treatment may have affected hormonal balance since the mammary gland and uterine tumours may be hormone-dependent tumours; however, the lack of any increase in mammary tumours in females argues against a direct oestrogenic action of atrazine.

A number of studies designed to investigate the possible estrogenic properties of atrazine have been provided. Neither atrazine, simazine nor the common metabolite, diaminochlorotriazine (DACT), at concentrations up to 100 mM, competed with radioactive oestradiol binding (5 nM) to oestrogen receptors (ER) in extracted uterine tissue under equilibrium conditions (at 4°C). The triazines were about 10^5 less potent than oestradiol itself in causing 50% reduction in labelled oestradiol binding to ER (at 25°C). Results from three types of *in vivo* studies in ovariectomised rats suggested that none of the three chloro-s-triazines possessed any significant intrinsic oestrogenic activity but that they are capable of weak inhibition of oestrogen-stimulated responses in the rat uterus; this inhibition may play a role in the changes in reproductive endocrine function previously observed in female SD rats. (It was claimed that these findings differentiate the triazines from eg. DDT, methoxychlor, chlordecone.)

In conclusion, the following points should be noted:

- The earlier onset in mammary tumours was not seen in male SD rats, in male or female Fischer rats, or male or female CD-1 mice.
- It is likely that the response observed in SD female rats only occurs above a certain threshold.
- The background incidence of mammary tumours is significantly higher in female SD rats than in female Fischer 344 rats eg. NCI data (1980) indicate a 36.4% historical control incidence for mammary tumours in SD rats and a 17.9% incidence in Fischer rats (with other historical control data reporting even higher incidences for SD rats).
- The available evidence suggests that neither atrazine nor its metabolites are genotoxic in animal cells (see also Brusick, 1993).
- In humans, menopausal women develop episodes of declining oestrogen secretion and longer periods of low oestrogen levels, in contrast to the situation in ageing SD rats. Therefore, it would appear that the atrazine response in SD rats is not an appropriate surrogate for the assessment of human risk for mammary tumour development.

Therefore, the mammary tumour findings in these new toxicology studies do not raise significant new concerns about the current uses of atrazine with respect to human health.

Epidemiological studies at the University of Alabama on atrazine production plant workers are inconclusive; an atrazine-linked increase in non-Hodgkin's lymphoma and soft tissue sarcoma cannot be completely ruled out. However, two published papers on agricultural risk factors for NHL and leukaemia, reporting results of four case-control interview studies in the USA (Nebraska, Iowa, Minnesota and Kansas), did not provide any evidence that atrazine use is associated with NHL or leukaemia in white males.

When administered subchronically to female Sprague-Dawley rats, the metabolite, **diaminochlorotriazine**, increased both the length of the oestrous cycle and the incidence of persistent oestrus (NOEL was 0.5 mg/kg bw/d), with relatively similar effects on sex hormone levels. Hormone level investigations were not conducted following dosing with any other metabolites. One carcinogenicity study in SD rats was conducted with **hydroxyatrazine**, without any increase in the incidence of mammary tumours.

12.3 Genotoxicity

In a large range of studies *in vivo* and *in vitro* in bacteria, *Drosophila* and mammalian cells, across all genotoxic endpoints (gene mutation assays; chromosomal effects assays; other gentoxic effects; and cell transformation assays), predominantly negative results were obtained and the weight of evidence to date is that atrazine is not genotoxic. Tests for induction of micronuclei and SCEs in rats and mice dosed for 13 weeks with atrazine-containing drinking water (up to 100x levels found in contaminated US water) were negative. Concerns have been expressed about the genotoxicity of N-nitrosoatrazine (NNAT) but available evidence points to rapid decomposition of synthetic NNAT in the environment; no data was cited which indicated that it was formed in the environment after atrazine use.

An hypothesis for oestrogen-dependent tumorigenesis relates to the observation that oestradiol metabolism proceeds primarily through two mutually exclusive pathways, pathway I to 2-hydroxyestrone (2-OHE1) or pathway II to 16-alpha-OHE1. It has been claimed that 2-OHE1 has minimal oestrogenic activity and is non-genotoxic whilst 16-alpha-OHE1, a fully potent oestrogen is genotoxic (Telang *et al*, 1992). It is suggested that substances which inhibit pathway I or elevate pathway II could increase tumour risk, while the converse would be the case for those factors which inhibit pathway II. Atrazine which, as discussed, is unlikely to be directly genotoxic *per se* has been hypothesized as possibly acting through this pathway ie. as having an indirect genotoxic and carcinogenic effect (Davis *et al*, 1994). However, there does not appear to be experimental evidence to support this hypothesis to date. Subsequent to completion of the draft atrazine review, several publications on *in vitro* data collected in cell culture studies and on epidemiological data in women,

discount this hypothesis [Safe S (1997) Is there an association between exposure to environmental oestrogens and breast cancer. Env Hlth Perspect 105, Suppl 3, 675-678; Ursin G, London S, Stanczyk F, Gentzschein E, Paganini-Hill A, Ross RK & Pike MC (1997) A pilot study of urinary estrogen metabolites (16 -OHE1 and 2-OHE1) in postmenopausal women with and without breast cancer. Env Hlth Perspect 105, Suppl 3, 601-605].

12.4 Reproduction and development

No teratogenic effects were noted in 2- and 3-generation reproduction studies with atrazine or in developmental studies with atrazine and each of its four metabolites.

A developmental study in rats given water representative of groundwater contaminated with pesticide/fertiliser mixtures at 1x, 10x and 100x actual concentrations (containing 0.5, 5 and 50 ng/mL atrazine) did not report any significant adverse effects. There were no developmental effects in 2- and 3-generation rat dietary studies in rats at the highest doses tested, even though there was some maternal toxicity reported in the 2-generation study.

12.5 Immunotoxicity

In one specific study on the immunotoxic potential of atrazine (Fournier *et al*, 1992), transient and reversible immunosuppression of humoral-mediated and cell-mediated responses and activated macrophage phagocytic activity could not be attributed to the direct chemical-related effect of sublethal exposure to an atrazine formulation after sublethal exposures.

12.6 NOEL considerations

The following Table briefly summarised the NOELs (and LOELs) from repeated-dose studies with atrazine; note that only those deemed adequate for regulatory purposed are included ie. the Table is not a complete listing of all the studies provided.

Atrazine

Study	NOEL mg/kg bw/d	LOEL and Toxic Effect at this Dose
NZW rabbit 21-day dermal	10 (F)	Reduced bodyweight gain & inc. cholesterol at 100 mg/kg.
	100 (M)	Dec. bodyweight, food consumption, erythroid parameters & WBCs, inc. spleen weight & some clin. chem. changes at 1000 mg/kg
SD rat 90-day dietary	est. 20	Dec. food intake & bodyweight, renal pelvic calcium deposition, splenic haematopoiesis at 100 mg/kg
SD rat 3-month dietary	est. 5	Red. weight gain & food consumption at 50 mg/kg
SD-derived rat 3-month dietary	0.6 (M) 3.35 (F)	Reduced bw at 3.3 mg/kg. Reduced bodywt gain & food intake, & splenic haemosiderin deposition at 35.3 mg/kg
beagle dog 90-day dietary	est. 5	Reduced testicle weights & anaemia at 15.8 mg/kg (no NOEL established if reduced bodyweight & food consumption at 5 mg/kg taken into account)
CD-1 mouse 21/22-month dietary	est. 45	inc. female mortality, reduced bodyweight, macroscopic kidney pathol. at 1000 ppm (150 mg/kg). Increase in alveolar cell tumours at 10 & 300 but not 3000 ppm - also largely within historical controls
CD-1 mouse 91-week dietary	1.2 (m) 1.6 (f)	Decrease in bodyweight/bodyweight gain at 38.4 (m) and 47.9 (f) mg/kg No evidence of carcinogenicity up to 385 (m) and 482 (f) mg/kg
SD rat 24-month dietary	0.4-0.6 (f) 2.4-3.3 (m)	mammary tumours at 2.8-4.5 mg/kg dec. bodyweight, behav. effects, palpable tissue masses, muscle degen. at 17.9-24.2 mg/kg
SD rat (f) *in utero* exposure then 65 wk diet	0.7 (f)	Mammary tumour incidence not increased.
SD rat (m) 52 wk dietary	2.3 (m)	Red.bodyweight & food consumption (m & f), reduced erythroid params (f), inc. serum cholesterol (f) at 3.5 (f) & 23.6 (m) mg/kg
SD rat 2-yr dietary	3.0-3.5	Red. bodyweight, inc. mortality, earlier onset mammary tumours at 20.7-21.6 mg/kg (no overall increase in incidence at term)
F344 rat 2-yr dietary	4.0-4.3	Red. bodyweight gain at 11.9-12.4 mg/kg No inc. in any tumour types
F344 rat 2-yr dietary	*ca.* 3.5	Slightly red. bodyweight gain at 200 ppm (*ca.* 9.0-11.4 mg/kg) No increase in any tumour types

Study	NOEL mg/kg bw/d	LOEL and Toxic Effect at this Dose
SD rat (f) 1-year dietary	4.1	Earlier onset of mammary tumours at 23.9 mg/kg
beagle dog 1-yr dietary	4.97	Reduced bodyweight & food consumption, moribundity, cachexia & ascites, clin. chem. and organ weight changes, and cardiotoxicity at 33.7 mg/kg
CR rat 2-gener'n dietary	2.73 (m) 3.45 (f)	Red. food consumption and bodywt gain at high dose of 500 ppm; no reproductive effects.
rat oral teratology	100	embryotoxic effects at the next highest dose of 500 mg/kg
SD rat oral teratology	10	Fetotoxicity (ossification delays) and maternotoxicity at 70 mg/kg
SD rat oral teratology	25	Minor skeletal variations and maternotoxicity (food consumption, bodywt) at 100 mg/kg
NZW rabbit oral teratology	5	No teratogenicity. Embrotoxicity and fetotoxicity at 75 mg/kg

See Attachment 9 for a full tabular lisiting of all toxicology studies assessed.

None of the recently-submitted studies has provided any lower NOEL for atrazine than the current lowest NOEL of 0.5 mg/kg/d. This was based on results of a 2-year dietary rat study (Mayhew, 1986) in which 10 ppm was taken as the no-effect-level for mammary tumours in female rats. While there was a possible small increase in mammary tumours at this dose [total numbers of tumours/rat were 55/66, 68/64, 91/68 and 130/65, while the percentage of female rats with mammary tumours (all types) was 53%, 61%, 69% and 72% at the control, 10, 70, 500 and 1000 ppm respectively], trend analysis indicated 10 ppm as a NOEL. Furthermore, other submitted studies (two studies in SD rats, two in Fischer rats) indicate that 70 ppm (3.5 mg/kg bw/d) was a NOEL for mammary tumours.

Atrazine metabolites

The accompanying Table lists the studies conducted with atrazine metabolites which are considered suitable for regulatory purposes; a complete summary list of all toxicology studies assessed in this report may be found at Attachment 9.

Study	NOEL mg/kg	LOEL and Toxic Effect at this Dose
Desethylatrazine		
Tif:RAIf rat 3-month dietary	3.2-3.35	Red. weight gain & food consumption (both sexes), min. haematol changes,AP increase and liver weight inc. (females) at 35.2-38.7 mg/kg
beagle dog 13-week dietary	3.7-3.8	Possible cardiac effects, renal tubular epithelial hyperplasia/basophilia at 28.8-32.2 mg/kg
rat oral teratology	30	maternal & fetal NOEL, based on reduced food intake, inc. in embryonic resportions, & no's of incompletely ossified elements at $\geq$100 mg/kg
rat oral teratology	5 (maternal) 25 (fetal)	Dec. food intake and bodywt at 25 mg/kg. Inc. fused sternebrae, poor ossific'n at 100 mg/kg
Desisopropylatrazine		
Tif:RAIf rat 3-month dietary	3.2-3.3	Thyroid gland activ'n and hypertrophy of TSH-producing cells in pituitary (m), extramedull. haematopoiesis in liver & spleen (f), inc. rel. liver weight (f) at 34.9-37.5 mg/kg
beagle dog 13-week dietary	3.8	Reduced bodyweight parameters & food intake, heart weight decrease at 33.3 mg/kg. Anaemia?
rat oral teratology	5	Maternal and fetal NOEL, based on reduced food consumption and bodywt gain, and an increase of fused sternebrae at 25 mg/kg. No terata.
Diaminochlorotriazine		
SD rat 4-week dietary	0.89-0.92	Red.bodyweight gain & food intake, and haematol. changes at $\geq$ 45.1-48.7 mg/kg
Beagle dog 4-week diet	13.9-14.1	Soft/mucoid/few faeces, red. bw, food consumption at 21-27.2 mg/kg
SD rat 90-day dietary	0.7	Oestrous-cycle effects at 7.6 mg/kg
Beagle dog 52-week diet	3.2-3.9 (m) 2.7-3.8 (f)	Inc. mortality, cardiovasc., haematol., biochem. effects, organ weight changes, fluid accumul'n, & liver, testes, bone marrow and thymus pathol. at 750 ppm (22.0-31.7 mg/kg)
SD rat oral teratology	2.5	Maternal and fetal NOEL, based on slight reduced food intake & bodywt gain, and inc's in skeletal variations at 25 mg/kg

Study	NOEL mg/kg	LOEL and Toxic Effect at this Dose
Hydroxyatrazine		
SD rat 90-day dietary	6.3(m) 7.35 (f)	Nephrotoxicity at 18.9 (m) and 22.7 (f) mg/kg
Beagle dog 13-week dietary	5.8 (m) 6.2 (f)	Nephrotoxicity at 59.6 (m) and 63.9 (f) mg/kg
SD rat 2-year dietary	0.962 (m) 0.475 (f)	Nephrotoxicity at 7.75 (m) and 1.17 (f) mg/kg
SD rat oral teratology	25	Maternal and fetal NOEL, based on reduced bodywt gains and food intake, and incomplete skeletal ossific'n at 125 mg/kg. No terata.

The lowest NOEL reported for **desethylatrazine** was 3.5 mg/kg bw/d, based on findings in two 3-month dietary studies, one in rats and one in dogs. The LOEL in the rat study was 35.2-38.7 mg/kg, with reductions in bodyweight gain and food consumption, minimal haematology changes and an increase in liver weight and serum AP. In the dog study, the LOEL was 28.8-32.2 mg/kg, with possible cardiac effects and renal tubular epithelial hyperplasia/basophilia.

The lowest NOEL reported for **desisopropylatrazine** was 3.2 mg/kg bw/d, based on findings in a rat 3-month dietary study. The LOEL in this study was 34.9-37.5 mg/kg, with thyroid gland activation and hypertrophy of TSH-producing cells in the pituitary (males), extramedullary haematopoiesis in liver and spleen (females), and increases in relative liver weight (females) at 34.9-37.5 mg/kg.

The lowest NOEL reported for **diaminochlorotriazine** was 0.7 mg/kg bw/d, based on findings in a rat 3-month dietary study. The LOEL in this study was 7.6 mg/kg, with oestrous-cycle effects at this dose.

The lowest NOEL reported for **hydroxyatrazine** was 0.5 mg/kg bw/d, based on findings in female rats in a 2-year dietary study. The LOEL in this study was 1.17 mg/kg, with nephrotoxicity at this dose.

12.7 Exposure

12.7.1 Food

Atrazine is used in high volumes, predominantly as a herbicide in preparation for plantings for coarse grains and sugarcane, with minor uses in plantation forestry and legumes.

The 1992 Australian Market Basket Survey (AMBS) (National Food Authority, Australian Govt Publishing Service) conducted assays for atrazine and simazine in meat and cereal foods. Because of their use pattern (just before or

after crop emergence) it was considered unlikely that residues would be present in food. No residues of either herbicide were detected.

This finding is in agreement with US data; in over 30 years of use, atrazine has not been detected in edible portions of plants or livestock nor has it been detected in market-basket surveys (Richards *et al*, 1995).

Thus it may be concluded that exposure of the population to atrazine in food is very unlikely.

12.7.2 Drinking water

The AMBS results for foodstuffs and the fact that atrazine is both mobile and reasonably stable in the environment, indicates that any non-occupational exposure to atrazine is likely to occur through drinking water.

In the USA, the NTP (National Toxicology Program) has conducted subchronic, reproductive, developmental and genotoxicity studies using drinking water containing pesticide/fertiliser mixtures representative of established groundwater contamination in California and in Iowa, with testing at 0.1x, 1x, 10x and 100x the median concentration found in the groundwater (atrazine at 0.5, 5 and 50 ng/mL). In 26-week toxicity studies, in neither B6C3F1 mice nor F344/N rats were there any clear adverse effects on clinical signs, body weight, water consumption, clinical pathology, neurobehavioural tests, the reproductive system, organ weights, or histopathological findings. *In vivo* genetic toxicology assays were conducted on peripheral blood erythrocytes from the female mice (induction of micronuclei) and on splenocytes from male rats and female mice (induction of micronuclei and SCEs) at the 13-week interim evaluation of the 26-week toxicity study. Results of tests for induction of micronuclei in erythrocytes of female mice treated with CAL water were negative. With the IOWA mix, significant increases were seen at 10x and 100x concentrations but they were within the historical control range of micronuclei. SCE frequencies in splenocytes of male rats and female mice were marginally increased by the CAL mixture but neither species exhibited increased frequencies of micronucleated splenocytes. None of these changes were considered to be of biological significance. In a continuous breeding study (2-generation) in CD-1 mice there were no significant effects on any parameter measured, even at doses of IOWA and CAL mixtures at 100x mean groundwater contamination levels. There was no testicular or epididymal pathology and oestrous cyclicity was not affected. Similarly, in a teratology study in Sprague-Dawley rats there were no significant effects on any parameter measured, at any dose of IOWA and CAL mixture tested.

An evaluation and summary of measured levels of atrazine in drinking water in Australia may be found in the Environmental Assessment. In summary, atrazine is commonly found in surface and ground waters, particularly within plantation forestry and irrigated agriculture areas. It has been found at levels in the order of 100 µg/L in irrigation drainage water from a rice growing area with

some maize, but in natural surface waters, it is generally below 10 µg/L. A median concentration of 8.1 µg/L was found in Tasmanian streams draining forestry plantations on the day of application. Only limited groundwater monitoring has been conducted but it has been detected at concentrations in the order of 1 µg/L. Groundwater samples often contain detectable levels of the metabolites desethylatrazine and/or desisopropylatrazine, at levels of the same order of magnitude as parent atrazine. Some of the highest groundwater concentrations observed were known to be of point-source origin, arising from improper pesticide handling practices or accidents. It may be noted that intake at 10 µg/L in water would result in an estimated intake approx. 5.7% of the current health ADI for atrazine.

Much of the above data was collected prior to introduction of a national strategy to minimise the levels of atrazine in water; the target date by which all atrazine product labels had to carry information to reflect the new controls was December 1995. Components of the strategy include:

- no mixing/loading or application within 20 metres of wells, sink holes, waterways etc.
- no application within 60 metres of natural or impounded lakes or dams
- no use in channels or drains (note: it was was widely used for irrigation channel hygeine)
- no use in industrial and non-agricultural situations
- reduced application rates
- a water monitoring program
- the establishment of a committee to plan and review the monitoring program.

The success of this strategy in reducing atrazine contamination of water remains to be seen.

A comparison and summary of Australian and some international guideline values for atrazine in drinking water is provided in Attachment 1. Australia's current standards are as follows:-

Health Value $= \frac{0.005 \text{ mg/kg bw/day} \times 70 \text{ kg} \times 0.1}{2 \text{ L/day}}$

$= 0.02$ mg/L

where:

- 0.005 mg/kg bw is the ADI, calculated from the NOEL using a safety factor of 100
- 70 kg is taken as the average wt of an adult
- 0.1 is based on 10% of the ADI
- 2 L/day is the estimated (maximum) amount of water consumed by an adult

Guideline Value = 0.0005 mg/L; if atrazine is detected at or above this value, the source should be identified and action taken to prevent further contamination.

The current standard is based on measurement of atrazine alone, whereas, as noted above, metabolites, when measured, have commonly been detected at levels of the same order of magnitude as parent atrazine. Since the metabolites are in the main no less toxic than atrazine (diaminochlorotriazine and hydroxyatrazine have lowest NOELs of 0.7 and 0.5 mg/kg bw/d respectively, equivalent to the NOEL for atrazine), these metabolites need to be considered in the residue definition. In view of the toxicity of atrazine and its metabolites, their persistence in the environment (including ground and surface water), and the level of not-unreasonable public concern about the presence of any level of pesticides in drinking water, it would be prudent public policy to do this. Furthermore, whilst there is no basis for concern about human health effects at current levels of contamination which have been reported, consideration should be given to strengthening the national strategy to minimise the levels of atrazine in water. Additional steps could include a ban on the use of atrazine for irrigation channel 'hygiene' and in agricultural or plantation forestry in water catchment areas.

12.8 Comment on public submissions

Relevant public submissions to the toxicological assessment have been summarised (Attachment 10). Most of the issues and concerns raised have been addressed in this Discussion. In response to the claim that current testing protocols may not detect the consequences of fetal exposure to endocrine disrupting chemicals, it should be pointed out that a number of reproductive and developmental toxicity studies were provided, including studies with contaminated drinking water (at up to 100x the levels found in ground water samples). Reasonably extensive research from the NTP in the USA with pesticide/fertiliser mixtures significantly answers concerns that atrazine has not been tested in combination with other chemicals. Specific concerns about immune sytem effects have to some extent been addressed by a relatively recent publication (Fournier *et al*, 1992; Section 10). Concerns about the potential of atrazine or N-nitrosoatrazine to cause genetic damage have been addressed (See Section 10). Claims that technical-grade atrazine may contain

hexachlorobenzene and tetrachloro-dibenzofuran as microcontaminants is not borne out by declarations of composition and batch analyses, but is a matter which needs to be checked by the NRA.

In its submission supporting the continued use of atrazine, a forestry organisation made the point that, with the increasing pressure to establish timber plantations rather than log native forests, atrazine provided an effective and cheap control of weeds during the first 18 months of establishment of eucalyptus plantations. Alternative herbicides are either significantly more expensive or not suitable for eucalypts.

13. CONSIDERATION OF PUBLIC HEALTH STANDARDS

13.1 Approval status

There are no objections to the continued approval of atrazine. The weight of evidence suggests that it is not genotoxic carcinogen and that in the Sprague-Dawley strain of rats, an earlier onset of mammary tumours at high doses in some toxicology studies is due to a strain specific hormonal effect. The pattern of oestrogen levels in ageing SD rats differs from another rat strain tested (F344) and from that in humans and the atrazine effect in SD rats is unlikely to be an appropriate surrogate for the assessment of human risk for mammary tumour development.

It is considered that, apart from significantly stricter controls over uses in riparian zones (see below under ‘Drinking Water Guidelines’) its current approved uses should continue.

13.2 NOEL/ADI considerations

Based on an assessment of the available data, no change to the current NOEL for atrazine is warranted. The NOEL is 0.5 mg/kg bw/d (10 ppm) in a 2-year Sprague-Dawley rat study, based on a LOEL of 70 ppm (2.8-4.5 mg/kg bw/d), with a statistically-significant increase in mammary tumour incidence at this dose. Whilst the mammary tumours are not considered to be relevant to human health, the response reflects an hormonal interaction and can be taken as an appropriately conservative endpoint for setting the ADI.

On the basis that the metabolites of atrazine (viz. desethylatrazine, desisopropylatrazine, diaminochlorotriazine and hydroxyatrazine) have similar and overlapping toxicities to the parent triazine, it would be prudent public health policy to consider altering the residue definition for atrazine to include parent compound plus these four metabolites. Thus, the ADI would be based upon a combined total of atrazine plus its four closely related triazine metabolites. This proposal was taken to the Department's Advisory Committee

on Pesticides and Health (ACPH) for consideration - its summary recommendations are reproduced below.

In its comments on the draft ECRP report, Novartis indicated that the combination of atrazine and its 3 chlorometabolites would be appropriate, but that the toxic effects of hydroxyatrazine (renal effects) were different from those of atrazine and thus it was not appropriate to include it in a residue definition; nevertheless, there are overlapping toxicities.

Regardless of these considerations, it should be noted that, based on available food monitoring data collected both nationally and internationally, exposure of the population to atrazine in food is very unlikely.

13.3 Drinking Water Guidelines

As noted above, exposure of the population to atrazine in food is very unlikely. However, the fact that atrazine is both mobile in the soil and reasonably stable in the environment indicates that non-occupational exposure to atrazine, if it occurs, is likely to occur through contamination of drinking water. Thus, drinking water standards need to be addressed.

Consistent with the above proposal for modifying the residue definition based on toxicological grounds, consideration should be given to amending the Australian drinking water guidelines to include the closely related metabolites with parent atrazine in the definition of atrazine; this action would have the equivalent effect of lowering the Guideline Value[1] (0.0005 mg/L) for atrazine alone since, in water samples in which atrazine is detected, one or more metabolites are commonly detected but disregarded in the current Standard.

This issue was also referred to the ACPH for consideration and advice.

Considerations of the Advisory Committee on Pesticides and Health (ACPH)

The following reproduces in full the draft summary and conclusions of the 12th meeting 5th February 1997) of the Department of Health and Family Services' ACPH (unratified as at the date of inclusion of the text in this report); the full report of the committee's deliberations on atrazine may be found at Attachment 13.

The Committee:

- CONSIDERED the Existing Chemicals Review Program assessment of the toxicological data for atrazine;
- NOTED that:

[1] The level, at or above which, action should take place to identify the source and prevent further contamination

- the toxicology database for atrazine and its metabolites (desethylatrazine, desisopropylatrazine, diaminochlorotriazine and hydroxyatrazine) is extensive;
- atrazine has low acute toxicity;
- in repeat-dose feeding studies with atrazine there were consistent, but relatively non-specific, toxic effects across all species tested;
- an early onset of mammary tumours was seen in female Sprague-Dawley (SD) rats, but not in male SD rats, female Fischer rats or either sex of CD-1 mice;
- atrazine or its metabolites appear not to be genotoxic in animal cells;
- the metabolites, desethylatrazine, desisopropylatrazine, diamino-chlorotriazine and hydroxyatrazine, generally have similar and overlapping toxicities to parent atrazine;
- *in-vitro* and *in-vivo* studies suggest that the triazines possess no intrinsic oestrogenic activity but are capable of weak inhibition of oestrogenic-stimulated responses in the SD rat uterus;

- DISCUSSED a proposal to revise the current acceptable daily intake (ADI) and residue definition for atrazine to include parent atrazine plus its four metabolites; desethylatrazine, desisopropylatrazine, diamino-chlorotriazine and hydroxy-atrazine;

- AGREED that there be no change in the existing ADI for atrazine of 0.005 mg/kg bw/day, based on a no-observable-effect-level (NOEL) of 0.5 mg/kg bw/day for mammary tumours in female rats observed in a 2-year dietary SD rat study

- whilst mammary tumours were not considered to be relevant to human health, the response reflects a hormonal interaction and this was considered to be an appropriately conservative endpoint for estimating the ADI.

- RECOMMENDED that the ADI be set for parent atrazine only;

- APPRECIATED the need to take into account toxicologically significant metabolites from an exposure risk assessment perspective;

- CONSIDERED that the inclusion the metabolites in the atrazine residue definition for food would be impractical as:

  - parent atrazine is likely to form the major component of the residue;
  - some of the metabolites are common to other triazine herbicides which could present enforcement difficulties; and
  - it would unnecessarily complicate regulatory analyses which may compromise routine residue monitoring.

- WAS AWARE that atrazine is fairly stable in ground water and that desethylatrazine, and hydroxyatrazine are major soil and water metabolites; and

- SUPPORTED modification of the basis for the atrazine guideline value in the *Australian Drinking Water Guidelines -1996* so that it applies to the total concentration of parent atrazine plus the metabolite desethylatrazine.

Conclusions

Thus the ACPH considered that for a number of reasons, especially that some of the metabolites are common to other triazine herbicides, the inclusion of metabolites in the atrazine residue definition for food would be impractical. This recommendation has been accepted by the Department.

However, recognising the need to take into account toxicologically-significant metabolites from an exposure risk assessment perspective, the ACPH supported the modification of the atrazine guideline value in the *Australian Drinking Water Guidelines - 1996*. They proposed that, rather than including all 4 metabolites (desethylatrazine, desisopropylatrazine, diaminochlorotriazine and hydroxyatrazine) as outlined above, only the atrazine-specific metabolites, desethylatrazine and hydroxyatrazine be included with atrazine in the definition for the guideline value.

The issue of which, if any, metabolites to include in the definition of atrazine for drinking water has been referred to the NH&MRC and ARMCANZ for consideration by the joint committee responsible for updating the *Australian Drinking Water Guidelines*.

14. RECOMMENDATIONS FOR PUBLIC HEALTH STANDARDS

Approval status

It is considered that, apart from significantly stricter controls over uses in riparian zones (see below under 'Drinking Water Guidelines') the current approved uses of atrazine should continue. Thus there are no objections to the continued approval of atrazine.

NOEL/ADI considerations

Based on an assessment of the available data, no change to the current NOEL for atrazine is warranted. The NOEL is 0.5 mg/kg bw/d (10 ppm) in a 2-year Sprague-Dawley rat study, based on a LOEL of 70 ppm (2.8-4.5 mg/kg bw/d), with a statistically-significant increase in mammary tumour incidence at this dose. Whilst the mammary tumours are not considered to be relevant to human health, the response reflects an hormonal interaction and can be taken as an appropriately conservative endpoint for setting the ADI.

The current ADI for atrazine of 0.005 mg/kg bw/d (based on the NOEL and using a safety factor of 100) is confirmed.

Drinking Water Guidelines

In view of the toxicity of atrazine and its metabolites, their persistence in the environment (including ground and surface water), and the level of not-unreasonable public concern about the presence of any level of pesticides in drinking water, consideration should be given to strengthening the national strategy to minimise the levels of atrazine in water. Additional steps could include a ban on the use of atrazine for irrigation channel 'hygiene' and in agriculture or plantation forestry in water catchment areas.

As outlined above (see under 'Consideration of Public Health Standards') it is recommended that the Australian drinking water guidelines be amended to include metabolites of atrazine together with parent atrazine in the definition of atrazine; this action would have the equivalent effect of lowering the Guideline Value[1] (currently 0.0005 mg/L) for atrazine alone since, in water samples in which atrazine is detected, one or more metabolites are commonly detected but disregarded in the current guideline.

This issue has been referred to the NH&MRC for consideration by a joint committee of the NH&MRC and the Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ) who conduct ongoing reviews of the guidelines. They have been requested to investigate the issue of which triazine metabolites should be included in the guidance value for drinking water.

Poisons scheduling

No change to the poisons schedule (S5 of the SUSDP) is warranted.

First aid and safety directions

The following pages detail recommended changes to the first-aid and safety directions for several atrazine products marketed in Australia. Safety directions will be finalised in conjunction with the occupational health and safety risk assessment.

Current safety directions

The current safety directions are as follows; no toxicology data have been submitted which would give any grounds for amending these SDs. However, since approval for home-garden uses of atrazine has been withdrawn in Australia, the home-garden entry for atrazine-containing products should be deleted from the *Handbook of First Aid Instructions and Safety Directions for Agricultural and Veterinary Chemicals (Including Pesticides)* (TGA & Worksafe Australia. AGPS).

Atrazine

WP SC WG all strengths
SC all strengths except where otherwise specified

Avoid contact with eyes and skin.	210 211
Do not inhale dust (WP, WG) (or) spray mist.	220 (221 WP WG) 223
When preparing spray and using the prepared spray	279 281 282
wear elbow-length PVC gloves.	290 294
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water.	350
After each day's use, wash gloves.	360 361

GR 170 g/kg or less with hexazinone 50 g/kg or less

Harmful if swallowed.	129 133
Will irritate the eyes and skin.	161 162 164
Repeated exposure may cause allergic disorders.	180
Sensitive workers should use protective clothing.	181
Avoid contact with eyes and skin.	210 211
Do not inhale dust.	220 221
When using the product, wear elbow-length PVC gloves, face-shield or goggles.	279 283 290 294 299
If product in eyes, wash it out immediately with water	340 343
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water.	350
After each day's use, wash gloves, face-shield or goggles and contaminated clothing	360 361 365 366

HG SC 350 g/L or less with amitrole 350 g/L or less

Will irritate the eyes and skin.	161 162 164
Avoid contact with eyes and skin.	210 211
Avoid inhaling spray mist.	219 223
When preparing spray and using the prepared spray wear rubber gloves.	279 281 282 290 312
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water.	350
After each day's use, wash gloves.	360 361

SC 250 g/L or less with dicamba 135 g/L or less

Will irritate the eyes and skin.	161 162 164
Avoid contact with eyes and skin.	210 211
When opening the container, preparing spray and using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat, and elbow-length PVC gloves.	279 280 281 282 290 292 294
Wash hands after use.	351
After each day's use, wash gloves and contaminated clothing.	360 361 366

Proposed additional safety directions

In addition, the following additional safety directions are proposed to cover several home garden products ('CRG Bantox AA Weedspray' and 'David Gray's Total Weed Killer' 200 g home-garden pack size; see below) which are not covered by existing entries. The SDs are the same as those for the entry *WP SC WG all strengths. SC all strengths except where otherwise specified*, with the requirement for 'elbow-length PVC gloves' (294) being replaced with 'rubber gloves' (312).

HG WP WG 400 g/kg or less with amitrole 400 g/kg or less

Avoid contact with eyes and skin.	210 211
Do not inhale dust or spray mist.	220 221 223
When preparing spray and using the prepared spray wear rubber gloves.	279 281 282 290 312
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water.	350

Current first aid instructions

The current entry is as follows:-

Atrazine If poisoning occurs, contact a doctor or Poisons Information Centre

No data have been submitted which would give any reason for amending these FAIs for atrazine products.

Atrazine-containing products

A number of atrazine products are available on the Australian market. Most of these have FASDs which are consistent with the above entries.

Corrections to the FASDs of the following products are recommended.
(**Note:** See above for the text wording for numerical SD codes listed below.)

Agspray Clear-It Total Herbicide

Safety Directions appearing on the current label are not consistent with the above and should be as follows:-

- 210 211 220 221 223 279 281 282 290 294 350 360 361

First-Aid Instructions are not worded correctly; they should be as follows:-

- If poisoning occurs, contact a doctor or Poisons Information Centre.
- If swallowed, and if more than 15 minutes from a hospital, induce vomiting, preferably using Ipecac Syrup APF.

CRG Bantox AA Weedspray

Safety Directions appearing on the current label for this home-garden product are not consistent with the above and should be as follows (consistent with the proposed SD entry):-

- 210 211 220 221 223 279 281 282 290 312 350 360 361

Note that 312 (rubber gloves) replaces 294 (elbow-length PVC) gloves in this situation.

Agchem Fyrbar Flowable Herbicide

Safety Directions appearing on the current label are not consistent with the above and should be as follows:-

- 210 211 220 223 279 281 282 290 294 350 360 361

David Gray's Total Weedkiller

Safety Directions appearing on the current label are not consistent with the above and should be as follows:-

- 210 211 220 221 223 279 281 282 290 294 350 360 361

First-Aid Instructions are not worded correctly; they should be as follows:-

- If poisoning occurs, contact a doctor or Poisons Information Centre.
- If swallowed, and if more than 15 minutes from a hospital, induce vomiting, preferably using Ipecac Syrup APF.

For the **home-garden** pack, the following Safety Directions are recommended:-

- 210 211 220 221 223 279 281 282 290 312 350 360 361

Note that 312 (rubber gloves) replaces 294 (elbow-length PVC) gloves in this situation (consistent with the proposed SD entry).

The first-aid instructions are correct for the home garden pack.

Atramet Combi SC Herbicide

Safety Directions appearing on the current label are not consistent with the above and should be as follows:-

- 210 211 220 223 279 281 282 290 294 350 360 361

First-Aid instructions as currently listed are more extensive than recommended; the following entry is sufficient:

- If poisoning occurs, contact a doctor or Poisons Information Centre.

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ATTACHMENTS

1. Drinking water quality guidelines for atrazine - international comparisons
2 Atrazine - toxicology data
3. List of clinical chemistry, haematology & urinalysis parameters
4. List of organs for organ weight determination & for histopath. examination
5. Oestrous cycle data in Sprage-Dawley and Fischer rats
6. Oestradiol levels in Female Sprague-Dawley and Fischer rats
7. Progesterone levels in Sprague-Dawley and Fischer rats
8. Incidences of mammary and pituitary tumours in female Sprague-Dawley and Fischer rats - combined results from two studies in each strain
9. Listing of submitted toxicology studies on atrazine and its metabolites
10. Public submissions to the atrazine ECRP review
11. Summary of toxicological hazard - November 1996
12. Summary of toxicological hazard - April 1995
13. ACPH12 - Draft meeting report.
14. Study summaries
15. Major degradation steps of atrazine in plants and animals
16. Metabolic pathway of atrazine in the goat

ATTACHMENT 1

Drinking water quality guidelines for atrazine - International comparisons

AUSTRALIA

Health Value = 0.005 mg/kg bw/day x 70 kg x 0.1
2 L/day
= 0.02 mg/L

where:

- mg/kg bw is the ADI, calculated from the NOEL using a safety factor of 100
- kg is taken as the average wt of an adult
- is based on 10% of the ADI
- L/day is the estimated (maximum) amount of water consumed by an adult

Guideline Value = 0.0005 mg/L; if atrazine is detected at or above this value, the source should be identified and action taken to prevent further contamination.

WHO

The WHO guideline value is 0.002 mg/L, determined using an additional safety factor of 10 for potential oncogenicity.

EUROPEAN COMMISSION

Directive 80/778/EEC of the European Community recommended member states to limit residues of pesticides and related products in drinking water to 0.1 µg/L (ie. 0.0001 mg/L). In setting this limit, analytical criteria (limit of detection) were decisive, and not the toxicological evaluation for individual substances.

Note: A comment on this limit for atrazine was made by Professor Hermann M Bolt, Director of the Department of Toxicology and Occupational Medicine, Dortmund, on the request of Ciba-Geigy AG, Switzerland. In a document dated July 10, 1993, an evaluation of atrazine data was conducted. His calculations were as follows:

Suggested Guideline = 0.0007 mg/kg bw/day x 70 kg x 0.1
2 L/day
= 0.0025 mg/L

where:

- mg/kg bw is the ADI, calculated from the NOEL of 0.7 mg/kg* using a safety factor of 1000
- kg is taken as the average wt of an adult
- is based on 10% of the ADI
- L/day is the estimated (maximum) amount of water consumed

*This value of 0.7 mg/kg bw/day was the estimated intake from a diet containing 10 ppm atrazine in the food; Australian authorities used the same NOEL of 10 ppm in the food (ie. the same toxicology study) but calculated the actual intake as closer to 0.5 mg/kg bw/d.

UNITED KINGDOM

The following information is based on information contained in the UK MAFF Evaluation on atrazine [(1) dated May 1992 and (2) dated July 1993)]. For surface and natural waters:-
Proposed ***Environmental Quality Standard*** = 2 µg/L (for atrazine and simazine combined)
Proposed ***Maximum Allowable Concentration*** = 10 µg/L (for atrazine and simazine combined)

The UK Water Supply (Water Quality) Regulations 1989 adopted the EC limit of 0.1 µg/L as the statutory level at the tap.

USA

The US EPA has established the following ***Health Advisory Levels*** (HALs) for atrazine:-

Exposure Duration	Popul'n Segment	HAL (ppb)	Safety Factor
1 day	child	100	100
10 day	child	100	100
7 year	child	50	100
7 year	adult	200	100
70 year	adult	3	1000

An additional 10-fold safety factor was used in calculating the lifetime HAL because the US EPA ranks atrazine as a class C (possible) carcinogen. For calculation of the lifetime (70 year) HAL, it is assumed that only 20% of atrazine exposure comes from drinking water and includes an additional 5-fold factor to account for this.

The maximum contaminant level (MCL) is a legally-enforceable drinking water standard; for atrazine it is equal to the lifetime HAL of 3 ppb ie. 0.003 mg/kg or 3 µg/kg.
Calculation:-

Lifetime HAL = $\frac{0.0005 \text{ mg/kg bw/day} \times 70 \text{ kg} \times 0.2}{2 \text{ L/day}}$

= 0.0035 mg/L

= 0.003 mg/L (3 µg/L) rounded

where:

- mg/kg bw is the Reference Dose (RfD), or ADI, calculated from the NOEL of 0.5 mg/kg (based on a multi-generation rat study) using a safety factor of 1,000
- kg is taken as the average wt of an adult
- is based on an allocation of 20% of the ADI
- L/day is the estimated (maximum) amount of water consumed

Note: In a document dated January 1994 ('Atrazine. Summary of Toxicology, Epidemiology and Other Data'), Ciba-Geigy Corporation, Greensboro, NC, claimed the following:-

Since setting the atrazine MCL, the EPA has re-evaluated the multi-generation study and raised the NOEL to 5 mg/kg/day. Since this is no longer the lowest NOEL, the RfD is now based on 3.5 mg/kg/day - the NOEL from the lifetime feeding study in the rats.

Ciba-Geigy have requested the EPA to re-evaluate the MCL for atrazine and base it on the RfD of 3.5 mg/kg/d, and not the former RfD of 0.5 mg/kg/day. However, to maintain stringent guidelines, Ciba proposed that three atrazine metabolites should be included in the new MCL.

NEW ZEALAND

Maximum Acceptable Value (MAV) - based on health considerations, the concentration of atrazine in drinking water should not exceed 0.002 mg/L (2 µg/L). The MAV was derived as follows:-

MAV = $\frac{0.5 \text{ mg/kg bw/day} \times 70 \text{ kg} \times 0.1}{2 \text{ L/day} \times 1000}$

= 0.002 mg/L

where:

- 0.5 mg/kg bw is the NOEL based on a carcinogenicity study in rats
- uncertainty factor is 1000 (100 for inter and intra-species variation, 10 to reflect the potential neoplasia)
- 70 kg is taken as the average wt of an adult
- 0.1 is based on 10% of the ADI
- 2 L/day is the estimated (maximum) amount of water consumed by an adult

Ref: Guidelines for Drinking-Water Quality. Ministry of Health Management for New Zealand, 1995

ATTACHMENT 2

Atrazine - toxicology data details

Submission No/Ref.	Company	Submission date	Submission details
1096	Ciba-Geigy	May 1986	supplementary data
503	Ciba-Geigy	Feb 1987	comment on chronic rat study
458, 784	Koor Intertrade	Aug 1988	TGAC approval
1226	Ciba-Geigy	Nov 1988	Supplementary data
940, 984, 3509, 3911, 3930, 4160, 4278, 4396, 3193	Ciba-Geigy	Oct 1990	Supplementary data
124	Amalgamated Chemicals	Oct 1990	TGAC approval
6738	Macspread	Sept 1990	EUP registration
9160	M&CT	Dec 1992	TGAC approval
9928	Makhteshim-Agan	Aug 1993	TGAC approval
10644	Chemspray	Oct 1994	EUP registration
7564, 9657, 9889, 10315, 10344, 10382, 10447, 10470, 10561, 10755	Ciba-Geigy	Nov 1994	Supplementary data
10839, 10977	Sandoz	Aug 1995	Supplementary data
10929	Ciba-Geigy	Feb 1995	ECRP review
11237	David Gray. Chemical Recovery . Kilford & Kilford (for Cyndan Chem's).	Dec 1995 Jan 1996 Dec 1995	ECRP review
11238	Makhteshim-Agan	Jan 1996	ECRP review
11239	Davison Industries	Jan 1996	ECRP review
11245	Ciba-Geigy	Feb 1996	ECRP review
11250	Farmoz Chemicals	Feb 1996	ECRP review
11251	Nufarm Ltd	Feb 1996	ECRP review
11252	Makteshim-Agan	Feb 1996	ECRP review
11267	Ciba-Geigy	Mar 1996	ECRP review
11292	Ciba-Geigy	Mar 1996	ECRP review

ATTACHMENT 3

List of clinical chemistry, haematology & urinalysis parameters

Clinical Chemistry	Haematology	Urinalyses
albumin	clotting parameters	appearance
alkaline phosphatase	(clotting time,	specific gravity
bilirubin (total)	prothrombin time)	glucose
calcium	erythrocyte count	ketones
chloride	haematocrit	sediment
cholesterol (total)	(packed cell	(microscopic)
cholinesterase activity	volume)	occult blood
creatinine (blood)	haemoglobin	pH
gamma-glutamyl transpeptidase	leucocyte	protein
globulin	differential count	volume
glucose (blood)	leucocyte total	bilirubin
LDH (serum lactate	count	urobilinogen
dehydrogenase)	platelet count	
phosphorus	reticulocyte count	
potassium	MCH	
protein (total)	MCHC	
SGPT (serum alanine	MCV	
aminotransferase)	blood smear	
SGOT (serum aspartate		
aminotransferase)		
sodium		
triglycerides		
urea nitrogen (blood)		
CPK (creatinine phosphokinase)		

ATTACHMENT 4

List of organs for organ weight determination and for histopathological examination

Organs Weighed	Tissues Examined		
adrenals brain gonads heart kidneys liver spleen thyroid (w/parathyroid)	adrenals aorta blood smear bone bone marrow brain (3 levels) cecum colon duodenum epididymes eyes eyes (optic nerve) gall bladder Harderian glands head - 3 sections (nasal cavity, para-nasal sinus, tongue, oral cavity, naso-pharynx, inner-ear)	heart ileum jejunum kidneys lacrimal gland liver lungs lymph nodes mammary gland muscle (smooth) muscle (skeletal) nerve peripheral) oesophagus ovaries pancreas pituitary	prostate rectum salivary gland seminal vesicle skin spinal cord (cervical thoracic, lumbar) spleen sternum stomach testes thymus thyroid (w/parathyroid) trachea urinary bladder uterus vagina zymbal's gland gross lesions

ATTACHMENT 5

Oestrous Cycle Data in Sprague-Dawley and Fischer Rats

ATTACHMENT 6

Oestradiol Levels in Female Sprague-Dawley and Fischer Rats

ATTACHMENT 7

Progesterone Levels in Female Sprague-Dawley and Fischer Rats

ATTACHMENT 8

Incidences of Mammary and Pituitary Tumours in Female Sprague-Dawley and Fischer Rats - Combined Results from Two Studies in Each Strain

ATTACHMENT 9

Listing of submitted toxicology studies on atrazine and its metabolites

Atrazine

Study	NOEL mg/kg/day	LOEL and Toxic Effect at this Dose
Short-term Repeat Dose		
Wistar rat 4-week dietary	-	Dose-dependent red'n in food consumption from 100 mg/kg
SD-derived juvenile rat 14-day gavage	-	Slight dec. bodyweight & several organ weights, inc. ALT at 25 mg/kg
SD female rat 14-day gavage	-	Oestrogen levels decreased at 200, but not 100 mg/kg
NZW rabbit 21-day dermal	10 (f) 100 (m)	Reduced bodyweight gain & inc. cholesterol at 100 mg/kg. Dec. bodyweight, food consumption, erythroid parameters & WBCs, inc. spleen weight & some clin. chem. changes at 1000 mg/kg
Subchronic Studies		
SD rat 90-day dietary	est. 20	Dec. food intake & bodyweight, renal pelvic calcium deposition, splenic haematopoiesis at 100 mg/kg
SD rat 3-month dietary	est. 5	Red. weight gain & food consumption at 50 mg/kg
SD-derived rat 3-month dietary	0.6 (m) 3.35 (f)	Reduced bodywt at 3.3 mg/kg. Reduced bodywt gain & food intake, & splenic haemosiderin deposition at 35.3 mg/kg
beagle dog 90-day dietary	est. 5	Reduced testicle weights & anaemia at 15.8 mg/kg (no NOEL established if reduced bodyweight & food consumption at 5 mg/kg taken into account)
Chronic Studies		
mouse 18-month dietary (two cross-bred strains)	-	no tumours (single dose level of 82 ppm)
CD-1 mouse 21/22-month dietary	est. 45	inc. female mortality, reduced bodyweight, macroscopic kidney pathol. at 150 mg/kg. Inc. alveolar cell tumours at 10 & 300 but not 3000 ppm - also largely within hist. controls

ATTACHMENT 9 (cont).

CD-1 mouse 91-week dietary	1.2 (m) 1.6 (f)	Decrease in bodyweight/bodyweight gain at 38.4 (m) and 47.9 (f) mg/kg No evidence of carcinogenicity up to 385 (m) and 482 (f) mg/kg
albino rat 2-yr dietary	3.65(m) 5.05 (f)	Decrease in bodyweight & food consumption at *ca.* 35 (m) and 44 (f) mg/kg
albino rat 2-yr dietary	-	Inadequate IBT study but decreased erythroid parameters at 1000 ppm (35.9-89.5 mg/kg).
SD rat 24-month dietary	0.4-0.6 (f) 2.4-3.3 (m)	mammary tumours at 2.8-5.8 mg/kg dec. bodyweight, behav. effects, palpable tissue masses, muscle degen. at 17.9-24.2 mg/kg
F344/LATI rat 2-yr diet	-	No increase in mammary tumours (f) at 375 or 750 ppm. (See text for other possible tumours)
SD rat (f) *in utero* exposure then 65 wk diet SD rat (m) 52 wk dietary	0.7 (f) 2.3 (m)	Mammary tumour incidence not increased. Red.bodyweight & food consumption (m & f), reduced erythroid params (f), inc. serum cholesterol (f) at 3.5 (f) & 23.6 (m) mg/kg
SD rat 2-yr dietary	-	Reduced bodyweight & fluid-filled uteri at low dose of 3.3-3.7 mg/kg. Earlier onset (not increased incidence) of mammary & pituitary tumours at 23-27 mg/kg
SD rat 2-yr dietary	3.0-3.5	Reduced bodyweight, inc. mortality, earlier onset mammary tumours at 20.7-21.6 mg/kg (no overall increase in incidence at term)
F344 rat 2-yr dietary	4.0-4.3	Red. bodywt gain at 11.9-12.4 mg/kg. No inc. in any tumour types.
F344 rat 2-yr dietary	*ca.* 3.5	Slightly red. bodyweight gain at 200 ppm (*ca.* 9.0-11.4 mg/kg). No increase in any tumour types.
SD rat (f) 1-year dietary	4.1	Earlier onset of mammary tumours at 23.9 mg/kg
beagle dog 1-yr dietary	est. 7.5	Dec. bodyweight & food consumption , inc. adrenal weight (and possibly ovaries and uteri) at est. 75 mg/kg
beagle dog 1-yr dietary	4.97	Reduced bodyweight & food consumption, moribundity, cachexia & ascites, clin. chem. and organ weight changes, and cardiotoxicity at 33.7 mg/kg
Reproduction Studies		
CR rat 3-gener'n dietary	est. 5-10	No effect at highest dose tested of 100 ppm
CR rat 2-gener'n dietary	2.73 (m) 3.45 (f)	Reduced food consumption and bodywt gain at high dose of 500 ppm; no reproductive effects.
CD-1 mice drinking water	-	No effects in a drinking water study with pesticide/fertiliser mixtures

ATTACHMENT 9 (cont'd)

Developmental Studies

rat oral teratology study	100	Embryotoxic effects at the next highest dose of 500 mg/kg
rat dietary and SC teratology	1000 (po) 200 (sc)	No effects at highest dose tested. Dec. litter size, inc. resorptions at 800 mg/kg.
SD rat oral teratology	10	Fetotoxicity (ossification delays) and maternotoxicity at 70 mg/kg
SD rat oral teratology	25	Minor skeletal variations and maternotoxicity (food consumption, bodywt) at 100 mg/kg
NZW rabbit oral teratology	5	No teratogenicity. Embrotoxicity and fetotoxicity at 75 mg/kg
F344 rat drinking water teratology	-	No effects in a drinking water study with pesticide/fertiliser mixtures
Sheep dietary teratology	-	Not teratogenic; embryotoxic at 30 mg/kg

Desethylatrazine

Study	NOEL mg/kg/day	LOEL and Toxic Effect at this Dose
Subchronic Studies		
SD rat 13-week dietary	-	Red. bodyweight at ≥500 ppm (32-35.5 mg/kg) and mild anaemia at 1000 ppm (66.8-69.8 mg/kg)
Tif:RAIf rat 3-month dietary	3.2-3.35	Red. weight gain & food consumption (both sexes), min. haematol changes, AP increase and liver weight inc. (females) at 35.2-38.7 mg/kg
beagle dog 13-week dietary	3.7-3.8	Possible cardiac effects, renal tubular epithelial hyperplasia/basophilia at 28.8-32.2 mg/kg
Developmental Studies		
rat oral teratology	30	maternal & fetal NOEL based on reduced food intake, inc. in embryonic resportions, & no.s of incompletely ossified elements at ≥100 mg/kg
rat oral teratology	5 (mat.) 25 (fetal)	Dec. food intake and bodywt at 25 mg/kg. Inc. fused sternebrae, poor ossific'n at 100 mg/kg

Desisopropylatrazine

Study	NOEL mg/kg/day	LOEL and Toxic Effect
Subchronic Studies		
rat 90-day dietary	-	Reduced bodyweight gain at lowest dose of 500 ppm (est. 25 mg/kg)
Tif:RAIf rat 3-month dietary	3.2-3.3	Thyroid gland activ'n and hypertrophy of TSH-producing cells in pituitary (m), extramedull. haematopoiesis in liver & spleen (f), inc. rel. liver weight (f) at 34.9-37.5 mg/kg

ATTACHMENT 9 (cont'd)

Study	NOEL mg/kg/day	LOEL and Toxic Effect
beagle dog 13-week dietary	3.8	Reduced bodyweight parameters & food intake, heart weight decrease at 33.3 mg/kg. Anaemia?
Teratology Studies		
rat oral teratology	5	Maternal and fetal NOEL, based on reduced food consumption and bodywt gain, and an increase of fused sternebrae at 25 mg/kg. No terata.

Diaminochlorotriazine

Study	NOEL mg/kg/day	LOEL and Toxic Effect at this Dose
Short-term Repeat Dose Studies		
SD rat 4-week dietary	0.89-0.92	Red.bodyweight gain & food intake, and haematol. changes at $\geq$ 45.1-48.7 mg/kg
SD rat (f) 14-day gavage	-	Red. thymus weight (abs & rel), red. spleen weight, reduced LH levels at all doses ($\geq$ 100 mg/kg)
Beagle dog 4-week diet	13.9-14.1	Soft/mucoid/few faeces, red. bw, food consumption at 21-27.2 mg/kg
Subchronic Studies		
SD rat 13-week dietary	-	Reduced bodyweight gain and mild anaemia at both tested doses, 34.3-34.7 and 70.6-74.2 mg/kg
SD rat 90-day dietary	0.7	Oestrous-cycle effects at 7.6 mg/kg
Chronic Studies		
Beagle dog 52-week diet	3.2-3.9 (m) 2.7-3.8 (f)	Inc. mortality, cardiovasc., haematol., biochem. effects, organ weight changes, fluid accumul'n, & liver, testes, bone marrow and thymus pathol. at 750 ppm (22.0-31.7 mg/kg)
Developmental Studies		
SD rat oral teratology	2.5	Maternal and fetal NOEL, based on reduced food intake & bodywt gain, and inc's in skeletal variations at 25 mg/kg

Hydroxyatrazine

Study	NOEL mg/kg/day	LOEL and Toxic Effect
Subchronic Studies		
SD rat 90-day dietary	6.3(m) 7.35 (f)	Nephrotoxicity at 18.9 (m) and 22.7 (f) mg/kg
Beagle dog 13-week dietary	5.8 (m) 6.2 (f)	Nephrotoxicity at 59.6 (m) and 63.9 (f) mg/kg
Chronic Studies		
SD rat 2-year dietary	0.962 (m) 0.475 (f)	Nephrotoxicity at 7.75 (m) and 1.17 (f) mg/kg
Developmental Studies		
SD rat oral teratology	25	Maternal and fetal NOEL, based on reduced bodywt gains and food intake, and incomplete skeletal ossific'n at 125 mg/kg. No terata.

ATTACHMENT 10

Public Submissions to the Atrazine ECRP Review

Specific public health issues outlined below are addressed in the main body of the report and/or in the "Discussion'.

(Submission dated 20 Oct. 1995) [DHFS file 95/12001; submission no. R11179]

This submission is from a resident of an isolated community in NW Tasmania. Whilst it is a personal submission, it was also written on behalf of, and with the blessing of, the Tasmanian Cleanwater Network. In 1993, the Forestry Commission (now Forestry Tasmania) used atrazine which contaminated the Creek which supplies water to half the 100 or so of the residents. Apparently, 2 and a 1/2 years after spraying, levels were still not below levels of detection. The author details her personal health history and attributes some problems to atrazine contamination of water.

It was stated that "during the debate between the Forestry Commission and our community, it was made quite clear that the reason for using atrazine hinged on its price. Clearly the cost to the innocent victims of such a decision are not considered in the equation".

Reference is made to Australia's 'sloppy drinking water standards [which] state very clearly that "atrazine should not be present in drinking water". Clearly, it is not possible to get it out once it is there .... we know what causes it (use of the substance) and we know how to remedy the situation (stop it being used)'.

Following the above comments (contained in a covering letter), a 13-page technical document was submitted, citing occupational health, public health and environmental and animal health references. The following lists the main human health concerns outlined:

- epidemiological studies suggesting that exposure to herbicides (including triazines) was associated with an increase in certain tumour types (ovarian cancer, non-Hodgkin's lymphoma);

- N-nitrosoatrazine (NNAT) may be formed from atrazine in soil and groundwater or in the acid environment of the stomach. NNAT has been demonstrated to cause elevations in chromosome breakage in human lymphocyte cultures exposed to concentrations as low as 0.1 ng/mL, as well as having mitogenic properties (causing significant elevation of the mitotic index) (Meisner, Roloff & Belluck, 1993).

- atrazine is reported to have reproductive and endocrine-disrupting effects; current toxicity test protocols may not detect the consequences of fetal exposure to endocrine-disrupting chemicals which would not be recognised until young adulthood and reproduction.

- there has been a regulatory concern about cancer induction but there need to be an adequate set of tests developed to examine chemicals for their ability to act as xenoestrogens.

(Submission received at NRA on 31 July 1995) [DHFS file 95/12001; submission no. R11134]

This letter expressed concern about the widespread contamination of river (including the Namoi) and groundwater in the Gunnedah region; the source of this information was the 1994/95 Dept of Land and Water Conservation Water Audit for the region. Of particular concern was that detectable levels were reported after a period of 5 years of drought, when up to 68% of dryland farming area had been out of production and that after good rainfall and increased agricultural production (hence herbicide use), there would be even greater translocation of atrazine and contamination of waterways.

(Submission received at NRA on 3 Aug. 1995) [DHFS file 95/12001; submission no. R11134]

The organisation making this submission have thousands of hectares of *Eucalytus globulus* and 4,000 ha of *Pinus radiata* in Western Australia, 90% of which is on ex-pasture land; their aim is to increase the hectarage of hardwood plantation by the year 2000 to supply a pulp mill to be located in the region.

Atrazine is used for plantation preparation and firebreak maintenance. It is estimated that each ha of land under trees will receive an average of 0.26 kg atrazine/year throughout a 10-year rotation, although it is noted that the average application rate for plantation establishment is 2.1 kg/ha and for firebreak maintenance (only about 20% of the plantations), 3.0 kg/ha. This plantation forestry use may be compared with other agricultural crop use of atrazine which can be up to 3.00 kg/ha each year.

The authors claim that effective weed control cannot be achieved without a pre-emergent herbicide and "without effective weed control in the first year, there will be no plantation".

Testing of streams for surface water runoff was conducted in 1989, 1992 and 1994 (up to 8 sites each year). Some readings greater than 2 ppb (the WHO limit) were recorded in the middle of winter after spraying for plantation preparation (ie. first year of plantation establishment) but were well below this limit by early spring of the first year and did not approach these concentrations in subsequent years in which only firebreaks were sprayed. Of the 127 sites (as at the date of the report; 26 July 1995), only one has fed into an impoundment used for human consumption.

(Submission received at NRA on 11 Aug. 1995) [DHFS file 95/12001; submission no. R11134]

The organisation making this submission expressed concern about atrazine because of its widespread distribution in the environment and reports of its reproductive and hormone-disrupting effects in laboratory animals, wildlife and/or humans. It is

suggested that it is an 'organochlorine chemical' with experimental evidence, either *in vitro* or *in vivo*, of oestrogenic activity.

Of particular concern was the use of atrazine by all major forestry corporations in Tasmania in preparing land cleared of native bush for plantations. It was claimed that despite the precations of replacing arial boom spraying with tractor-drawn boom sprays and increasing buffer zones along side permanent waterways from 10 to 20 m, there was still unacceptable contamination of the Lorinna water supply. Apart from concerns about human health, the effect on stream primary productivity was questioned.

It was reported that atrazine has been banned in Germany (March 1991), and temporarily banned in Italy in 1990 (extended in 1991).

(Submission received at NRA on 4 Oct. 1995) [DHFS file 95/12001; submission no. R11165]

The author stated that, if the NRA applies the precautionary principle in the matter of chemical use in the environment, it expects that the NRA will withdraw approval for atrazine use in Australia. However, it stated that conservation and environmental organisations and many people in the community have a low level of confidence in the ability of Government environmental, health and safety agencies to protect the interests of the wider community when there is a conflict with the economic interests of manufacturers or users.

The following lists some general remarks which were made:

- much of the data to support atrazine has been generated by the manufacturers of atrazine and hence there is a conflict of interest in expecting a corporation to provide evidence that would adversely affect the market potential of their own product;
- Ciba-Geigy has not proven that atrazine does not cause or increase the risk of cancer;
- safety research is restricted to a few species, which may not be analogous to humans;
- no comprehensive research has been done to test atrazine in synergy with its metabolites, degradates, other chemical pesticides, fertilizers, or other possible carcinogens in the environment;
- in addition to lethal or clinical effects which are observed in standardized testing, sub-clinical and behavioural effects, immune system effects, or effects which may not be manifest for generations or decades are a legitimate concern.

A number of more specific comments were made, including the following:

- a large number of products containing atrazine are freely available over the counter in Australia;

- a paper (Yoder *et al*, 1973) was cited as indicating that more chromosomal aberrations were seen in agricultural workers exposed to herbicides, including atrazine, during mid-season spraying period than during the off season;
- hexachlorobenzene is a by-product of atrazine production (Verschueren, 1983);
- tetrachloro-dibenzofuran has been found in atrazine (Uhler, 1992);
- N-nitrosoatrazine (NNAT) which may be formed from atrazine in soil and groundwater Wolff NL *et al*, 1976; Weisenburger *et al*, 1987) or in the acid environment of the stomach (Ruddell *et al*, 1976) has been demonstrated to cause elevations in chromosome breakage in human lymphocyte cultures exposed to concentrations as low as 0.1 ng/mL (Lorraine *et al*, 1993). (Note that it was not possible to find this reference - it appears that the reference should be to Meisner *et al*, 1993)

It was stated that "the distress derived from knowingly, albeit involuntarily, drinking contaminated water or consuming contaminated food because there is no option, is real and should be given serious credence" and that "if these concerns cannot be addressed by decision makers and manufacturers to the satisfaction of the community, there is adequate justification for exclusion of a manufactured chemical compound from the environment". There is no doubt that regulators do have to seriously take into consideration the anxiety that may result from the knowlege that one's water supply contains measurable levels of a pesticide, no matter that the available scientific evidence may indicate that the chemical in question does not constitute any measurable risk to health.

submission dated 27 June 1995

The organisation making the submission advised that there was increasing pressure to establish timber plantations rather than log regenerated native forests. It is necessary to control weeds during the first 18 months of establishing eucalytus plantations, best achieved through chemical control, since tillage is not conducive to soil conservation and disturbs tree roots. It was noted that atrazine are applied only once in the life of forestry plantations, and that not all planting areas require treatment.

The detection limit for atrazine was stated to be 0.05 µg/L. It was claimed that the cases of water contamination which occurred in Tasmania were but 2 cases in 60 other forest-type applications which did not result in any operational concerns. Nevertheless, atrazine use has been embargoed until the outcome of the ECRP review has been announced.

Weed control in conifer plantations is not such a problem at the moment since hexazinone can be used as a pre- or post-emergent herbicide over the top of the pines.

An active research program has been initiated to look at alternatives to chemicals and alternative site preparation techniques, in order to reduce the reliance on pesticides.

Attachment 11: SUMMARY OF TOXICOLOGICAL HAZARD

Date of Preparation:	Updated November 1996
Chemical name:	Atrazine
Worst oral LD50 in rats:	1869 mg/kg
Worst oral LD50 in other species:	1750 mg/kg in mice
Worst dermal LD50:	>3100 mg/kg in rats
Worst inhalation LC50:	>5000 mg/m^3 (4 h, nose only) in rats
Skin irritation:	none in rats, rabbits or humans
Eye irritation:	none or only slight in rabbits
Skin sensitisation:	reported as a non-sensitiser, sensitiser and a strong sensitiser in three seaparate studies in guinea pigs. negative in humans

Remarks:

T-value: 180

NOEL: 0.5 mg/kg bw/d (10 ppm) in a 2-year Sprague-Dawley rat study, based on a LOEL of 70 ppm (2.8-4.5 mg/kg bw/d), with a statistically-significant increase in mammary tumour incidence at this dose.

Attachment 12: SUMMARY OF TOXICOLOGICAL HAZARD

Date of preparation: April 1995

Chemical name: Atrazine/Dicamba

These results were obtained with 'Marksman Herbicide' (Sandoz), containing 250 g/L atrazine and 130 g/L dicamba

Worst oral LD50 in rats: 5897 mg/kg

Worst oral LD50 in other species:

Worst dermal LD50: >2000 mg/kg in rats

Worst inhalation LC50: >5000 mg/m^3 in rats

Skin irritation: slight in rabbits

Eye irritation: slight in rabbits

Skin sensitisation: nil in guinea pigs

Remarks:

T-value:

NOEL:

Attachment 13

Draft report of the considerations of the 12th meeting of the Advisory Committee on Pesticides and Health (ACPH) of the Department of Health & Family Services, held on 5th February, 1997

PURPOSE

The Committee considered the toxicology and public health assessment of atrazine and its major metabolites (desethylatrazine, desisopropylatrazine, diaminochlorotriazine, and hydroxyatrazine), conducted as part of the Existing Chemicals Review Program (ECRP).

BACKGROUND

Atrazine is a triazine herbicide used pre-emergence and early post-emergence for selective control of broad-leaf and grassy weeds in various food crops (such as corn and sorghum), forestry plantations and in non-crop situations. Atrazine has been in use in Australia for more than 20 years.

Atrazine is one of 80 agricultural and veterinary chemicals identified for review under Australia's ECRP. Atrazine was selected on the grounds that it may contaminate ground and surface water and could have endocrine-disruptor and carcinogenic potential. Following a data call-in period, a number of submissions on atrazine were received from industry, users, and the public. All submitted toxicology data and many recent publications have been evaluated in detail.

The toxicology of atrazine was first considered by the National Health and Medical Research Council (NHMRC) in 1985. The toxicology of atrazine has been evaluated and reviewed on a number of occasions since then. This has occurred for a number of reasons:

- the fact that atrazine had some IBT-generated toxicological studies in its data package which required verification or replacement;
- a review of some 400 agricultural chemicals (including atrazine) as part of Australia's Technical Grade Active Constituent (TGAC) Clearance Scheme (1985-1992); and
- a number of supplementary submissions containing new toxicity studies, many of which focused on the carcinogenicity potential of atrazine.

In addition, there has been a number of applications for clearance of atrazine from new sources of manufacture (ie. new Technical Grade Active Constituents) and/or new end-use products (EUP) containing atrazine.

Atrazine currently has an Acceptable Daily Intake Level (ADI) of 0.005 mg/kg bw/d, based on a No Observable Effect Levels (NOEL) of 0.5 mg/kg bw/d, established in a 2-year rat study.

DISCUSSION

The Committee noted the main toxicological findings of atrazine. The key findings are outlined below:

The overall pathway for metabolism of atrazine is consistent across mammalian species, although quantitative differences exist. Atrazine is almost completely absorbed from the gastrointestinal tract, rapidly metabolised in the liver, then excreted from the body (mainly in the urine), with no significant accumulation and without metabolism in other tissues. The main metabolic pathway for atrazine is stepwise N-dealkylation via desisopropylatrazine and/or desethylatrazine to the major metabolite, DACT. The triazine ring remains intact.

Atrazine has low acute toxicity and does not cause skin sensitisation in human skin. In repeat-dose feeding studies, the consistent toxic effects noted in mice, rats and dogs were relatively non-specific and included reduced bodyweight gain, reduced food consumption and some liver enlargement at high doses. Inhibition of the blood forming system was seen across species following treatment at high doses of atrazine. This effect was reversible.

The atrazine metabolites, desethylatrazine and desisopropylatrazine were similar to, to up to twice as acutely toxic as atrazine, whilst diaminochlorotriazine (DACT) and hydroxyatrazine were similar to, or possibly marginally less acutely toxic than atrazine. However, DACT was more toxic than atrazine in repeat-dose studies, in terms of mortalities and gross clinical signs. In repeat-dose feeding studies with desethylatrazine, desisopropylatrazine, DACT and hydroxyatrazine, there were similar findings to atrazine viz. reduced bodyweight gain and food consumption, mild anaemia and some liver enlargement.

Twenty long term toxicity studies on atrazine were provided, including 3 in mice, 14 in rats and 3 in dogs. In addition, long term studies using DACT (one-year dietary study in dogs) and hydroxyatrazine (2-year dietary study in rats) were evaluated. Studies indicated that in Sprague-Dawley (SD) rats, but not in mice or Fischer rats, there was an earlier onset (and much less consistently across studies, an increase in incidence) of mammary tumours, a common tumour type in this particular strain of rats. This response was not considered to be indicative of a cancer risk to humans, since: atrazine does not damage genetic material and is therefore unlikely to initiate cancer; and the mammary tumours in SD rats appear to be unusually susceptible to age-related hormonal changes unlike those which occur in humans during ageing.

Some non-cancer findings in chronic studies with atrazine included possible cardiovascular effects, with atrial thrombi observed at high doses (1500 and 3000 ppm) in a mouse study, and atrial fibrillation, ECG changes and gross and microscopic cardiac lesions in a dog study (1000 ppm). Possible kidney toxicity, with macroscopic kidney pathology (granular/irregular surface and/or white/pale appearance) in a mouse study, and pelvic calculi/microcalculi in one of a number of rat studies was reported. Somewhat reduced survival of females at the high doses of 1000 ppm and 3000 ppm in two mouse studies and in one of a number of rat studies was

reported; in the other chronic rat studies, mortalities were either not affected or there was a small increase in survival in males.

Some non-cancer findings in chronic studies noted after dietary DACT administration included cardiovascular effects in dogs (sino-atrial arrest at 2500 ppm and atrial haemorrhagic lesions at 1500 ppm). The kidneys and lower urinary tract were target organs of hydroxyatrazine toxicity.

No birth defects were noted in 2- and 3-generation reproduction studies with atrazine or in developmental studies with atrazine and each of its four metabolites.

In a large range of studies *in vitro* and *in vitro* studies across all genotoxic endpoints (gene mutation assays; chromosomal effects assays; other genotoxic effects; and cell transformation assays), predominantly negative results were obtained and the weight of evidence is that atrazine does not interact with genetic material. Desethylatrazine, desisopropylatrazine, DACT and hydroxyatrazine were negative in gene mutation assays, *in vivo* chromosomal effects assays, and assays for DNA damage.

In a specific study on the immunotoxic potential of atrazine, transient and reversible immunosuppression of humoral-mediated and cell-mediated responses and activated macrophage phagocytic activity could not be attributed to the direct chemical-related effect of sublethal exposure to an atrazine formulation.

The lowest NOEL for atrazine is 0.5 mg/kg bw/d, based on results of a 2-year dietary rat study in which 10 ppm was taken as the no-effect-level for mammary tumours in female rats. Whilst the mammary tumours are not considered to be relevant to human health, the response reflects an hormonal interaction and is therefore an appropriately conservative endpoint for assessing the ADI.

The lowest NOEL reported for desethylatrazine was 3.5 mg/kg bw/d, based on reductions in bodyweight gain and food consumption, minimal haematology changes and an increase in liver weight and serum alkaline phosphatase (3-month dietary study in rats) and possible cardiac effects and renal tubular epithelial hyperplasia/basophilia (3-month dietary study in dogs). The lowest NOEL reported for desisopropylatrazine was 3.2 mg/kg bw/d, based on thyroid gland activation and hypertrophy of TSH-producing cells in the pituitary (male rats), extramedullary haematopoiesis in liver and spleen (female rats), and increases in relative liver weight (female rats) (3-month dietary study). The lowest NOEL reported for DACT was 0.7 mg/kg bw/d, based on oestrous-cycle effects (3-month dietary study in rats). The lowest NOEL reported for hydroxyatrazine, a plant metabolite of atrazine, was 0.5 mg/kg bw/d, based on nephrotoxicity in rats (2-year dietary study).

On the basis that the metabolites of atrazine (viz. desethylatrazine, desisopropylatrazine, diaminochlorotriazine and hydroxyatrazine) have similar and overlapping toxicities to the parent triazine, the Chemicals Unit recommended that the residue definition for atrazine should include parent compound plus these four metabolites. Thus, the current ADI of 0.005 mg/kg bw/d should be based upon a combined total of atrazine plus its four closely related triazine metabolites.

The Committee considered that the existing atrazine ADI of 0.005 mg/kg bw/d, based on the NOELs for mammary tumours in female rats, remains appropriate. With regard to the inclusion of the atrazine metabolites in the ADI a member noted that desisopropylatrazine and DACT are common metabolites to other triazine herbicides, and their inclusion would present enforcement difficulties and add to costs of routine analysis..

The Committee was aware that atrazine is used mainly as a pre-emergent or early post-emergent herbicide, and there is very low potential for residues to occur in foodstuffs (and have not been found in Australian food residue surveys). On this basis, the Committee concluded that it would be impractical to include the metabolites within the residue definition.

However, the Committee noted that the most likely exposure route for the public is through contamination of drinking water, due to atrazine's relative stability in the environment and soil mobility. The Committee supported the application of appropriate restrictions on atrazine's use around waterways, catchments etc. (the riparian zone) to avoid water contamination. Given that atrazine and its desethyl and hydroxylated metabolites are likely to be encountered in combination when in contaminated potable water, the Committee recommended that it would be reasonable for the potable water guidelines residue definition to include atrazine and these metabolites, and that the combined intake be taken into consideration in conducting risk assessments.

RESOLUTION NO 12/7

The Committee:

- AGREED that there be no change in the existing ADI for atrazine of 0.005 mg/kg bw/day, based on a no-observable-effect-level (NOEL) of 0.5 mg/kg bw/day for mammary tumours in female rats observed in a 2-year dietary SD rat study

whilst mammary tumours were not considered to be relevant to human health, the response reflects a hormonal interaction and this was considered to be an appropriately conservative endpoint for estimating the ADI.

- RECOMMENDED that the ADI be set for parent atrazine only;

- APPRECIATED the need to take into account toxicologically significant metabolites from an exposure risk assessment perspective;

- CONSIDERED that the inclusion the metabolites in the atrazine residue definition for food would be impractical as:

- parent atrazine is likely to form the major component of the residue;
- some of the metabolites are common to other triazine herbicides which could present enforcement difficulties; and

- it would unnecessarily complicate regulatory analyses which may compromise routine residue monitoring.

- WAS AWARE that atrazine is fairly stable in ground water and that desethylatrazine, and hydroxyatrazine are major soil and water metabolites; and

- SUPPORTED modification of the basis for the atrazine guideline value in the *Australian Drinking Water Guidelines -1996* so that it applies to the total concentration of parent atrazine plus the metabolites desethylatrazine, and hydroxyatrazine.

ATTACHMENT 14

STUDY SUMMARIES

2. TOXICOKINETICS AND METABOLISM

2.1 *In vitro* Studies

2.1.1 Subcellular fractions

a) ***Dauterman WC & Muecke W (1974) In vitro Metabolism of Atrazine by Rat Liver. Ciba-Geigy Agrochemical Division, Basle. Pesticide Biochem Physiol 4: 212-219***

The metabolism of atrazine and 6 possible metabolites by rat liver subcellular fractions was studied *in vitro*. The dealkylation reaction was predominant in the microsomal fraction, and conjugation with glutathione in the soluble fraction.

N-Dealkylation was associated with the microsomes and the isopropyl group was more readily cleaved than was the ethyl substituent. Conjugation with reduced glutathione was associated with the cytosol and was a slower reaction than N-dealkylation. These researchers found no evidence for the direct formation of 2-hydroxy-s-triazines *in vitro* (unlike Bakke et al., 1972; Section 2.2.1d).

b) ***Adams NH, Levi PE & Hodgson E (1989) In Vitro Metabolism of Triazine Herbicides in Vertebrates. North Carolina State University, Raleigh, NC, USA. Report date May 1989***

The metabolism of atrazine (99%), simazine (98.3%) and terbutryn (98.7%) was studied with *in vitro* incubations with hepatic microsomal or hepatic supernatant preparations. Species studied were rats (Sprague-Dawley and Fischer), mice (ICI), goats, sheep, pigs, NZ White rabbits and chickens. The principal metabolites with all 3 herbicides and all species were 4- or 6-monodealkylated 2-chloro or 2-thiomethyl-s-triazines ie. all species produced the de-isopropyl and the desethyl metabolites (2-chloro-4-amino-6-ethylamino-s-triazine and 2-chloro-4-amino-6-isopropylamino-s-triazine) of atrazine. The level of the metabolite with neither ethyl or isopropyl groups (2-chloro-4,6-bisamino-s-triazine) was detected but was too low to quantitate. No dechlorinated or ring cleavage products were noted. Although there was considerable variation among species in the rates of metabolism and ratios of principal metabolites, strain or sex-related differences were not observed in Sprague-Dawley and Fischer rats.

The observations that atrazine inhibits the P-450 mediated O-demethylation of p-nitroanisole in a dose-dependent manner, and that there is an absolute requirement for NADPH, suggest that the N-dealkylation reactions of triazines are mediated by the cytochrome P-450-dependent monooxygenase system.

c) ***Muecke W (1993) The Metabolic Behaviour of N-Nitrosocompounds in the Rat: A Case Study. Ciba-Geigy Ltd, Basle, Switzerland***

This report appeared to be a published paper but neither the reference or date were apparent. Because of their established or presumed toxicological significance, nitroso derivatives of atrazine were studied, even though the formation of such compounds in soil, water, plants or animals has never been observed/reported.

The study showed that in liver fractions from male Tif: RAIf(SPF) rats of about 200 g bodyweight, atrazine is preferentially degraded by a NADPH-dependent microsomal system leading to N-dealkylation products, whereas conjugation with GSH is of lower importance.

Nitrosoatrazine (GS 25435) is quantitatively metabolised to the glutathione conjugate in the presence of GSH in systems containing cytosol or microsomes; only in the absence of GSH and the presence of NADPH is the substrate dealkylated (about 30%). *In vivo* experiments with nitrosoatrazine administered by the oral and intravenous routes (completely orally absorbed) confirmed that the dominant metabolite was the mercapturic acid derivative of nitrosoatrazine. Radioactive nitrosoatrazine and nitrosohydroxyatrazine were rapidly eliminated from organs and tissues (with the exception of erythrocytes for nitrosoatrazine, a rodent specific feature for chlorotriazines).

In conclusion, nitrosoatrazine is exclusively metabolised by the GSH pathway while nitroso-hydroxyatrazine is very efficiently denitrosated. Hydroxyatrazine is dealkylated by a NADPH-dependent enzyme system to desethyl- and desisopropyl hydroxyatrazine. For nitrosohydroxyatrazine (CGA 61242), microsomal N-denitrosation is the dominant process, with subsequent dealkylation of the hydroxyatrazine.

2.1.2 Isolated hepatocytes

a) ***Simoneaux B & Thede B (1988) Comparative Metabolism of Atrazine by Mammalian Tissue Cultures: Preliminary Report. Ciba-Geigy, Greensboro, NC, USA. Report No. ABR-88070. Date 16 May 1988 (no GLP or QA statement)***

The biological phase of this work was mainly conducted in the Dept of Environmental Toxicology, UCLA at Davis; the human tissue studies were performed at SRI International, Menlo Park, California. Atrazine metabolite studies were conducted at Ciba-Geigy, Greensboro. Hepatocytes were isolated from Fischer rat, Sprague-Dawley rat, Alpine goat and human liver tissue and incubated with [^{14}C]atrazine (95.8 µCi/mg; radiochemical purity 98.7%) for metabolism studies, and with unlabelled atrazine (97% purity) for cell cytotoxicity studies.

Atrazine at 25 ppm did not appear to significantly affect cell function of hepatocytes isolated from any of the 4 species studied. Assays included investigation of the effect on cell attachment, 7-ethoxycoumarin metabolism (O-deethylase and conjugation activity), total DNA, ATP levels and LDH activity.

Following radiolabelled incubations, Fischer rat, goat and human hepatocytes produced similar patterns of metabolites whereas the pattern arising from incubation with hepatocytes from Sprague Dawley rats was significantly different. The major compounds isolated from Fischer rat, goat and human hepatocyte incubations were unmetabolized atrazine and the two monodealkylated derivatives, desethylatrazine (G 30033) and desisopropylatrazine (G 28279). In contrast, Sprague Dawley hepatocytes further metabolised these derivatives to the didealkylated metabolite, diaminochlorotriazine (G 28273) and a collection of unidentified polar metabolites. Approximate estimates of rates of metabolism suggested that the hepatocytes from the two rat strains had similar rates of metabolism, human hepatocytes had a significantly lower rate, and goat hepatocytes a significantly higher rate.

This study was not well conducted, insofar as there were only a limited number of incubations conducted. This is unfortunate, in view of the suggestion from this work that atrazine metabolism in the SD rat may differ from that in Fischer rats and humans, and which might have a bearing on the effect of atrazine on the endocrine system in SD rats compared with that of Fischer rats.

b) ***Thede B (1988) Comparative Metabolism of Atrazine by Mammalian Hepatocytes: Progress Report. Ciba-Geigy, Greensboro, NC, USA. Report No. ABR-88139. Date 13 Oct. 1988 (no GLP or QA statement)***

The biological phase of this work was conducted in the Dept of Environmental Toxicology, UCLA at Davis (Sprague Dawley (SD) rat and goat studies) and in the Dept of Target Organ Toxicity, SRI International, Menlo Park, California (Fischer rat, guinea-pig, Rhesus monkey and human studies). Atrazine metabolite studies were conducted at Ciba-Geigy, Greensboro. Hepatocytes were isolated from CD-1 mice, Fischer rats, Sprague Dawley rats, guinea pigs (strain not stated), Alpine goat, Rhesus monkeys and human liver tissue, and incubated with [^{14}C]atrazine (95.8 µCi/mg; radiochemical purity 98.7%) for metabolism studies, and with unlabelled atrazine (97% purity) for cell cytotoxicity studies.

Atrazine at 25 ppm did not appear to significantly affect cell function of hepatocytes isolated from any of the 4 species studied. Variations in results of cell function assays were consistent with problems associated with cell isolation than with xenobiotic cytotoxicity. Assays included investigation of the effect on cell attachment, 7-ethoxycoumarin metabolism (O-deethylase and conjugation activity), total DNA, ATP levels and LDH activity.

Following radiolabelled incubations, CD-1 mice, Fischer rat, SD rat, and Rhesus monkey hepatocytes rapidly metabolised atrazine to the didealkylated chloro-metabolite of atrazine, diaminochlorotriazine (G 28273), then further metabolised this to a variety of Phase II (conjugated) metabolites; all three species displayed very different arrays of Phase II metabolites.

The primary products of the guinea pig, goat and human hepatocyte incubations were the two monodealkylated derivatives, desethylatrazine (G 30033) and desisopropylatrazine (G 28279), with minor evidence of further metabolism to the didealkylated metabolite, diaminochlorotriazine (G 28273). The different species appear to share common pathways, with different kinetics in each step of those pathways eg. rats appear to preferentially deisopropylate atrazine whereas CD-1 mice and humans preferentially de-ethylate it. The ratio of G 30033 (N-deethylated product) to G 28279 (N-deisopropylated product) in 24 h incubation samples was approx. 2:1 in mouse and 3:1 in human hepatocytes, but 1:3 in SD rat and 1:2 in Fischer rat hepatocytes. Guinea pig, goat and monkey hepatocyte incubations produced roughly equal amounts of the two monodealkylated metabolites.

G 28273 (didealkylated atrazine) accounted for 50-70% of total radiolabel in 24 h incubations with hepatocytes from CD-1 mice, rats (both strains) and Rhesus monkeys, but only 3-10% in incubations with guinea pig, goat and human hepatocyte cultures.

Species and strain differences were also apparent in Phase II metabolism - whilst SD and Fischer rats and CD-1 mice rapidly formed the didealkylated chlorometabolite of atrazine, all three displayed very different arrays of Phase II metabolites. In cation exchange column chromatography, early eluting peaks (acid or neutral polar metabolites) accounted for the majority of Phase II metabolites in SD rats whereas a very different pattern was seen in CD-1 mice and humans.

The results of this study appear to contradict the findings of the previous study (Simoneaux & Thede, 1988) insofar as that study suggested that major compounds isolated from Fischer rat, goat and human hepatocyte incubations were unmetabolized atrazine and the two monodealkylated derivatives, desethylatrazine (G 30033) and desisopropylatrazine (G 28279) whilst Sprague Dawley hepatocytes further metabolised these derivatives to the didealkylated metabolite, diaminochlorotriazine (G 28273). Yet the present study indicated that not only hepatocytes from SD rats but also from CD-1 mice, Fischer rats, and Rhesus monkeys rapidly metabolised atrazine to the didealkylated chloro-metabolite of atrazine, diaminochlorotriazine.

However, both studies (Simoneaux & Thede 1988 and this study) suggest, that in human hepatocytes, the primary metabolites are the two monodealkylated derivatives, desethylatrazine (G 30033) and desisopropylatrazine (G 28279), with minor evidence of further metabolism to the didealkylated metabolite, diaminochlorotriazine (G 28273); this is in contrast to SD rats in which atrazine is rapidly metabolised to the didealkylated chloro-metabolite,

diaminochlorotriazine. Thus, there could be some doubt as to the appropriateness of SD rats in particular, as models for the toxicity of atrazine to humans.

2.2 *In Vivo* Studies in Animals

Atrazine is a relatively old herbicide, approved at a time when regulatory requirements for toxicokinetic studies were less stringent and when experimental methodology for the conduct of such studies was more limited than now eg. some techniques employed in earlier studies contributed to direct in vitro degradation of atrazine, leading to incorrect assumptions about metabolic transformations occurring in vivo. This, coupled with the fact that multiple metabolites are generated from atrazine in plants and animals, has meant that many of the available company studies on the absorption, distribution, metabolism and elimination of atrazine have been somewhat piecemeal and confusing. A relatively recent study (Paul, Dunsire & Hedley, 1993) (see 2.2.3a below) has gone a long way towards comprehensively assessing atrazine toxicokinetics in rats.

2.2.1 Single-dose oral studies in rats

a) ***Hazelton Labs (1960) Atrazine-C^{14} Metabolism Study. Hazelton Labs Inc., Palo Alto, CA. Project no: not specified. Report date 15 July 1960***

Long-Evans rats (2/sex) in glass metabolism cages received single oral doses of ^{14}C-labelled atrazine (in 0.5% methylcellulose; between 41.4 to 59.8 µCi administered per animal, with specific activity of the compound = 18.32 µCi/mg), and faeces and urine were sampled at 6, 12, 24, 36 and 48 h. At 48 h, blood samples were collected by cardiac puncture, and a wide range of body tissues were sampled.

At 48 h, 67-72% of the total dose had been excreted by each of the 4 rats, 52-57% in urine, 12-15% in faeces, and 0.04-0.09% as exhaled CO_2. Highest tissue residues of radioactivity were in the blood (6.49-8.39 ppm atrazine equivs), liver and kidneys (2.88-3.81 ppm), with remaining tissues having levels between 0.1 to 2.05 ppm.

b) ***Bakke JE & Robbins JD (1967) Metabolism of Atrazine and Simazine by the Rat. Metabolism and Radiation Research Laboratory, Animal Science Research Division, USDA, North Dakota, USA***

Two groups of 14 male rats (strain not stated) were dosed by stomach tube with 0.53 mg atrazine or 0.57 mg simazine; triazines were administered as ^{14}C-ring-labelled compounds in ethanol solution. Urine and faeces were sampled daily for 3 days, and tissues were sampled after sacrifice at 2, 4 or 8 days, from 4 animals/group. CO_2 was sampled from 2 animals/ group which were housed in glass metabolism cages. Ethylamino side-chain labelled atrazine (0.58 mg) and simazine (0.49 mg) were also each administered to two rats, with housing in glass metabolic cages for CO_2 collection, as above.

There was complete recovery of atrazine radioactivity, regardless of label position. For ring-labelled atrazine, there was 65% in urine, 20% in faeces, 0.1% in CO_2 and 15.8% in body tissues and for ethylamino side-chain label, 21.7% in urine, 13.6% in faeces, 43.0% in CO_2 and 5.1% in body tissues. A qualitatively and quantitatively similar pattern was seen for simazine. Atrazine tissue residues (average ppm triazine equivalents) were liver (1.7), brain (1.1), heart (1.4), lung (2.0), kidney (1.7), digestive tract (0.9), omental fat (0.1) and leg muscle (0.5) after 8 days. There were 17, 19 and 22 urinary metabolites detected for simazine, atrazine and propazine (data cited from a prior study), respectively. The major urinary metabolite, accounting for 20-30% of the total activity, and most of the minor metabolites, were common to 3 triazines herbicides, atrazine, simazine and propazine; identification of metabolites was not made.

c) ***Cassidy JE & Caballa SH (1971c) Metabolism of Atrazine and its Metabolites in Female Rats. Ciba-Geigy, Ardsley, NY. Report No. GAAC-71005A. Report date 23 March 1971 [Abstract]***

Female rats (strain not stated) were given a single dose of ring-labelled ^{14}C-atrazine or 2-chloro-4,6-diamino-s-triazine (G 28273) at 1.5 mg/kg. Residues at 96 h after atrazine dosing (in ppm atrazine equivalents) were: liver (0.34), kidney (0.23), muscle (0.06), fat (0.12) and blood (0.34). After G 28273, residues were somewhat higher, up to 2-fold in muscle. Residues were 50% lower after 192 h. No significant labelled CO_2 was detected. More than 60% of radioactivity was excreted in urine.

After a single dose of radioactive hydroxyatrazine (G-34048), very low residues were found at 72 h (0.003 ppm in liver, <0.001 ppm in other tissues). When the aqueous solubles and insolubles of corn (treated with ^{14}C-atrazine) were administered to rats in a single dose, tissue residues were 0.001 pm or less after 72 h. It was concluded that metabolites of atrazine synthesized by corn would be rapidly eliminated from rats and would not result in significant tissue residues.

d) ***Bakke JE, Larson JD & Price CE (1972) Metabolism of Atrazine and 2-Hydroxyatrazine by the Rat. US Dept of Agriculture, Animal Science Research Div., Fargo, ND. J Agr Fd Chem 20(3): 602-607***

Fourteen Sprague-Dawley rats were each dosed by stomach tube with 0.53 mg [ring-^{14}C]atrazine or 0.50 mg [ring-^{14}C]2-hydroxyatrazine as a single oral dose. Urine and faeces were collected daily and CO_2 was collected in 2 animals for 72 h. Animals were sacrificed and tissues analysed at 2, 4 and 8 days.

At 72 h after dosing, radioactivity was recovered mainly in urine (65% for atrazine, 78% for hydroxyatrazine) and faeces (20%, 5.5%), with less than 0.1% of the dose of either compound in expired air, and less than 0.1% of hydroxyatrazine in body tissues. For atrazine, 15.8% of the dose remained in

the carcass; at the 72 h sacrifice time, the digestive tract had the highest levels, followed by kidney (4.0 ppm atrazine equivalents, on a freeze-dried basis), liver, lung, heart, brain, muscle and omental fat (0.5 ppm).

Nineteen urinary metabolites were detected in rats given atrazine, of which 4 were identified viz. hydroxyatrazine, 2-hydroxy-4-amino-6-isopropylamino-s-triazine, 2-hydroxy-4-amino-6-ethylamino-s-triazine, and ammeline (2-hydroxy-s-triazine), these four comprising about 47% of urinary radioactivity. The urine of rats given hydroxyatrazine contained hydroxyatrazine and its two mono-N-dealkylated analogues, 2-hydroxy-4-amino-6-isopropylamino-s-triazine ('desethylhydroxyatrazine') and 2-hydroxy-4-amino-6-ethylamino-s-triazine ('desisopropylhydroxyatrazine'), these three representing 88% of urinary radioactivity.

It was assumed that all of the 2-hydroxytriazine metabolites were not artifacts of the isolation procedure (but see Bohme & Baer 1967; Section 2.2.8a).

e) ***Miles JB & Orr G (1987) Characterization and Identification of Atrazine Metabolites from Rat Urine. Ciba-Geigy Corp., Greensboro, NC, USA. Report No. ABR-87115. Report date 17 Nov. 1987***

Five adult female Sprague Dawley rats each received a single dose (100 mg/kg bw), by oral gavage, of ^{14}C-labelled atrazine (>97% pure). Urine and faeces were sampled at 24, 48, and 72 h intervals.

A mean total of 47% of the dose was excreted in the urine, 50% in faeces, with 1.37% in blood and 6% in remaining tissues. Average total recovery of radioactivity was 104%. Isolation and characterisation of urinary metabolites indicated that about 60% of the radioactivity consisted of 4 metabolites, hydroxyatrazine, 2-hydroxy-4-amino-6-isopropyl- amino-s-triazine, 2-hydroxy-4-ethylamino-6-amino-s-triazine, and 2-hydroxy-4,6-diamino-s-triazine. The results of this study indicated that hydrolysis of the carbon-chlorine bond and N-dealkylation comprised the major path of metabolism.

Note: Subsequent studies indicated that the hydroxy metabolites identified in this study were artifacts of the acidic isolation procedure and that they are excreted into the urine as the corresponding 2-chloro-s-triazines.

f) ***Timchalk C, Dryzga MD, Langvardt PW, Kastl PE & Osborne DW (1990) Determination of the Effect of Tridiphane on the Pharmacokinetics of ^{14}C-Atrazine following Oral Administration to Male Fischer 344 Rats. Toxicol 61: 27-40***

Note: Tridiphane [2-(2,2,2-trichloroethyl)-2-(3,5-dichlorophenyl)-oxirane] is an herbicide often mixed with atrazine, due to its synergistic action.

Male Fischer 344 rats (4/group) received either an oral dose of 30 mg/kg [^{14}C]atrazine, or a non-labelled dose of 60 mg/kg tridiphane in corn oil followed by 30 mg/kg [^{14}C]atrazine after 2 h.

Urine and faeces were collected over 12 h intervals up to 24 h after dosing, and at 24 h intervals thereafter. Blood was sampled at 4, 8, 10, 12, 24, 36, 48 and 72 h *via* an indwelling jugular vein cannula. After 72 h, cages were rinsed and the wash analysed, and all rats were killed and the carcases sampled. Urinary metabolites were chemically identified.

There were no signs of toxicity. About 93% of radioactivity was recovered, with 66% in urine and 18% in faeces. Skin and carcass contained 1.5 and 4% of the dose, respectively (atrazine only), or 3.5 and 5%, respectively (atrazine and tridiphane). Plasma radioactivity followed compartment kinetics, reaching a peak 8-10 h after dosing, with absorption half lives of 2.6 and 3.3 h. Plasma clearance also had first-order kinetics, with half lives of 10.8-11.2 h.

In the 0-12, 12-24, 24-48 and 48-72 h intervals, urine excretion accounted for 36%, 21%, 8% and 1.3% of the total dose, respectively. Faecal elimination was 7%, 8% and 3% over the 0-24, 24-48 and 48-72 h intervals. The major urinary metabolite was 2-chloro-4,6-diamino- 1,3,5-triazine (64%, 67%). Other metabolites tentatively identified were S-(2,4-diamino-1,3,5-triazin-6-yl)-mercapturic acid, S-(2-amino-4-methylethylamino-1,3,5-triazin-6-yl)- mercapturic acid, 2-chloro-4-amino-6- ethylamino-1,3,5- triazine, and 2-chloro-4-amino-6-methylethylamino-1,3,5-triazine. No unmetabolized atrazine was detected in urine.

It was concluded that tridiphane did not affect atrazine pharmacokinetics or metabolism in rats.

2.2.2 Multiple-dose oral studies in rats

a) ***Murphy TG & Simoneaux BJ (1985) Metabolism of ^{14}C-Atrazine in Orally Dosed Rats. Ciba Geigy, Greensboro, NC. Report No. ABR-85104. Report date 6 Dec. 1985***

Thirty six Male Harlan Sprague-Dawley rats (3/time point) received 0.4 or 4.0 mg/kg/day [^{14}C]atrazine (>97.5% radiochemical purity) for 7 days. Urine and faeces were collected at 24 h intervals. Animals were sacrificed on days 5, 7, 9, 10, 14 and 18 after treatment.

Dose recoveries were 104 and 93% for low and high doses, respectively. On average, 70% of the dose was recovered in urine, 25% in faeces, and 2-3% in tissues.

Tissue radioactivity peaked at day 8 (high dose) and day 10 (low dose). Highest tissue levels at day 8 were 1.63 µg/g (RBCs), 1.06 µg/g (liver) and 0.74 µg/g (kidney) at the low dose, and 21.66 µg (RBCs), 6.40 µg/g (liver) and 5.28 µg/g (kidney) at the high dose.

Depletion half lives from all tissues except RBCs, muscle and brain was estimated to be 4 days. Brain had an estimated depletion half life of 10 days, and for RBCs 25-30 days, reflecting covalent binding to rodent RBCs. Erythrocytes, muscle and liver contained 2-3% of the total dose 18 days after the last dose.

b) ***Thede B (1987) Study of delta-^{14}C-Atrazine Dose/Response Relationship in the Rat. Ciba-Geigy Corp., Greensboro, NC, USA. Report No. ABR-87087. Report date 23 Oct. 1987***

Female Sprague-Dawley Charles River CD albino rats (14/group) were intubated with 0, 1, 3, 7, 10, 50 and 100 mg/kg/day ^{14}C-triazinyl-labelled atrazine (97.9%) in a corn starch/polysorbate-80 aqueous suspension for 10 days. Urine and faeces were sampled daily, and blood was sampled at 8 intervals up to sacrifice. At sacrifice, body tissues were analysed for radioactivity. Sacrifice was performed 3 h after the tenth dose in one group, and 72 h after the tenth dose in the other groups.

In rats sacrificed 3 h after the tenth dose, overall average recovery of radioactivity was 90%, with 70% in urine and 13% in faeces. Some 72 h after the tenth dose, 94% of total radioactivity was recovered, 76% in urine and 15% in faeces. These proportions were independent of dose levels in the range 1 to 100 mg/kg/day.

Plasma concentrations of radioactivity were linearly proportional to dose and conformed to first order kinetics. Plasma concentrations reached a plateau after 8 or 9 days, and declined exponentially after dosing with a half life of 38.6 h. Erythrocyte concentrations rose daily, but the estimated steady-state plateau was at 30 days. Decline after dosing was exponential, with a half life of 8 days.

Erythrocytes (1.6% of total dose) and liver (0.6% of total dose) exhibited highest radioactivity levels. Mammary tissue (pectoral, inguinal) concentrations after the tenth dose were linearly related to plasma concentrations.

The overall conclusion was that subchronic administration did not alter the pattern or first order kinetics of atrazine observed in single-dose experiments.

c) ***Orr GR, Simoneaux BJ & Davidson IWF (1987) Disposition of Atrazine in the Rat. Ciba-Geigy Corp., Greensboro, NC, USA. Report No. ABR 87048. Report date 23 Oct. 1987***

Charles River CD rats from CR Breeding Labs, Wilmington, Maryland (5/sex/group) received either a gavage dose of 1 or 100 mg/kg ring-^{14}C-labelled atrazine in 1:1 water:ethanol ($\geq$ 98% radiochemical purity), or a gavage dose of unlabelled atrazine (in 3% cornstarch, 0.5% v/v Tween 80) at 1 mg/kg/day for 14 days, with a labelled dose on day 15. Urine and faeces were sampled at intervals up to 7 days after dosing. All animals were killed after 7 days and a wide range of tissues sampled for radioactivity. Metabolism cages were washed and the wash mixture analysed for radioactivity.

The findings of this study are also included in a summary by Orr (1987) evaluated elsewhere (see 2.2.3b) In brief, total radioactivity recoveries for the 3 groups ranged from 88-103% (mean 98%), with 74% excreted in urine and 18.8% in faeces. The observation that the percentage of the dose recovered in urine and faeces was constant regardless of dose, gender or dosage regime suggested complete oral absorption, with biliary excretion being the origin of faecal radioactivity.

Tissue levels 7 days after dosing were 7.0, 4.7%, and 4.6% of the dose for low dose, high dose and repeat dose animals, respectively; there were no sex differences. Tissue levels were highest in erythrocytes (0.6 ppm), whereas fat, blood plasma and uterus contained the lowest levels. Low-dose, subchronically treated rats displayed lower tissue concentrations than rats given a single low dose (average 0.67:1), indicating some accumulation from subchronic low dosing (with unlabelled atrazine). Tissue concentrations were lower after subchronic dosing than after a single low dose, possibly indicating tissue saturation. Urinary excretion kinetics best fitted a linear 2-compartment open model, with elimination half lives of 6.9 and 31.1 h. The half life of renal elimination from the central compartment was 12.4 h.

d) ***Gojmerac T & Kniewald J (1989) Atrazine Biodegradation in Rats - a Model for Mammalian Metabolism. Bull. Environ. Contam. Toxicol. 43:199-206***

Male Fischer rats received an oral dose of 15 or 30 mg pure atrazine (60, 120 mg/kg/day) in paraffin oil daily for 7 days. Liver, kidney and brain tissues were sampled at 24 h sacrifice, and analysed for residues of atrazine and 5 atrazine metabolites.

Unchanged atrazine was detected in all tested tissues, with concentrations (pooled data) of 4, 2.3 and 0.8 ppm in kidney, liver and brain respectively (60 mg/kg/day), or 7.5, 3.10 and 1.7 ppm respectively (120 mg/kg/day). Desethylatrazine was detected in brain and kidney. Desisopropylatrazine was detected in liver and kidney. Hydroxyatrazine and hydroxy-diaminoatrazine were found only in the liver, and hydroxy-desethylatrazine only in the kidney. None of the atrazine hydroxy analogues were detected in the brain.

2.2.3 Oral ADME studies in rats

a) ***Paul HJ, Dunsire JP & Hedley D (1993) The Absorption, Distribution, Degradation and Excretion of [U-^{14}C]Triazine in the Rat. Ciba-Geigy Ltd, Basle, Switzerland. Lab: Inveresk Research International Ltd, Tranent, Scotland. IRI Project no. 153138; IRI Report no. 9523. Date 7 Dec. 1993. (GLP; USA, EC, Japan)***

Study Group	No. Animals	Dose (mg/kg)	Brief Description of Study
E	3/sex	1.0	blood collection at different times
	3/sex	99.3	blood collection at different times
F	12 males	0.9	tissue collection at different times
	12 males	107.1	tissue collection at different times. Collection of urine and faeces (3 animals) at different times.
G	4 males	1.0	urine, bile and faeces collection at different times (bile cannulae), & GI tract and carcass after 2 days.
L	4 males	98.8	whole body autoradiography at different times.

A series of studies using [U-^{14}C]atrazine (Batch no. GAN-XVIII-77-5; 53.51 µCi/mg; >99% radiochemical purity) and unlabelled atrazine (Batch no. AMS 126/105; 99.0% purity) was conducted in Sprague Dawley rats from Charles River, UK. The test substance, in 0.5% w/v carboxymethylcellulose (CMC) sodium salt and 0.4% Tween 80, was administered by gavage in single doses in a series of experiments outlined in the Table above.

Absorption

In bile-duct cannulated animals (Group G) given single oral 1 mg/kg doses and killed at 2 days, recoveries of radioactivity were as follows: urine (64.7%); bile (7.3%); tissues, not including gastrointestinal (GI) tract (15.7%); faeces (3.5%); GI tract (1.3%); cage wash (5.6%); for a total recovery of 98.2% of the dose. Since about 88% of the dose was in urine, bile and tissues whilst faeces and GI tract/contents only contained about 4.8% at 48 h, it is apparent that atrazine undergoes close to complete oral absorption. Based on AUCs from blood kinetic data, the extent of absorption was independent of sex and dose level.

Distribution

In Group F animals, tissue residues at the low dose were determined at 2 h (time of maximum radioactivity levels in the blood), at 48 h, 168 h and 336 h post-dose. At the high dose, they were determined at 24 h (time of maximum radioactivity levels in the blood), at 72 h, 168 h and 336 h post-dose.

At the blood radioactivity peak (2 h) after the low-dose (0.9 mg/kg), highest levels were in kidneys, liver and RBCs (1.172, 0.862 and 0.710 ppm atrazine equivalents, respectively). By 7 days, respective levels were 0.044, 0.086 and 0.316 ppm ie. RBC levels remained high whereas all other tissues were in the range 0.007-0.086 ppm. At the high dose (91.4 to 109.8 mg/kg), RBC levels were higher than other tissues at all time points; at the blood radioactivity peak (24 h) levels in kidneys, liver and RBCs were *ca*. 27, 29 and 65 ppm atrazine equivalents, respectively). By 7 days, respective levels were 2.663, 3.75 and 41.724 ppm ie. RBC levels remained high whereas all other tissues were in the range 0.15-5.65 ppm.

Whole-body autoradiography (Group L animals) was performed at 24 h, 72 h, 168 h and 336 h. At 24 h, absorption from the small intestine was almost complete, with wide distribution of the absorbed material. At 72 h, tissue levels were lower, with only low GI levels. At the two later times, only well-perfused tissues (eg liver, kidney, lungs, heart, spleen) had apparent radioactivity, presumably due to the presence of erythrocyte binding.

Metabolism

Urine of high-dose male rats revealed about 26 metabolite fractions by 2-D TLC. The major metabolite was diaminochlorotriazine (G 28273) (about 26% of the administered dose), with a minor amount of desisopropylatrazine (G 28279). There was evidence for the presence of desethylatrazine (G 30033) and CGA 10582 (2-acetylcysteinyl-4,6-diamino-1,3,5-triazine. Similar results were seen at the low dose; quantatively, the major metabolite fraction was reduced.

The faeces of high-dose males had about 12 metabolites; unchanged parent, diaminochlorotriazine and desethylatrazine accounted for about 0.24%, 1.3% and 0.14% of the dose respectively. At the low dose, parent (0.12% of the dose) and diaminochlorotriazine (0.38%) were identified, with no other fraction accounting for >0.7% of the dose.

The bile from low-dose males had about 9 metabolites/metabolite fractions ranging from 0.1-1.6% of the dose. A ‘major’ fraction was diaminochlorotriazine, ‘minor’ fractions desethylatrazine and desisopropylatrazine.

Elimination

Information on elimination of a low 1 mg/kg dose over 24 h is presented above (see under 'Absorption'). Excretion of a high (91.4 mg/kg) dose over 168 h was measured in a subset of Group F animals. Recovery of the administered dose was: urine (66.2%); faeces (19.7%); cage wash (4.6%); for a total recovery of 90.4%. Excretion was rapid, with 85% of the dose being recovered within 48 h. Mean total residues accounted for *ca*. 5.4% of the dose at 7 days.

Assuming first-order kinetics, the terminal elimination of radioactivity from plasma at the low dose had a half-life of approx. 88 h (calculated for the period 48-336 h) cf. 300 h in erythrocytes and whole blood, and 65-208 h in the remaining tissues and organs, the longer times reflecting the greater presence of RBCs (see under 'Distribution'). At the high dose, plasma radioactivity had a terminal half-life of approx. 59 h, RBCs and whole blood approx. 300 h, and remaining tissues and organs, 123-300 h. Whereas elimination of radioactivity from organs, tissues and body fluids was biphasic, monophasic elimination was apparent from erythrocytes.

ADME Summary

In SD rats, atrazine undergoes close to complete absorption from the GI tract after oral administration and is rapidly eliminated, predominantly in the urine. Bile is a minor elimination route. Apart from erythrocytes, tissue residues were low, and there was no evidence of accumulation, except for erythrocytes, due to s-triazine binding to haemoglobin (Hb). This binding is specific for rodent Hb due to its structure, and is not found in other species (Hamboeck et al, 1981). The binding of s-triazine metabolites to rodent haemoglobins appears irrelevant to other species. Atrazine undergoes almost complete metabolism; the major path is stepwise N-dealkylation via desisopropyl atrazine or desethylatrazine to the major metabolite, diaminochlorotriazine. The triazine ring remains intact.

b) ***Orr G (1987) A Summary of the Disposition, Kinetics and Metabolism of Atrazine in the Rat. Ciba-Geigy Corp., Greensboro, NC. Study No. ABR-87116 Date 17 Nov. 1987***

After single oral doses of 1 and 100 mg/kg bw [^{14}C]atrazine (triazine ring labelled) in Sprague Dawley Charles River CD rats (5/sex/dose), the preferred route of excretion in rats was urine, regardless of sex, dose rate or subchronic dosing (one group treated for two weeks with 1 mg/kg unlabelled atrazine, followed by a single radioactive dose).

Kinetics

Over 7 days, about 74% of the total dose was excreted in urine, and 19% in faeces. These results indicate complete oral absorption, with faecal radioactivity arising from biliary excretion. The kinetics of elimination were consistent across studies and followed first-order kinetics from a 2-part open

system, with a whole body half life of elimination of 31.3 ± 2.8 h (beta-phase). The apparent volume of distribution (Vd) was 4.15 L/kg bw.

Distribution

In subchronic oral dosing studies, tissue concentrations varied in less than direct proportion to the dose rate but no sex-related differences were observed. Highest residues were in erythrocytes then liver, while fat, plasma and uterus contained the lowest levels. High levels in rat erythrocytes were ascribed to covalent binding of the triazine ring to exposed cysteine sulfhydryls in the rat haemaglobin chain. Tissue concentrations of radioactivity from atrazine declined exponentially after dosing, and were linearly proportional to plasma concentrations.

Metabolism

The accompanying Figure presents the proposed metabolism of atrazine in rats. Atrazine is readily dealkylated to give either of the possible monodealkylated metabolites (desethylatrazine, desisopropylatrazine; no's 13 & 14) which in turn can be further dealkylated, conjugated with reduced glutathione, or excreted directly. The didealkylated-s-triazine (diaminochlorotriazine, or DACT; no. 15), the major metabolite, is readily excreted but also may be subject to GSH conjugation. Glutathione conjugates of atrazine, desethylatrazine and desisopropylatrazine (ie. no's 16, 17 and 18) may suffer loss of any remaining alkyl substituents to give the conjugate of DACT (no. 19) or may enter into the mercapturic acid pathway directly. At some point during the metabolism of these sulfur-containing conjugates, they are acted upon by a carbon-sulfur lyase to give the 2-mercapto-s-triazines (no's 20, 21, 22 & 4). These can be excreted or methylated to give 2-methylthio-s-triazines (no's 23, 24, 25 & 26) which can be excreted directly or further oxidised to give the corresponding S-oxides (no's 26, 27, 28 & 29); these are the electrophiles putatively involved in the covalent binding of s-triazine residues to rodent haemoglobin.

A minor alternative pathway involves the oxidation of a primary carbon on the side chain of atrazine or either of the monodealkylated metabolites to a carboxylate, to give metabolites (no's 30, 31, 32 & 33) which are excreted without further modification.

A discussion of results of previous studies indicated that the hydroxylation of the C-2 chlorine, as described by Miles & Orr (1987) (see Section 2.2.1e) was an artifact of the formic acid elution used in the preliminary clean-up step. The demonstrated conversion of authentic 2-chloro-s-triazines to the corresponding 2-hydroxy compounds by the cation exchange column and conditions employed by Bakke et al. (1972) (Section 2.2.1d) confirmed that the appearance of 2-hydroxy metabolites in this study was also an artifact of the procedures used ie. metabolic hydrolysis of the carbon-chlorine bond did not occur.

In conclusion, the main metabolic pathway was considered to be oxidative removal of alkyl side chains, with 2-chloro-4,6-diamino-s-triazine being the major metabolite. The 2-carbon-chlorine bond is stable to enzymic hydrolysis but is subject to conjugation via GSH transferase. Sulfur-containing metabolites are acted on to give 2-sulfhydryl-s-triazines which in are subject to methylation followed by oxidation to the corresponding S-oxides. Oxidation of the primary positions of the alkyl side chains is a minor alternative metabolic route.

2.2.4 Single-dose dermal studies in rats

a) ***Williams SC & Marco GJ (1983a) Dermal Absorption of ^{14}C-Atrazine by Rats. Ciba-Geigy, Greensboro, NC, USA. Report No. ABR-83005. Project no. 101950. Date 16 May 1983***

Groups of Sprague-Dawley albino rats (Harlan, Madison, WI) received a dermal application of [U-ring-^{14}C]atrazine (17.2 µCi/mg) in ethanol for 2, 4, 8, 24 and 48 h at doses of 0.25 or 2.5 mg/rat; animals (4/sex/dose at each time point) were about 200 g at study commencement. The solution was applied to a 1.5 cm^2 area of shaved back skin, and the rear legs shackled to prevent scratching. Whilst not expressly stated, it appears that the site was left uncovered.

A preliminary study (2.5 mg/rat applied for 72 h) showed that there was very little radioactivity excreted in CO_2 and volatiles, with 105.8% of applied radioactivity recovered (>50% in excreta, 6.2% in tissues, less than 14% in both cage wash and carcass, and less than 14% on the treated skin area).

Since atrazine tended to flake off the skin at the high dose, only the low-dose data was considered in detail. Total low-dose recoveries averaged 101.4% at 72 h; at this time, recoveries (% of applied radioactivity) were as follows: urine (33.4-35.8%); faeces (11.9-10.5%); skin wash (27.6-29.2%); dissolved skin site (0.63-0.84%); tissues (5.5-6.6%); blood (0.86-1.05%); carcass (12.4-13.87%); and cage wash (5.3-7.3%). There was no apparent sex difference in absorption. Half-lives for absorption were calculated as 38.9 h (low-dose males) and 43.0 h (females).

Comment: This study did not appear to be well conducted and is of limited value compared with other more detailed dermal absorption studies.

b) ***Williams SC & Marco GJ (1983b) Excretion Rate of ^{14}C-Atrazine from Dermally Dosed Rats. Ciba-Geigy USA Report No. ABR-83081 Report Date 20 Oct. 1983***

Four groups of two female Harlan Sprague-Dawley rats were each dermally dosed with 0.015, 0.25, 2.5 or 5.0 mg/kg ^{14}C-labelled atrazine (17.2 µCi/mg), dissolved in tetrahydrofuran. The compound was applied to 1.5 cm^2 of shaved back skin, and urine and faeces were sampled every 24 h up to 144 h when all animals were sacrificed for tissue sampling. The area of application was selected to minimise oral ingestion and the rear legs were shackled to prevent scratching.

The main route of excretion was via urine and ranged from 36.9-58.8% (from low to high dose respectively), with the largest amount excreted during the first 48 h, and reaching a plateau at 120 h. Corresponding faecal values were 14.9-20.6%, with most of the dose excreted within 48 h (low to high dose respectively). Total excretion to 144 h averaged 51.8% (low dose) to 79.4% (high dose). In the final 24 h collection period (120 to 144 h), an average of less than 4% of the total dose was excreted (regardless of the dose). About 10% (low dose) or 1% (high dose) remained on and in the skin. Comparison of the amount excreted with dose indicated a proportional relationship. On the basis of atrazine excreted, it appears that the extent of dermal absorption was reasonable (at least 50% absorption at a low dose of 15 µg/kg to almost 80% at 5 mg/kg), the extent increasing with increasing dose.

c) ***Murphy TG & Simoneaux BJ (1987) Dermal Absorption of ^{14}C-Atrazine in the Rat. Ciba-Geigy, Greensboro, NC, USA. Report No. ABR-87098. Project no. 101950. Date 6 Nov. 1987***

Groups of male Charles River Sprague-Dawley albino rats received a dermal application of [U-ring-^{14}C]atrazine for 2, 4, 10 or 24 h at 0.1, 1 or 10 mg/rat. Atrazine was prepared as a simulated '4L' formulation. The mixture was applied to 10 square centimetres of shaved back skin, and covered with filter paper and foil. In another experiment, atrazine was prepared as a simulated '80W' in water formulation, applied at 10 mg atrazine/rat for 10 h.

Total dose recoveries ranged from 94-103%. After 10 h exposure (4L formulation), total amounts absorbed (excreta, blood, carcass, washed skin) were 26.9%, 21.6% and 10.8% for the low, mid and high dose levels respectively, with less than 1% excreted. The rate of atrazine absorption was inversely related to the dosage level.

After 24 h exposure, total amounts 'absorbed' were 25.7, 30.3% and 9.8% for the low, mid and high dose levels, respectively. Less than 3% of the dose (all levels) was excreted in a 24 h period, mainly via urine. For all 3 doses at both application times, 60-80% was removed by detergent wash of the skin and most of the radioactivity considered to be absorbed was found in the dissolved skin; these skin residues did not decline significantly with time after application.

The total percent absorbed at 10 h for the 4L and 90W formulations was 10.8% and 9.1%, with no differences in results between the two preparations.

In conclusion, most 'absorbed' radioactivity from dermally-applied atrazine formulations was found in dissolved skin after removal of surface radioactivity by detergent washing. Less than 3% of the dose (all levels) was excreted in a 24 h period, this being mainly in urine.

d) ***Chengelis CP (1994) A Dermal Radiotracer Absorption Study in Rats with ^{14}C-Atrazine. Ciba-Geigy Corp., Greensboro, USA. Lab: WIL Research Labs, Ashland, Ohio. WIL Study No. 82048. Study completion date 22 June 1994. (GLP; US EPA)***

This study was conducted in general accordance with US EPA Pesticide Assessment Guidelines, Section 85-2. Groups of male CD rats (from Charles River Breeding Labs, Portage, Michigan) received a dermal application of [^{14}C]atrazine (98.9% radiochemical purity) for a 0.5, 1, 2, 4, 10 or 24 h exposure period at doses of 0.1, 1 and 10 mg/rat (4/group), with euthanasia at the end of the exposure period. Separate groups were exposed for either 10 or 24 h then terminated at 24, 48 and 72 h post-exposure. Atrazine was prepared as a simulated '4L' formulation. The mixture was applied to 10 square centimetres of shaved back skin, and covered with filter paper and foil. Resultant targeted doses were equivalent to approx. 10, 100 and 1000 µg/cm^2, actual average amounts were 9.13, 94.3 and 936 µg/cm^2.

Low levels of radioactivity were found in blood within 1 h at all dose levels but absorption was slow and maximum levels occurred near 72 h after cessation of exposure (for 10 or 24 h). Highest blood concentrations were 0.046, 0.401, and 1.351 µg/g at the low-, mid- and high doses respectively. Absorbed atrazine was readily eliminated, primarily in the urine and secondarily in the faeces.

The amount of absorbed atrazine was determined by a direct procedure (summation of amounts in urine, faeces, carcass and blood) and an indirect procedure involving calculation of unabsorbed atrazine. As the dose level increased, the percent of dose absorbed decreased; after a 10 h exposure, an average of 1.88% of the low dose was absorbed, 0.34% of the mid dose and 0.08% of the high dose (direct measurement); after 24 h exposure, the equivalent amounts were 7.88%, 9.11% and 0.14%.

As noted above, however, absorption continued to increase after cessation of exposure (10 and 24 h periods), apparently due to release of material from the skin into the circulation. In animals killed at 72 h after 10 h exposure (ie. 82 h after the start of the application period), amounts absorbed at the low-, mid- and high doses were 21.6%, 13.3% and 6.5% of the dose, whilst in those killed 72 h after 24 h exposure (ie. 96 h after the start of the application period), amounts absorbed at the low-, mid- and high doses were 24.4%, 26.2% and 5.8% of the dose.

At all times, significant amounts not removable by washing were associated with the skin site. For all dosage levels the amounts in the skin increased only slightly over 24 h exposure eg. at the low dose 24.2% and 30.8% of the dose was associated with the skin after 0.5 and 24 h exposure respectively, at the mid dose, 27.1% and 31.6%, and at the high dose, 11.4% and 14.2%. In the post-exposure phase, skin levels decreased to much lower levels.

Total dose recoveries ranged from 90 to 101%, average 96.2% (low dose), 91 - 105%, average 98.4% (mid dose) and 98-107%, average 102.9% (high dose). Amounts excreted in urine were 3 to 4-times the amounts in faeces.

Application time (hrs)	Dose (µg/cm 2)	percentage of applied dose (at 72hrs after end of exposure) in:-						
		Skin wash	Skin	Blood	Urine	Faeces	Carcass	
10	9.13	64.84	5.11	0.34	13.88	4.34	3.00	92.8
10	94.3	73.94	3.44	0.17	8.28	2.04	2.83	92.3
10	936	87.66	2.25	0.09	4.26	1.30	0.87	98.6
24	9.13	61.06	4.90	0.254	15.51	5.72	2.93	92.2
24	94.3	65.2	2.73	0-.26	15.99	6.53	3.46	95.5
24	936	90.8	2.6	0.08	3.77	1.12	0.84	101.1

In summary, topically-applied atrazine rapidly saturated the skin site in rats and once present, was slowly released into the circulation. Because of this rapid skin site saturation, after 10 and 24 h exposure, similar levels of systemic absorption were seen. Whilst the percent of dose absorbed decreased with increasing dose, actual amounts absorbed continued to increase. The urine was the predominant route of excretion.

After 10 h exposure, total amounts absorbed (excreta, blood, carcass, washed skin) were 27%, 22% and 11% for the low, mid and high dose levels, respectively. The rate of atrazine absorption was inversely related to the dosage level. After 24 h exposure, total amounts absorbed were 26%, 30% and 10% for the low, mid and high dose levels, respectively.

Most absorbed radioactivity was found in dissolved skin after washing. Skin residues did not decline with time after application. Less than 3% of the dose (all levels) was excreted in either a 10 or 24 h period, mainly via urine.

2.2.5 ADME studies in goats

a) ***Madrid SO & Nichols M (1987a) Distribution and Characterization of Radioactivity of ^{14}C-G 28273 in a Lactating Goat. Ciba-Geigy Corp. Agricultural Division, Vero Beach, Florida. Study no. M6-101-47A. Report no. ABR-87064. Report date 29 Oct. 1987***

and

b) ***Huhtanen K (1987a) Characterization of Tissue Residues from a Lactating Goat Treated with ^{14}C-G 28273. Ciba-Geigy Corp. Agricultural Division, Vero Beach, Florida. Study no. M6-101-47A. Report no. ABR-87104. Report date 13 Nov. 1987***

Since this is a study on the toxicokinetics and metabolism of the atrazine metabolite, **2,4-diamino-6-chloro-s-triazine** (DACT), and not atrazine itself, it is not reported in detail but the results are summarised.

Approximately 7.5 mg/day of [U-^{14}C]DACT (GAN-XI-52; 39.9 µCi/mg; 98.8% radiochemical purity) was orally administered in gelatine capsules to a 34 kg lactating goat for 10 consecutive days; this was equivalent to a feeding level of 5.81 ppm in a daily feed intake of 1.29 kg. Urine, faeces and milk were collected daily for 4 days, with sacrifice 24 h after the last dose, for measurement of radioactivity in blood, gastrointestinal (GI) contents, bile, and 7 other tissues.

Some 77.3% of the total administered radioactivity was accounted for (exhaled CO_2 was not collected), of which >86% was in urine and faeces. Urine contained 57.9% of the total dose, faeces 8.4%, indicating urinary excretion predominated over the faecal route. Milk contained 2% of the total administered; the plateau level in milk was 0.133 ppm and most extractable radioactivity was in whey, predominantly as unchanged DACT.

Urinary metabolites were very polar; neither DACT, its glutathione conjugate, nor 2,4-diamino-6-hydroxy-s-triazine were found. Extractable faecal radioactivity (44-64% of total faecal activity) was mainly DACT; metabolites were those found in urine. The biphasic extraction data for tissues and blood was similar to that for faeces; the majority of metabolites in tissues and blood were bound to proteins. Approximately 70% of the non-extractable residue solubilised by protease treatment from liver, muscle and blood was 2,4-diamino-6-mercapto-s-triazine.

c) ***Monford MS (1991) Metabolism of [Triazine-^{14}C]Hydroxyatrazine. Radioanalysis Report for the Detection and Quantity of Radioactivity Present in the Tissues, Blood, Feces, Urine and Milk in Lactating Goats. Ciba-Geigy Corp. Agricultural Division, Vero Beach, Florida. Study no. M91-101-001A. Report no. ANPHI-91004 Report date 4 Nov. 1991***

and

d) ***Pickles M (1991) Biological Report for the Metabolism of [Triazine-^{14}C] Hydroxy-atrazine in Lactating Goats. Ciba-Geigy Corp. Agricultural Division, Vero Beach, Florida. Study no. F-00123. Report no. BIOL-91010. Report date 1 Nov. 1991***

Since this is a study on the toxicokinetics of the atrazine metabolite, **hydroxyatrazine**, and not atrazine itself, it is not reported in detail but the results are summarised.

The oral administration of approx. 150 mg/day of [triazine-^{14}C]hydroxyatrazine (JAK-VI-11; 46.3 µCi/mg; 97.3% radiochemical purity; 95.6% chemical purity) in gelatine capsules to two 17-month-old lactating goats for 4 consecutive days was considered equivalent to feeding levels of 143 and 83 ppm in a daily feed intake of 1.5 kg; doses were 4.7 and 4.52 mg/kg/d. Urine, faeces and milk were collected daily for 4 days, with sacrifice at 5.92 and 6.68 h after the last dose, for measurement of radioactivity in blood, GI contents, bile, and 7 other tissues.

72.3 to 78.9% of the total administered radioactivity was accounted for (exhaled CO_2 was not collected), of which >98% was in urine, faeces and GI contents. Urine contained 67% of the total dose, faeces 14.75%, bile 0.009%, indicating urinary excretion predominated over the faecal route. Milk contained <0.4%, fat 0.45%, and muscle, 0.6% of total recovered radioactivity. (The fat and muscle figures were calculated for all body fat and all skeletal muscle, on the basis that fat and muscle comprised 40% and 4% of total bodywt, respectively.)

Both treated goats remained healthy and stable in body weight throughout the study.

2.2.6 ADME studies in hens

a) ***Madrid SO & Nichols M (1987b) Distribution and Characterization of Radioactivity of 14C-G 28273 in Laying Chickens. Ciba-Geigy Corp. Agricultural Division, Vero Beach, Florida. Study no. M6-101-46A. Report no. ABR-87063. Report date 29 Oct. 1987***

and

b) ***Huhtanen K (1987b) Characterization of Tissue Residues from Laying Chickens Treated with 14C-G 28273. Ciba-Geigy Corp. Agricultural Division, Vero Beach, Florida. Study no. M6-101-46A. Report no. ABR-87111. Report date 17 Nov. 1987***

Since this is a study in hens on the toxicokinetics and metabolism of the atrazine metabolite, **2,4-diamino-6-chloro-s-triazine** (DACT), and not atrazine itself, it is not reported in detail but the results are briefly summarised.

DACT was fed orally to two laying hens at 0.55 mg/day (a level equiv. to about 5-6 ppm in the diet) for 14 consecutive days. Levels in yolks ranged from undetectable to 0.363 ppm, in whites from 0.004 to 0.206 ppm. Greater than 81% of the dose was in excreta (sacrifice of chickens occurred 24 h after last dose). At least 10 compounds were identified in liver extracts, of which one was 2,4-diamino-6-mercapto-s-triazine.

2.2.7 Intravenous ADME studies in monkeys

a) ***Hui X, Wester RC & Maibach HI (1995a) Interim Report: Disposition of Atrazine in Rhesus Monkeys Following Intravenous Administration. Ciba-Geigy Corp., Greensboro, NC. Lab: Dept of Dermatology, University of California. Report no. UCSF 95SU04. Study completion date 19 June 1995 (no GLP; conducted under Good Scientific Practices; QA Statement)***

[U-Ring-^{14}C]atrazine (98.1% radiochemical purity; 96.5% chemical purity; 50.8 µCi/mg) was intravenously administered to 4 female Rhesus monkeys (20-30 years old) at a dose level of 0.26 $\pm$ 0.01 mg (13.35 $\pm$ 0.37 µCi); vehicle was 2:1 saline: propylene glycol. The principal route of elimination of radioactivity was the urine; at 12 h, 24 h and 168 h, 44.3%, 62.5% and 84.8% was recovered. The peak faecal elimination was found in the 48-72 h collection period (7.4%), with 11.7% of the label recovered in the faeces over 168 h. At this time, total radioactivity excreted was 98.9 $\pm$ 5.9% of the dose (urine, faeces, cage wash).

The time-course of radioactivity in the plasma was best described by a two-compartment pharmacokinetic model, with parameters as follows: area under the curve (AUC; 0.42 $\pm$ 0.03 µg/h/mL); maximum plasma concentration (Cmax; 0.0385 $\pm$ 0.0006 µg/mL); plasma clearance (Cl; 601.08 $\pm$ 38.07 mL/hr); distribution-phase half-life (t1/2 ; 1.51 $\pm$ 0.12 h); elimination-phase half-life (t1/2 ; 17.71 $\pm$ 2.16 h); volume of distribution at steady state (Vss; 13601 $\pm$ 788 mL). No indication was given as to the body weights of the animals used, but assuming a 20-25 kg range, theVss of approx. 13 L does not indicate an extensive distribution or concentration of atrazine in tissues and organs.

b) ***Simoneaux BJ, Brady JF, Cheung MW & Yokley RA (1996a) Interim Report: Disposition of Atrazine in Rhesus Monkeys Following Intravenous Administration. Ciba-Geigy Corp., Greensboro, NC. Report no. Report no. ABR-95131. Study completion date 29 Jan. 1996 (GLP; US EPA)***

The balance data and excretion kinetics part of this study are covered in the previous report (Section 2.2.7a); this study covers investigation of metabolites by the Biochemistry Group, Ciba-Geigy Crop Protection. [U-Ring-^{14}C]atrazine (98.1% radiochemical purity; 96.5% chemical purity;

50.8 µCi/mg) was intravenously administered to 4 female Rhesus monkeys (20-30 years old) at a dose level of 0.26 ± 0.01 mg (13.35 ± 0.37 µCi); vehicle was 2:1 saline: propylene glycol.

Two major metabolites found in urine were desethylatrazine (G 30033) and diaminochloro- triazine (G 28273); no atrazine or desisopropylatrazine (G 28279) was detected. Plasma contained atrazine and these metabolites as well as desisopropylatrazine (G 28279). The expected glutathione pathway metabolite, the mercapturic acid of atrazine (atrazine mercapturate), was detected primarily in urine collected to 24 h. Research was continuing on the quantitation of mercapturates of G 30033, G 28273 and G 28279. A proposed metabolic pathway is presented (see Figure).

2.2.8 ADME studies in other species/Comparative studies

a) ***Boehme C & Baer F (1967) The Transformation of Triazine Herbicides in Animals. Cosmet Toxicol 5: 23-28***

Groups of male albino rats and rabbits each received a dose of one of 5 triazine herbicides (simazine, atrazine, propazine, prometon, prometryn). Rats were dosed at a level of 50 to 200 mg/kg bw in peanut oil, and rabbits received a dose of 600-1000 mg/kg by oesophageal probe. Urine was sampled for metabolites.

Dealkylation was the most important metabolic pathway for the 5 triazines. Oxidation of the alkyl side chains to carbonic acids occurred to a small degree. Five metabolites were identified for atrazine in rat urine. All retained the triazine ring intact.

The authors, who used organic extraction and normal phase chromatography rather than acidic conditions, did not observe the C-2 hydroxylation reaction subsequently described by Bakke and coworkers (Bakke et al., 1972) (see section 2.2.1d). Propazine was a possible second metabolite.

b) ***Roger JC, Caballa SH & Knaak JB (1973) Metabolism of 14C-Atrazine in Goat, Sheep and Rat. Ciba-Geigy, Agricultural Division, Ardsley NY. Report No. GAAC-73038. Report Date 11 May 1973***

In this preliminary study, a lactating goat, a ewe and 2 rats were fed ring-labelled ^{14}C-atrazine at 5 ppm in the diet for 10 days. A 'metabolite' common to all species was found in protease-digested liver (but not in the liver of a rat only treated for 24 h). The metabolite was suggested as possibly being a mixture of conjugates of atrazine and various dealkylated atrazine derivatives. Sheep and goat liver could contain more of the conjugate of atrazine whilst the rat liver could contain mostly the conjugate of the metabolite 2,4-diamino-6-chloro-s-triazine. The metabolic pattern in goat and sheep urine were similar although absolute proportions of the metabolites varied. In rat urine, the metabolic pattern was less complex, possibly arising from didealkylated atrazine. It appears that the ruminants dealkylate atrazine slowly, rats more

rapidly, thus favouring conjugation of dealkylated derivatives rather than atrazine itself, as in the ruminants.

c) ***Erickson MD et al. (1979) Determination of s-Triazine Herbicide Residues in Urine: Studies of Excretion and Metabolism in Swine as a Model to Human Metabolism. J Agr Fd Chem. 27: 743-746***

Pittman-Moore miniature pigs (3-5 months old) were dosed under anaesthesia via stomach tube with 0.1 g atrazine technical in ethanol. Urine was sampled and analysed. Atrazine and its metabolites were detected in urine for about 24 h. Desethylatrazine
[2-chloro-4-(amino-6-(isopropylamino)-s-triazine] was identified as an atrazine metabolite.

d) ***Thede B (1989) Nature of Atrazine Residues in Animals: An Overview. Ciba-Geigy Corp., Greensboro, NC. Lab/Study no. ABR-89053. Study completion date Aug. 1989***

This is an overview document summarising atrazine metabolism in animals.

The profile of metabolites of atrazine (and other closely related chlorotriazine herbicides) isolated from animals is quite complex. However, there are two metabolic pathways, one the dealkylation of the alkylamino side chains, the other, glutathione conjugation at the chloro position, with stepwise degradation of the glutathione moiety. The overall pathway is consistent across species although quantitative differences may exist due to species variations in the kinetics of individual steps. The liver is the primary site of xenobiotic metabolism, with pathway intermediates being isolated from this tissue. Metabolites isolated from tissues, excreta, milk and eggs are primarily pathway end-products. With the exception of the liver and kidney, all tissue concentrations of metabolites were lower than blood levels. All observations indicate that atrazine is rapidly metabolised in the liver, then excreted from the body with no significant accumulation and without metabolism in tissues.

e) ***Capps T (1989) Atrazine: Nature of Plant Metabolites in Animals (Animal Metabolism). Ciba-Geigy Corp., Greensboro, NC. Lab/Study no. ABR-89065. Study completion date Aug. 1989***

This report is an overview document summarising metabolism and excretion in animals of crop biosynthesized metabolites.

Studies in which animals have been fed plants treated with radioactive atrazine or fed directly with major plant-generated metabolites show that atrazine and chorotriazines lead to tissue residues whereas residues from feeding hydroxyatrazine are 10 to 20-fold less than from feeding atrazine; hydroxyatrazines are very polar and generally pass through animals unchanged, being eliminated very rapidly.

In corn treated with atrazine, hydroxyatrazine metabolites predominate. In a feeding study in goats fed with corn (containing 0.012 ppm ^{14}C-residues) or corn silage (containing 0.946 ppm) for 5 days, residues in milk were 0.0001 ppm and 0.002 ppm ^{14}C-residues respectively. In a study in which dry corn plants at the dent stage (containing 0.32 ppm ^{14}C-residues) were fed to goats for 6 days, maximum levels in milk were 0.003 ppm.

Atrazine-treated sorghum fodder (containing 1.47 ppm 14C-residues) was fed to goats for 8 days; maximum ^{14}C-residues in milk were 0.002 ppm.

In chickens fed for 4 days with corn grain containing 0.047 ppm ^{14}C-atrazine equivalents, 92% of recovered radioactivity was found in excreta; egg yolks contained 0.01 ppm, whites 0.008 ppm atrazine equivalents.

This review covered the following studies (submission no. R11245; data submission date Jan. 1996):-

Sumner, Caballa & Cassidy (1971) Metabolism of delta-^{14}C-2-Hydroxy-4-Ethylamino-6-Isopropylamino-s-Triazine in a Cow. Ciba-Geigy Corp., Greensboro, NC. Report no. GAAC-71047. Report date 6 Aug.1971

Cassidy & Caballa (1971a) Metabolism of Atrazine Metabolites in Corn by Goats - Part I Silage. Ciba-Geigy Corp., Greensboro, NC. Report no. GAAC-71021. Report date 9 June 1971

Cassidy & Caballa (1971b) Metabolism of Atrazine Metabolites in Corn by Goats - Part II Grain. Ciba-Geigy Corp., Greensboro, NC. Report no. GAAC-71021. Report date 30 June 1971

Roger & Knaak (1972) Metabolism of delta-^{14}C-Atrazine Metabolites in Sorghum Fodder by a Goat. Ciba-Geigy Corp., Greensboro, NC. Report no. GAAC-72088. Report date 21 July 1972

Roger , Caballa & Knaak (undated) Metabolism and Balance Study in Goats given delta-14C-Atrazine in Capsules or in the Feed. Ciba-Geigy Corp., Greensboro, NC. Lab/Study no. GAAC-72131

Madrid & Nichols (1987a) Distribution and Characterization of Radioactivity of 14C-G-28273 in a Lactating Goat. Ciba-Geigy Corp., Greensboro, NC. Lab/Study no. ABR-87064 (see 2.2.6a above)

Sumner, Caballa & Cassidy (undated) Metabolism of Atrazine in the Cow. Ciba-Geigy Corp., Greensboro, NC. Lab/Study no. GAAC-71049R Study completion date ?

Simoneaux (1989) Fate of Biosynthesized ^{14}C-Atrazine Metabolites in Lactating Goats. Ciba-Geigy Corp., Greensboro, NC. Lab/Study no. ABR-89054. Study completion date July 1989

Simoneaux (1989a) Atrazine - Nature of the Residue: Further Characterization of Metabolites Present in Feces of an Atrazine Dosed Goat. Lab/Study no. ABR-89026 Study completion date May 1989

Simoneaux (1989b) Atrazine - Nature of the Residue: Further Characterization of Metabolites Present in Urine and Tissues of an Atrazine Dosed Goat. ABR-89027 Study completion date May 1989

Emrani (1989) Fate of Corn Biosynthesized Metabolites of delta-^{14}C-Atrazine in Chickens. ABR-89006. Study completion date Aug. 1989

Ballantine (1989) Estimated Dietary Exposure of Hydroxyatrazine Metabolites to Man. Ciba-Geigy Corp., Greensboro, NC. Lab/Study no. ABR-89067. Study completion date Aug. 1989

Arising from the US EPA Atrazine Data Call-In Notice of 2 Nov. 1988 and company discussions with the EPA about the potential for dietary exposure of humans to hydroxyatrazine metabolites from plants, Ciba-Geigy presented a worst-case dietary exposure estimate for hydroxyatrazine plant metabolites. Metabolism of atrazine in plants proceeds via 3 pathways:

- dealkylation of the sidechain chlorotriazine ring alkyl groups;
- enzyme-mediated s-glutathione conjugation and displacement of the chloro group from the triazine ring as well as s-glutathione conjugation of the dealkylated chlorotriazine metabolites; and
- hydrolysis of chlorotriazine to hydroxytriazine.

Calculations were based upon the assumption that the ^{14}C-radioactivity found in the plant and animal substrates (fed on the plants) is comprised solely of hydroxyatrazine metabolites. These levels were then multiplied at least 1.5-fold (including levels in liver, the organ containing the highest residue levels in animals), more in some food commodities, to account for uncertainty in exposure estimates. Using the US EPA's Tolerance Assessment System to calculate TMRCs (Theoretical Maximum Residue Concentrations), the calculated TMRC for hydroxyatrazine metabolites in the US population was 0.00067 mg/kg/day; this figure is 13.3% of the reference dose (RfD or ADI) for atrazine (0.005 mg/kg/d).

2.3 Studies in Humans

2.3.1 Kinetics and metabolism following oral dosing

a) ***Davidson JWF (1988) Metabolism and Kinetics of Atrazine in Man. Ciba-Geigy Project No. 101947. Bowman Gray School of Medicine, USA***

Six adult volunteers ingested a single oral dose of atrazine at a dose level of 0.1 mg/kg. Urine was collected and analysed for 168 h after dosing. Blood samples were collected and analysed at 0, 2, 3, 4, 5, 6, 8, 24, 32, 72 and 168 h after dosing.

Atrazine and metabolite G-28279 (2-amino-4-chloro-6-ethylamino-triazine) were detected in whole blood at levels below quantitation. Metabolites G-30033 (2-amino-4-chloro- 6-isopropylamino-s-triazine) and G-28273 (2,4-diamino-6-chloro-s-triazine) were identified in blood for up to 24 h. G-3033 appeared rapidly, peaking near 2 h and then rapidly declined with a half-life of 2.8 h; it probably resulted from liver P-450 oxidative dealkylation of atrazine; this metabolite is renally eliminated and also further dealkylated to G-28273 (peaking in blood at about 5 h), which also appears in urine. This possibly also undergoes further metabolism (conjugation or dechlorination). Both metabolites disappeared from the blood with first order kinetics.

Atrazine was undetected in urine whereas 3 chloro metabolites G-3033, G-28279 and G-28273 were detected. These 3 metabolites accounted for 5.4%, 1.4% and 7.7% of the dose, respectively, a total of 14.5% of the dose. Other metabolites have not been detected or quantified. Since significant amounts of atrazine were not found in blood or urine, this leaves 85% of the dose unaccounted for. Possibly atrazine is incompletely absorbed from the GI tract; however, in rodents the extent of absorption is >70%, even when administered in the diet. Another possibility is complete ring cleavage and metabolism to CO_2 and N_2; however, the evidence from studies in rodents, goats, chickens or plants is that the triazine ring is biologically stable. Other possibilities include extensive biliary excretion or else, there are many other metabolites excreted into the urine which have not been identified and quantified.

2.3.2 *In vitro* percutaneous absorption

a) ***Jack L (1994) The In vitro Percutaneous Absorption of Formulated [U-^{14}C]Triazine G 30027 (Atrazine) and [U-^{14}C]Triazine G 27692 (Simazine) through Human and Rat Abdominal Epidermis. Ciba-Geigy Ltd, Basle, Switzerland. Lab: Inveresk Research International, Tranent, Scotland. IRI Project no. 154697; Report no. 10702. Report date 16 Dec. 1994 (GLP)***

This study was conducted according to UK MAFF Pesticide Safety Directorate Guideline Working Document 5/9 (Nov. 1992). Radiolabelled [U-^{14}C]atrazine (Batch no. CL-XXIX-44.3; 98% radiochemical purity) was mixed with formulation ingredients for 'Gesaprim 90WDG' and 'Gesaprim 500FW'. Full-thickness human abdominal skin, cleaned of subcutaneous fat and muscle,

was obtained at autopsy (donor's age and sex recorded) and stored at *ca*. -20^{o}C until required. Rat abdominal skin was from rats (both sexes) supplied by Charles River, Margate, UK, also stored frozen before use. Before use, epidermis was carefully peeled away from the underlying dermis. An automated flow-through diffusion cell system was used, with a skin surface temp. of *ca.* 30-32^{o}C.
Three dose levels of Gesaprim 500FW were tested, 1:1, 1:100, and 1:200 in water (human and rat epidermis). Similarly, three dose levels of Gesaprim 90WDG were tested, 1:3, 1:100, and 1:200 in water (rat epidermis only). The compositions of the undiluted formulations are given in Attachments 3-1 and 3-2.

Following testing of barrier integrity (using tritiated water), 50 µL of the appropriate formulation was applied to the stratum corneum surface of the skin sample (donor chamber left open to the atmosphere) and the receptor side perfused (1.5 mL/h) with ethanol:water (50:50), with collection of hourly fractions (0 - 6 h) then 2-hourly fractions (6 - 48 h). At the end of the 48 h, the skin surface was washed to recover remaining material, then skin samples were solubilised to recover bound material.

Atrazine in Gesaprim 500FW was poorly absorbed through human and rat epidermis *in vitro*, at each of 3 dose levels of atrazine, 0.4, 0.9 and 37 mg/cm^2. The quantity absorbed by human skin was independent of the dose applied and remained constant at about 24 µg equiv/cm^2. Over 48 h, only 0.06%, 2.57% and 5.96% of the high-, mid- and low doses were absorbed through human skin. The human epidermis was less penetrable than the rat epidermis as the absorption rates were 7- and 2-fold lower at the high and mid/low dose than those in the rat epidermis. In the rat, the amount absorbed at the high-dose (viz 124 µg equiv/cm^2) was 2-fold higher than at the mid- and low dose (viz. 53 and 62 µg equiv/cm^2), although the high-dose concentration applied was 40- and 90-fold higher than the mid- and low doses.

When formulated as Gesaprim 90WDG, radioactive atrazine penetrated rat epidermis faster than from the Gesaprim 500FW formulation, as indicated by the two-fold greater absorption rate (26 µg/cm^2/h *vs* 11.55 µg/cm^2 at the high dose of 35-37 mg/cm^2 atrazine).

Most of the radioactivity applied as Gesaprim 500FW was recovered in the skin wash (66-107% for both rat and human), with between 1% of the high-dose and 6-15% of the mid- and low doses being retained in the epidermis of both species. Following Gesaprim 90WDG application to rat epidermis, means of 43% and 44% were recovered in skin wash and solubilised skin respectively; high levels associated with the skin were considered to be unabsorbed material which was not removed by washing due to the nature of the solid deposit formed.

In conclusion, atrazine in a suspension formulation and in a prepared water-dispersible granule formulation was poorly absorbed through both rat and human epidermis.

b) Ademola JI, Sedik LE, Wester RC & Maibach HI (1993) In Vitro Percutaneous Absorption and Metabolism in Man of 2-Chloro-4-ethylamino-6-isopropylamine- s-triazine (Atrazine). Department of Dermatology, University of California, School of Medicine, San Francisco. Arch Toxicol 67(2): 85-91

The percutaneous absorption of atrazine in human skin from four sources was examined utilizing a flow-through *in vitro* diffusion system. About 16.4% of the applied dose was absorbed by the skin. Radioactivity in the receptor fluid at 20 h was less than 5% of the administered dose. The highest concentration was found in the skin supernates, in which 12.0% of the dose was recovered. Some metabolites of atrazine were identified by thin layer and high pressure liquid chromatography after extraction of receptor fluid and the skin supernates. Two metabolites, desisopropylatrazine and DACT were found in the receptor fluid and the skin supernates. An additional metabolite, desethylatrazine, was found in the skin supernates. Since desisopropylatrazine represented about 50% of the total metabolites formed during percutaneous absorption, cleavage of the N-isopropyl to the amino product was a key step in the metabolism of atrazine. Further metabolism may proceed by cleavage of the N-deethyl group to give totally dealkylated atrazine. The biotransformation of atrazine was studied in skin microsomal fraction supplemented with an NADPH-generating system. In analogy to metabolism during percutaneous absorption, atrazine was metabolized to its deisopropyl and deethylpropyl derivatives. In addition, 2-hydroxy derivatives of atrazine were reported to be formed by skin microsomal fractions.

2.3.3 *In vivo* percutaneous absorption

a) ***Hui X, Wester RC & Maibach HI (1995b) Interim Report: In vivo Percutaneous Absorption of Atrazine in Man. Ciba-Geigy Corp., Greensboro, NC. Lab: Dept of Dermatology, University of California. Report no. H832-11835-01. Study completion date 25 Oct. 1995 (no GLP; conducted under Good Scientific Practices; QA Statement)***

[^{14}C]Atrazine as a simulated Aatrex 4L formulation, protected by a non-occlusive cover, was applied to a 25 cm^2 area of skin on the ventral forearm of male volunteers for 24 h, with removal of radioactivity by washing. At 168 h, the site was stripped with tape for residual radioactivity, with collection of urine and faeces throughout. Two doses were used, 6.7 µg/cm^2 (containing a total of 0.167 $\pm$ 0.002 mg [^{14}C]atrazine; 6.452 $\pm$ 0.065 µCi) in 4 volunteers, and 79 µg/cm^2 (containing 1.975 $\pm$ 0.026 mg [^{14}C]atrazine; 24.689 $\pm$ 0.326 µCi) in 6 volunteers. Radiochemical purity was 98.4% (low dose) and 98.0% (high dose), while respective chemical purity was 94.3% and 96.3%. At the low dose, dose accountability was 101%, with 5.63 $\pm$ 3.04% of the applied dose absorbed, all of which was excreted in urine and faeces. At the high dose, dose accountability was 92%, with 1.18 $\pm$ 1.03% absorbed and excreted in urine and faeces. The principal route of excretion was the urine (8.3-times as much in urine as in faeces (cf. an iv study in monkeys, in which the ratio was

very similar viz. 7.23:1). The average half-life of renal excretion was 17.5 ± 5.4 h and 24.5 ± 9.0 h for the low- and high-dose groups, respectively. Negligible radioactivity was found in tape stripping samples, indicating that, under the conditions of the study, skin or stratum corneum is not an important reservoir for atrazine following dermal exposure. Mean flux rates, at the low- and high doses, of 1343 ± 719 and 1077 ± 944 dpm/cm^2/h were not significantly different.

The extent of *in vivo* percutaneous absorption in man of 5.63% and 1.18% at topically applied doses of 6.67 and 79 µg/cm^2 may be compared with rat data of 24.4% and 26.2% for 10 and 100 µg/cm^2, respectively. On this basis, it was claimed that the extent of percutaneous absorption in man was much less than in rats. However, in a submitted rat dermal absorption study (26.9% and 21.6% 'absorbed' after 10 h at 10 and 100 µg/cm^2), most of the radioactivity considered to be absorbed was found in the dissolved skin, with less than 1% of the dose excreted (Murphy & Simoneaux, 1987: Section 2.2.4c). From this comparison, it does not appear appropriate to conclude that the extent of percutaneous absorption in man is much less than in rats, although rats appear to retain much more in the skin (not removable by surface washing) than do humans.

However, data from this study may be comparedwith that obtained in a rat study [Chengelis, 1994; see Section 2.2.4 (d)]. A comparison indicates that dermal absorption probably is greater in rats than in humans, on the basis of greater excretion of an dermal dose (applied for 24 h) in urine and faeces (see Table). However, caution should be exercised in any quantitative comparison since in rats, application was on back skin and in humans, on the ventral forearm; differences in absorption by anatomical region have been noted for a number of pesticides.

	Rat[1]		Human[2]	
Applied dose (µg/cm^3)	9.13	94.3	6.7	79
% of dose excreted in:-				
Urine	3.82%	7.22%	5.03%	1.11%
Faeces	0.46%	1.33%	0.61%	0.07%

Atrazine applied as simulated Aatrex 4L formulation.

1. Rat: 24-h application, 24-h collection: excretion data are higher for 72-h collection point, indicating continued absorption from a skin depot.
2. Human: 24-h application, 168-h collection data.

b) ***Simoneaux BJ, Brady JF, Cheung MW & Yokley RA (1996b) Interim Report: In vivo Percutaneous Absorption of Atrazine in Man. Ciba-Geigy Corp., Greensboro, NC. Report no. ABR-96003. Study completion date 29 Jan. 1996 (GLP; US EPA)***

The balance data and excretion data from this study are covered in the previous report (Section 2.3.3a); this study covers investigation of metabolites by the Biochemistry Group, Ciba-Geigy Crop Protection. ^{14}C-Atrazine, protected by a non-occlusive cover, was applied to a 25 cm^2 area of skin on the ventral forearm of male volunteers for 24 h, with removal of radioactivity by washing. At 168 h, the site was stripped with tape for residual radioactivity, with collection of urine and faeces throughout. Two doses were used, 6.7 µg/cm^2 (containing a total of 0.1667 $\pm$ 0.0017 mg ^{14}C-atrazine; 6.4518 $\pm$ 0.0652 µCi) in 4 volunteers, and 79 µg/cm^2 (containing 1.975 $\pm$ 0.0261 mg ^{14}C-atrazine; 24.6887 $\pm$ 0.3260 µCi) in 6 volunteers. Radiochemical purity was 98.4% (low dose) and 98.0% (high dose) whilst respective chemical purity was 94.3% and 96.3%.

Because of the low levels of radioactivity in the samples, it was difficult to quantitate levels of metabolites. Two metabolites found in urine were desethylatrazine (G 30033) and diaminochlorotriazine (G 28273). The expected glutathione pathway metabolite, the mercapturic acid of atrazine (atrazine mercapturate), was detected at trace levels. No atrazine or desisopropylatrazine (G 28279) was detected. Plasma contained atrazine and these metabolites as well as desisopropylatrazine (G 28279). Research was continuing on the quantitation of metabolites. It was suggested that the pathway for atrazine metabolism in monkeys is probably operative in man (see previous Figure).

3. ACUTE TOXICITY

3.1 Technical-Grade Active Constituent

3.1.1 Median lethal dose studies

A summary of findings of acute median lethal dose studies with atrazine is shown in the Table below.

Route	Species [Strain]	Sex	LD50 (mg/kg)	TGAC*	Reference
po	mouse [?]	M/F	1750		Ciba-Geigy 1957a
po	mouse [Tif.MAG]	M/F	3992		Ciba-Geigy 1975a
po	mouse [SD]	M/F	>10000	Agan	Sive 1976
po	mouse [HSD(ICR)]	M	>>1330		Kuhn 1988
po	rat [SD]	M/F	>8500		(?)
po	rat [?]	M/F	3080		Ciba-Geigy 1957b
po	rat [Wistar]	M/F	4200	Agan	Makhteshim 1973
po	rat [Wistar	M/F	2800	L'moet	Makhteshim 1973
po	rat [Tif.RAI]	M/F	1869		Ciba-Geigy 1975b
po	rat [Wistar]	?	1960		Amalgamated Chemicals 1984a
po	rat [SD]	M/F	3517		Kuhn 1991a
po	rat [Harlan SD]	M/F	*ca.* 5000		Jones 1994
ip	mouse [Tif.MAG]	M/F	626		Ciba-Geigy 1974
ip	rat [Tif.RAI]	M/F	235		Ciba-Geigy 1975c
ip	rat [SD]	M/F	5000 -10000 Agan	Sive 1976	
sc	mouse [CD-1]	M/F	>5000		(?)
sc	rat [CD/CRJ]	M/F	>5000		(?)
Dermal	rat [Tif.RAIf]	M/F	>3100		Ciba-Geigy 1976a
Dermal	rat [Harlan SD]	M/F	>2000	Agan	Dreher 1994a
Dermal	rat [[Tif.RAIf]	M/F	>2000		Hartman 1993
Dermal	rabbit (NZ White)¶	M/F	>4500	Agan	Van Beek & Williams 1973
			>4500	L'moet	
Inhalation (mist)#	rat [Tif.RAI]	M/F	> 710 mg/m^3		Ciba-Geigy 1973
Inhalation	rat [Tif.RAIf]	M/F	>5100 mg/m^3		Ciba-Giegy 1989a
Inhalation	rat [HSD:(SD)]	M/F	>5820 mg/m^3		Holbert 1991
Inhalation # (dust)	rat [SD]	M/F	>5130 mg/m^3 Agan		Blagden 1994

Where no reference given (?), data was from previous assessments on file but no reference cited.

¶ NZ - New Zealand

Inhalation studies are 4-h nose-only exposures [except for Holbert (1991) where whole-body exposure was used].

* Atrazine source: Unless otherwise stated, studies were on Ciba-Geigy atrazine: Agan = Agan Chemical Manufacturers Israel, L'moet = Ligtermoet Chemi NV, the Netherlands.

Symptoms common to oral and intraperitoneal administration were sedation, dyspnoea, exophthalmus, abnormal body positioning and ruffled fur. No symptoms were noted in the dermal or inhalation studies with the highest doses administered. No gross pathology was noted at sacrifice regardless of the route of administration.

Details of all studies are provided below. Unless otherwise stated, the observation period for median lethal dose studies was 14 days. The purity of the compound tested is indicated, if reported. Most studies were reasonably well reported and apparently well conducted; some studies had quality assurance statements or GLP certifications (see detailed acute toxicity study citations in reference list).

Information to identify each of the studies are given in the citations in the reference list.

Ciba-Geigy (1957a): The oral LD50 of atrazine (preparation G 30027; 5% and 10% suspension in gum arabic) in mice (strain?) of both sexes (16-23 g bodywt) was about 1750 mg/kg (8-day observation period).

Ciba-Geigy (1975a): The oral LD50 of atrazine (batch no. 6245; suspension in 2% CMC) in 5-10 week-old Tif.MAG (SPF) mice (both sexes) was determined as 3992 mg/kg; mice were starved one night before treatment and the observation period was 14 days. At all doses tested (1670 to 6000 mg/kg) signs within 2 h of treatment included sedation, dyspnoea, curved or ventral position and ruffled fur; the degree of sedation was dose-related. Animals which survived had all recovered within 7-8 days. There were no gross organ changes.

Sive (1976): Acute studies of technical atrazine (lot no. #7003 from Agan Ltd, Israel; 98% purity) gave oral LD50 values of >10 g/kg in Sprague-Dawley strain (?) mice of both sexes (15-25 g). In Sprague-Dawley rats (both sexes), the intraperitoneal LD50 of the same material was between 5-10 g/kg. In both mouse and rat tests, compound was administered as a 25% suspension in sterile water.

Kuhn (1988): This study was conducted according to US EPA Guidelines no. 81-2. HSD (ICR) strain male mice from Harlan Sprague-Dawley Inc., Houston, Texas (15/gp) were fasted for at least 16 h then given atrazine technical (FL-850612; Batch D3413J10; 97.7% purity) at a 4.44 or 13.32% w/v concentration in 0.5% CMC in water by gavage at doses of 0, 444, and 1332 mg/kg bw. The animals, 23.3 - 33.3 g when tested, were observed for at least 14 days. Clinical symptoms included decreased activity, ataxia, body tremors, discharge, piloerection, polyuria, ptosis and sensitivity to the touch; they occurred early and resolved by day 4 in 14/15 high-dose animals. Part from 2/15 low-dose animals with slight polyuria on at least one day, there were no signs at the low dose. There were no gross necropsy findings of note. Results indicated an LD50 of >>1332 mg/kg for male mice in this study.

Ciba-Geigy (1957b): The oral LD50 of atrazine (preparation G 30027; 10% and 20% suspension in gum arabic; purity not stated) in rats (both sexes; strain not stated; 120-140 g bodywt) was determined as about 3080 mg/kg (8-day observation period).

Makhteshim (1973): Acute studies of two samples of technical-grade atrazine (sourced from Agan Chemicals, Israel; purity unstated: and Ligtermoet Chemie NV, the Netherlands; 97% purity) gave oral LD50 values of 4.2 and 2.8 g/kg, respectively, in Wistar-derived rats (95-174 g males, 84-211 g females). Compound was administered as a 20 or 30% suspension in CMC. Symptoms were generally in accord with findings seen with atrazine sourced from Ciba Geigy. Deaths occurred mostly within the first 2 days of dosing; animals became sick, had decreased locomotor activity, "lumpback" behaviour and rough coats. No abnormalities were reported at necropsy.

Ciba-Geigy (1975b): The oral LD50 of technical atrazine (batch no. 6245; suspended in 2% CMC) in Tif.RAI (SPF) rats of both sexes was determined as 1869 mg/kg (14-day observation period). Animals were starved one night before treatment. At all doses tested (600 to 6000 mg/kg) signs within 2 h of treatment included sedation, dyspnoea, exophthalmos, curved position and ruffled fur; the degree of sedation was dose-related. Animals which survived had all recovered within 7-8 days. There were no gross organ changes.

Amalgamated Chemicals (1984a): The acute oral LD50 of atrazine technical was 1960 mg/kg bw in SPF Wistar rats. Clinical signs were rapid abdominal pain, exophthalmus, gasping, ataxia, disturbed co-ordination and sedation - coma. About 50% of animals died within 24 h. Survivors showed weight losses. Decedents showed strong hyperaemia of the GI tract.

Kuhn (1991a): This study was conducted according to US EPA Guidelines no. 81-1. Sprague-Dawley HSD:(SD) strain rats from Harlan Sprague-Dawley Inc., Houston, Texas (5/sex/gp) were fasted for at least 16 h then given atrazine technical (FL-850612; Batch D3413J10; 97.7% purity) at a 40% w/v concentration in water by gavage at doses of 2000, 4000, 5050 and 5500 mg/kg. The animals were observed for at least 14 days. Results indicated an LD50 of 3520 and 3000 mg/kg for males and females respectively, or 3090 mg/kg overall. Clinical symptoms included decreased activity, ataxia, diarrhoea, emaciation, clinical lacrimation, nasal discharge, piloerection, polyuria, ptosis and salivation, with GI tract distended with gas, testes withdrawn into the abdominal cavity, and discolouration of GI tract contents ("red slurry and creamy paste") reported at necropsy.

Jones (1994): This study was conducted according to US EPA Guidelines 81-1. Following a range-finding study, Sprague-Dawley rats supplied by Harlan UK Ltd, Oxon (5/sex) of approx. 8-10 weeks of age were given a single oral dose of atrazine technical (Batch no. D-9481; purity not stated) as a suspension in arachis oil BP at 5000 mg/kg. Surviving animals were observed for 21 days, then underwent gross necropsy. Two (2) per sex died, one female on day 2 and all others between days 8-11. Signs of delayed toxicity were apparent from day 4 on and included hunched posture, decreased respiratory rate, and/or laboured respiration, lethargy, emaciation, tiptoe or splayed gait, red/brown staining around the snout and/or mouth, ataxia, and dehydration. All surviving animals gained weight during the second and third weeks and appeared normal by 14-19 days after dosing. At necropsy of animals which died, abnormalities

included haemorrhagic lungs, dark liver and kidneys, haemorrhage of the gastric mucosa, the non-glandular region of the stomach, and the small and large intestines; animals which survived appeared normal. The acute oral LD50 was approximately 5000 mg/kg

Ciba-Geigy (1974): The intraperitoneal LD50 of technical atrazine (batch no. Mg.5041; suspension in 2% CMC) in 5 to 10 week-old Tif.MAG (SPF) mice (both sexes) was determined as 626 mg/kg; the observation period was 7 days. At all doses tested (464 to 1000 mg/kg) signs within 2 h of treatment included sedation, exophthalmos, dyspnoea, curved position and ruffled fur; the intensity/extent of these symptoms were dose-related. Animals which survived had all recovered within 4-7 days. There were no gross organ changes.

Ciba-Geigy (1975c): The intraperitoneal LD50 of technical atrazine (batch no. Mg.6245; suspension in 2% CMC) in 5 to 10 week-old Tif.RAI (SPF) rats (both sexes) was determined as 235 mg/kg; the observation period was 14 days. At all doses tested (147 to 1000 mg/kg) signs within 2 h of treatment included sedation, exophthalmos, dyspnoea, curved or ventral position and ruffled fur; the intensity/extent of sedation and dyspnoea were dose-related. At the two highest doses (600 and 1000 mg/kg), chromodacryorrhoea and salivation were observed. Animals which survived had all recovered within 7-8 days. There were no gross organ changes.

Ciba-Geigy (1976a): The dermal LD50 of technical atrazine (lot no. 6654; suspension in 2% CMC) in Tif.RAIf (SPF) rats (both sexes) was >3100 mg/kg, the highest dose level used; application was on a shaved area of the back of 60 cm^2 under occlusive dressing for 24 h. There were no deaths over the 14-day observation period. Skin irritation was absent and there were no gross pathological changes at necropsy.

Dreher (1994a): This study was conducted according to US EPA Guidelines 81-2. Sprague-Dawley rats supplied by Harlan UK Ltd, Oxon (5/sex) of approx. 10-14 weeks of age were given a single 24 h semi-occluded dermal application of atrazine technical (Batch no. D-9481; purity not stated) at 2000 mg/kg. It was applied uniformly as a powder to an area of shorn skin (approx. 5 x 4 cm) which was moistened with arachis oil: a piece of surgical gauze was placed over the treatment area and semi-occluded with self-adhesive bandage secured with surgical adhesive tape. Animals were observed for 14 days, then underwent gross necropsy. There were no deaths, and no signs of systemic toxicity or skin irritation. All animals gained weight as normal. The acute dermal LD50 was >2000 mg/kg.

Hartmann (1993): This study was conducted according to OECD Guideline 402 and EC Guideline 92/69/EEC, B.3. Young Tif: RAI (SPF) rats supplied by Ciba-Geigy Animal production, Stein (5/sex) of approx. 10-14 weeks of age (222-255 g bodywt) were given a single 24 h semi-occluded dermal application of atrazine technical (Batch no. FL-881692; 97.1% purity) at 2000 mg/kg bodywt. It was applied uniformly at 4 mL/kg in 0.5% CMC in 0.1% aqueous polysorbate 80 to an area of shorn skin (approx. 10% of body surface); a piece

of surgical gauze was placed over the treatment area and semi-occluded with self-adhesive bandage secured with surgical adhesive tape, before removal by washing with water after 24 h. Animals were observed for 14 days, then underwent gross necropsy. There were no deaths, and no signs of systemic toxicity (apart from piloerection and hunched posture, common symptoms in acute dermal toxicity tests) or of skin irritation. All animals gained weight as normal. The acute dermal LD50 was >2000 mg/kg (both sexes).

Van Beek & Willems (1973): The dermal LD50 of two samples of technical atrazine (Agan Chemical Manufacturers, Israel; purity unstated: and Ligtermoet Chemie NV, the Netherlands; 97%) in NZ White rabbits (both sexes; 2.0-2.7 kg initial weight) was > 4.5 g/kg in both cases. Material was applied to shaved skin (approx. 10% of body surface area) for 24 h, under a cellulose sheet and wrapped in polyethylene foil. A slight and reversible erythema was observed at the application site, with slight scaliness at 2 weeks in the high-dose (4.5 g/kg) animals.

Ciba-Geigy (1973): The inhalation LC50 in Tif.RAI (SPF) rats of both sexes (170-180 g bodywt) was > 710 mg/m^3 (1 h exposure), the highest dose level used. A 20% aqueous suspension was used to produce an aerosol mist for nose-only exposure. The air concentration was gravimetrically determined. After a 7-day observation period animals were necropsied; there were no compound-related gross changes. The bulk of the particles were > 7 µm in size.

Ciba-Geigy (1989a): The test was conducted according to OECD Test Guideline 403. The acute LC50 of atrazine technical (batch no. GP-920901/FL 890322; 96-97% purity) was greater than 5100 mg/m^3, when applied to 8-9 week-old adult male and female albino rats (Tif: RAIF (SPF) hybrids of RII x RII/2) by the nose only for 4 h (median particle diameters 1.6-1.9 µm), followed by a 14-day observation period (5/sex/gp). The mass median aerodynamic diameter (MMAD) of the aerosolised powder was 1.6-1.9 µm, with a GSD of 1.5-1.6; 26-38% of the particles had a diameter smaller than 7 µm and 13-20%, less than 3 µm. The mean exposure concentration was 5148 $\pm$ 178 mg/m^3 (SD).

There were no deaths. Clinical signs were piloerection, hunched posture, dyspnoea and reduced spontaneous activity. In atrazine-exposed animals, males had a marginally lower body weight gain in the second observation week, the females in the first week. All exposed animals recovered within 5 days, and no treatment effects were noted at gross pathology.

Holbert (1991): This study was conducted according to US EPA Guideline 81-3. Young adult Sprague-Dawley HSD: (SD) rats with bodywts 248-268 g (males) and 190-223 g (females), supplied by Harlan Sprague Dawley Inc., Houston, Texas (5/sex), were exposed (whole-body exposure) to a dust of atrazine technical (FL-910737 ARS 14105; batch code GP-900317; 97.4% purity) for 4 hours. The mean achieved concentration was 5.82 mg/L, with a MMAD of 5.051 µm (1 h) and 4.687 µm (2.5 h) and a respirable fraction

(<1 µm) of 7.26%. Animals were observed for 14 days, then underwent gross necropsy. There were no deaths. Signs observed during the study were pilo-erection, reduced activity, lacrimation, nasal discharge, polyuria, ptosis and salivation. All animals gained weight normally and appeared normal by 2-3 days after dosing. No abnormalities were detected at necropsy. The acute inhalational LD50 of atrazine dusts was >5.82 mg/L (5820 mg/m^3).

Blagden (1994): This study was conducted according to US EPA Guideline 81-3. Young adult Sprague-Dawley rats of 237-269 g (males) and 196-218 g (females) bodywt, supplied by Charles River (UK) Ltd, Manston, Kent (5/sex), were exposed (nose only) to a dust of atrazine technical (Batch no. D-9481; purity not stated) for 4 hours. The mean achieved concentration was 5.13 mg/L, with a mass median aerodynamic diameter of 1.8 µm, and a respirable fraction (< 1 µm) of 24.5%. Animals were observed for 14 days, then underwent gross necropsy. There were no deaths. Signs observed during the study were wet fur, hunched posture, pilo-erection, and test-material staining on the snout and head. Incidents of ptosis and isolated incidents of red/brown staining around the snout. All animals gained weight normally and appeared normal by 2-3 days after dosing. No abnormalities were detected at necropsy. The acute inhalational LD50 of atrazine dusts was >5.13 mg/L (5130 mg/m^3).

3.1.2 Eye and dermal irritancy & sensitisation studies

A summary of the findings of eye and dermal acute irritancy and sensitisation studies are shown in the Table below.

Irritation Studies

Study	Species (Strain)	Sex	Finding	TGAC*	Reference
Eye	rabbit [Himalayan]	M/F	not irritating		Ciba-Giegy 1976b
Eye	rabbit [NZ White]	M/F	slightly irritating	Amalgamated 1984b	
Eye	rabbit [?]	?	not irritating	Agan	Rosenfeld 1984a
Eye	rabbit [NZ White]	M/F	mild irritant		Dreher 1994b
Skin	rat (?)	?	not irritating		(?)
Skin	rabbit [NZ White]	M/F	not irritating		Amalgamated 1990
Skin	rabbit [Himalayan]	M/F	mildly irritating		Ciba-Geigy 1976c
Study	**Species (Strain)**	**Sex**	**Finding**	**TGAC***	**Reference**
Skin	rabbit [Himalayan]	M/F	not irritating	Agan	Rosenfeld 1984b
Skin	rabbit [NZ White]	M/F	not irritating		Dreher 1994c
Skin	human	M	non irritant		Shelenski & Gittes 1965

Sensitisation Studies

Study	Species (Strain)	Sex	Finding	TGAC*	Reference
Skin	guinea pig [Pirbright]	M/F	sensitiser		Ciba-Geigy 1985a
Skin	guinea pig [white]	M/F	strong sensitiser		Ciba-Geigy 1985b
Skin	guinea pig [D-H#]	F	non-sensitiser	Agan	Dreher 1994d
Skin	human	M	non sensitiser**		Shelenski & Gittes 1965

Where no reference given (?), data was from previous assessments on file but no reference cited.

* Atrazine source: Unless otherwise stated, studies were on Ciba-Geigy atrazine: Agan = AganChemical Manufacturers, Israel.

Dunkin Hartley strain

** 0.5% w/v aqueous suspension of 80% wettable powder

Details of these studies are provided below. The purity of the compound tested is indicated, if reported. Most studies were reasonably well reported and apparently well conducted; some studies had quality assurance statements or GLP certifications (see detailed acute toxicity study citations in reference list).

Eye irritation

Ciba-Geigy (1976b): In albino Himalayan rabbits (3/sex; 1.5 to 2 kg bodyweight), 0.1 g atrazine (lot 6654) was applied into the conjunctival sac of the left eye which was then closed for one second. Half the rabbits had their eyes flushed (10 mL of lukewarm water) after about 30 sec. There was no evidence of any eye irritation.

Amalgamated Chemicals (1984b): Atrazine technical was a slight eye irritant in NZ white rabbits, when 0.1 g of the undiluted powder was instilled in the eye, without washing. Animals showed conjunctival irritation with redness and secretion during the first 12 h, which resolved after 24 h.

In rabbits, eye irritation was absent, with or without flushing the eye following the instillation of 0.1 g atrazine.

Rosenfeld (1984a): Tests in young adult albino rabbits (strain not stated) indicated that Atranex Technical (Agan chemical Manufacturers, Israel, lot/batch 7139; purity unstated) was virtually non-irritant in the eyes. Test article (0.1 g) was placed into one eye of each of six rabbits and examined for up to 72 h. Slight conjunctival redness (all eyes) and chemosis (2/6) had disappeared by 24 h. No systemic effects were observed. These results are in

accord with the findings from experiments with the atrazine sourced from Ciba Geigy.

Dreher (1994b): This study was conducted according to US EPA Guidelines 81-4. NZ White rabbits of approx. 12-20 weeks of age (2.5 to 2.68 kg), supplied by David Percival Ltd, Cheshire (6 animals), were given a single application of atrazine technical (Batch no. D-9481; purity not stated) to one eye. A quantity of 0.1 mL of test material (approx. 80 mg) was placed into the conjunctival sac of the right eye. Assessment of ocular irritation/damage were made at 1, 24, 48 and 72 h. No adverse corneal effects were noted but iridial inflammation was noted in 4 tested eyes at 24 h and in one eye at 48 h. All eyes were normal at 72 h. Atrazine technical was classified as a mild ocular irritant in rabbits, according to the modified Kay & Calandra classification system (Kay & Calandra, 1962).

Dermal irritation

Amalgamated Chemicals (1990): Atrazine technical was a slight skin irritant in NZ White rabbits when 0.5 g was applied to abraded, or unabraded back or flank skin under gauze, tape and bandage for 24 h, followed by 72 h observation. There was no irritation of intact skin, and only slight erythema in scarified skin after 24 h, which resolved by 48 h.

Ciba-Geigy (1976c): In albino Himalayan rabbits (3/sex; 1.5 to 2 kg bodyweight), 0.5 g atrazine (lot 6654) applied to intact and abraded skin (24-h contact under occlusive bandage) produced a reversible and barely perceptible erythema which was confined to previously abraded skin. It was classed as a mild irritant.

Rosenfeld (1984b): Tests in young adult albino rabbits (2.0-3.5 kg) indicated that Atranex (Agan Chemical Manufacturers, Israel; lot/batch no. 7139; purity not stated) was virtually non-irritant in the skin. It was applied as a paste under a 1 inch square piece of gauze to the clipped dorsal surface, and held in place by hypoallergenic tape and the trunk of the animal was wrapped with a perforated plastic sheet. After 4 h application time, there were no systemic effects and only very slight erythema at about 45 min after patch removal, with all test sites normal by 24 h. The primary irritation score for Atranex Tech. was 0.125 ie. it was not a primary irritant.

Dreher (1994c): This study was conducted according to US EPA Guidelines 81-5. NZ White rabbits of approx. 12-20 weeks of age (2.28 to 2.81 kg), supplied by David Percival Ltd, Cheshire (6 animals), were given a single 4 h semi-occluded dermal application of atrazine technical (Batch no. D-9481; purity not stated) at 2000 mg/kg bodywt to clipped intact skin. A quantity of 0.5 g of material moistened with 0.5 mL distilled water was applied uniformly under a 2.5 x 2.5 cm gauze patch, secured with a piece of surgical adhesive tape and the torso of each animal wrapped in an elastic corset. The patches were removed after 4 h and test material removed by gentle swabbing. Application sites were examined at 1, 24, 48 and 72 h following patch removal.

Animals were observed for 14 days, then underwent gross necropsy. There were no deaths, and no signs of systemic toxicity or skin reactions. Atrazine technical was classified as a non-irritant according to the Draize classification scheme.

Skin sensitisation

Ciba-Geigy (1985a): In a skin sensitisation test (OECD Guideline test no. 406) in Pirbright white guinea pigs (10/sex) of about 10-weeks of age (330 to 433 g), atrazine (lot 210200: 98.2% purity) was given intracutaneously during the "sensitization" period and adjuvant was added for "optimization". Later challenge (dermal application) led to a positive allergenic effect. Concentrations used (in 20% ethanol, in saline solution) were 0.1 mL of 0.1% solutions for intradermal application and 30% solutions for dermal application. Thus this test indicated a skin-sensitising (contact allergenic) potential of atrazine.

Ciba-Geigy (1985b): Technical grade atrazine (G 30027 tech; lot no. 407181; 98% purity) was tested in a Magnusson and Kligman skin sensitisation test (OECD Test Guideline no. 486) using female Pirbright White (Tif: DHP) strain guinea-pigs from Ciba-Geigy Tierfarm, Sisseln, Switzerland (10/sex/gp). For the first induction, atrazine was intradermally injected in oleum arachnis and in adjuvant saline mixture (1% concentration) and for the second induction, applied epidermally in vaseline (30%). Most animals (14/20 males and females at 24 hours and 13/20 at 48 h) showed a positive reaction after challenge with technical grade atrazine. Draize scores were 1-2 for both erythema and edema (slight - well defined). The proportion of animals with positive reactions (65-70%) corresponded to a maximisation grading of 4 ie. indicating that atrazine has a strong skin sensitising (contact allergenic) potential in albino guinea-pigs.

Dreher (1994d): This study was conducted according to US EPA Guidelines 81-6. Female Dunkin-Hartley albino guinea pigs of approx. 8-12 weeks of age (330-420 g), supplied by David Hall Ltd, Staffordshire (20 test and 10 control animals), were used for assessing the sensitising potential of technical atrazine (Agan Atranex Technical; Batch no. D-9481; purity not stated). The test material was freshly prepared at 25% w/v in arachis oil and in a 1:1 mixture of arachis oil and Freund's Complete Adjuvant (intradermal induction); at 50% w/w in arachis oil (topical induction); and 25% and 10% w/w in arachis oil (topical challenge). These concentrations were chosen on the basis of sighting tests. On the basis of a 0/20 sensitisation rate, atrazine technical was classified as a non-sensitiser to guinea pig skin in the Magnusson & Kligman Maximisation test.

Shelanski & Gittes (1965): Patch tests were conducted on fifty human subjects. Approximately 0.5 mL of the test material (atrazine 80W-Fl-2858 ARS 2447A-64; 0.5% w/v suspension in water) was placed on patches applied to the arms or backs of the subjects for 24 hours, after which the patches were removed and reactions, if any, were graded. After a 24-hour rest period, the subjects received a second patch application. This procedure was repeated

until a series of 15 consecutive exposures had been made. A 14-day 'rest' period was then allowed after which a challenge dose was applied. None of the fifty subjects reacted to any of the 15 applications or to the challenge application, indicating that atrazine was not a skin irritant nor a sensitiser.

Note: The composition of atrazine 80W-F1-2858 was not specified; elsewhere it was stated to be an 80% wettable powder formulation.

3.2 Acute Toxicity of Atrazine Metabolites

Acute oral toxicity studies were carried out on four atrazine metabolites, including the plant metabolite, hydroxyatrazine. The results are summarised in the following Table:-

Desethylatrazine (G 30033)

Study/route	Species (Strain)	Sex	LD50 (mg/kg)	Reference
Oral	rat [Harlan SD]	M	1890	Kuhn 1991b
		F	68	Kuhn 1991b
		M/F	1110	Kuhn 1991b

Desisopropylatrazine (G 28279)

Study/route	Species (Strain)	Sex	LD50 (mg/kg)	Reference
Oral	rat [Harlan SD]	M	2290	Kuhn 1991c
		F	810	Kuhn 1991c
		M/F	1240	Kuhn 1991c

Diaminochlorotriazine (G 28273)

Study/route	Species (Strain)	Sex	LD50 (mg/kg)	Reference
Oral	rat [Harlan SD]	M	>5050	Kuhn 1991d
		F	>5500	Kuhn 1991d
Oral	rat [Blu:SD]	M	*ca.*11300	Sabol 1991a
		F	*ca.* 5230	Sabol 1991a
		M/F	*ca.* 5460	Sabol 1991a
Oral	rat [Blu:SD]	M	*ca.* 3690	Sabol 1991b
		F	*ca.* 2360	Sabol 1991b
		M/F	*ca.* 2310	Sabol 1991b
Eye irritation	rabbit [NZ White]	M/F	minimal (wash)	Cannelongo 1979
			moderate (unwash)	Cannelongo 1979
Skin irritation	rabbit [NZ White]	M/F	slight (intact)	Sabol 1979
			mild (abraded)	Sabol 1979

Hydroxyatrazine (G 34048)

Study/route	Species (Strain)	Sex	LD50 (mg/kg)	Reference
Oral	rat [Harlan SD]	M/F	5050	Kuhn 1991e

All metabolites studies were on Ciba-Geigy material.

Individual study details are reported below. Most studies were reasonably well reported and apparently well conducted; some studies had quality assurance statements or GLP certifications (see detailed acute toxicity study citations in reference list).

Desethylatrazine (G 30033)

Kuhn (1991b): This study was conducted according to US EPA Guidelines no. 81-1. Young adult Sprague-Dawley HSD:(SD) strain rats from Harlan Sprague-Dawley Inc., Houston, Texas were fasted for at least 16 h then given desethylatrazine (G-30033) technical (FL-901515; Batch 7-27-830; 95.7% purity) at a 40% w/v concentration in 2.0% w/v CMC by gavage at doses of 250, 500, 2000, 3500 and 5050 mg/kg (5/sex/gp). The animals were observed for at least 14 days. Results indicated an LD50 of 1890 mg/kg for males and 668 mg/kg for females, with an overall LD50 of 1110 mg/kg. Clinical symptoms included decreased activity, ataxia, emaciation, lacrimation, nasal discharge, piloerection, polyuria, diarrhoea, salivation and ptosis, with GI tract distended with gas, discolouration of the liver and contents of the GI tract, and testes drawn into the abdominal cavity reported at necropsy. There was 100% mortality of both sexes at the two highest doses.

Desisopropylatrazine (G 28279)

Kuhn (1991c): This study was conducted according to US EPA Guidelines no. 81-1. Young adult Sprague-Dawley HSD:(SD) strain rats from Harlan Sprague-Dawley Inc., Houston, Texas were fasted for at least 16 h then given **desisopropylatrazine** (G-28279) technical (FL-901747; Batch 02-0919-1400; purity unstated) at a 25% w/v concentration in 2.0% w/v CMC by gavage at doses of 250, 500, 2000, 3500 and 5050 mg/kg (5/sex/group). The animals were observed for at least 14 days. Results indicated an LD50 of 2290 mg/kg bw for males and 810 mg/kg for females, with an overall LD50 of 1240 mg/kg. Clinical symptoms included decreased activity, ataxia, emaciation, lacrimation, nasal discharge, piloerection, polyuria, diarrhoea, salivation and ptosis, with GI tract distended with gas, discolouration of the liver and contents of the GI tract, and testes drawn into the abdominal cavity reported at necropsy. There was 90-100% mortality of both sexes at the two highest doses.

Diaminochlorotriazine (G 28273)

Kuhn (1991d): This study was conducted according to US EPA Guidelines no. 81-1. Young adult Sprague-Dawley HSD:(SD) strain rats from Harlan Sprague-Dawley Inc., Houston, Texas were fasted for at least 16 h then given diaminochlorotriazine (DACT) technical (FL-871776; Batch GP-720301; purity unstated) at a 40% w/v concentration in water by gavage at doses of 4000, 5050 and 5500 mg/kg (5/sex/group at the middle dose, 5 females/group at the other two doses). The animals were observed for at least 14 days. Results indicated an LD50 of >5050 mg/kg for males and >5500 mg/kg for females. Clinical symptoms included decreased activity, emaciation, lacrimation, nasal discharge, piloerection, polyuria, salivation and ptosis, with GI tract distended with gas and discolouration of the liver reported at necropsy. At the high dose, 1/5 animals (females) died.

Sabol (1991a): Young adult Sprague-Dawley strain Blu:(SD) rats from Blue Spruce Farms Inc., Altamont, NY, were fasted for at least 16 h then given

diaminochlorotriazine (DACT) technical (FL-781538; ARS 1857; purity unstated) at a 16.65% w/v slurry in corn oil by gavage at doses of 2463, 3457, and 4256 mg/kg (5 females, 10/sex, and 5 males used at the respective doses). The animals, of bodywts 200-280 g (males) and 200-225 g (females), were observed for at least 14 days. Combined deaths at the 3 dose levels were 3/5, 14/20, and 4/5. Results indicated approximate LD50's of 3690 mg/kg for males and 2360 mg/kg bw for females; the combined LD50 was calculated as 2310 mg/kg. Clinical symptoms included rapid breathing, constricted pupils, epistaxis, tremors, decreased activity, aggressiveness, ataxia, emaciation, piloerection, polyuria, haematuria, melanuria, corneal opacity, and ptosis. At necropsy, in addition to confirmation of the above clinical observations, findings included discolouration of the lungs and liver, GI tract distended with gas, discolouration of the contents of stomach, intestines and urinary bladder, testes withdrawn into abdominal cavity, discolouration of the stomach mucosa, stomach wall fragile, dilated renal pelvis, white paste and oil in pleural cavity, and serosal blood vessels pronounced on stomach. Whilst not commented on, the appearance of white paste and oil in the pleural cavity (one female at the mid dose) would suggest mis-dosing.

Sabol (1991b): Young adult Sprague-Dawley strain Blu:(SD) rats from Blue Spruce Farms Inc., Altamont, NY, were fasted for at least 16 h then given **diaminochlorotriazine** (DACT) technical (FL-781538; ARS 1857; purity unstated) at a 33.3% w/v slurry in corn oil by gavage at doses of 2491, 3457, 5050 and 7189 mg/kg (5/sex/group, except no females tested at the low dose). The animals, of bodywts 200-295 g (males) and 200-220 g (females), were observed for at least 14 days. Combined deaths at the 4 dose levels were 1/5, 2/10, 3/10 and 7/10. Results indicated approximate LD50's of 11,300 mg/kg for males and 5230 mg/kg bw for females; the combined LD50 was calculated as 5460 mg/kg. Clinical symptoms included respiratory gurgle, constricted pupils, epistaxis, tremors, bloody penis, decreased activity, emaciation, piloerection, polyuria, haematuria, melanuria, corneal opacity, and ptosis. At necropsy, in addition to confirmation of the above clinical observations, findings included bloody discharge around the anus, discolouration of the lungs and liver, GI tract distended with gas, discolouration of the contents of stomach, intestines and urinary bladder, testes withdrawn into abdominal cavity, discolouration of the stomach mucosa, adrenal glands and pancreas, thickening of stomach mucosa, and serosal blood vessels pronounced on stomach.

Cannelongo BF (1979a): An eye irritation study was conducted in NZ White rabbits using 2,4-diamino-6-chloro-s-triazine (FL 781538; ARS 1857; purity not stated); animals were from Ray Nichols Rabbitry, Lumberton, Texas. 100 mg of undiluted test material was placed into the right eye of 9 animals (3 males, 6 females); three treated eyes (female animals) were washed with deionised water for one minute, beginning 30 sec. After treatment, eyes were examined at 1, 24, 48 and 72 h, and then at 4, 7 and 10 days. DACT was classified as moderately irritating in non-washed eyes, and minimally-irritating in washed eyes.

Sabol (1979): NZ White rabbits (3/sex) from Ray Nichols Rabbitry, Lumberton, Texas, were used to test the primary skin irritation potential of **diaminochlorotriazine** (DACT) technical (FL-781538; ARS 1857; purity unstated). An area approximately 12 x 17 cm on the back of the trunk was clipped, and minor longitudinal and lateral abrasions were made on two of four skin test sites on each rabbit; they were deep enough to penetrate the stratum corneum but not the derma. Test material (0.5 g, moistened with 0.6 mL normal saline) was applied to each site beneath a 1 square inch surgical patch secured with adhesive tape, then the entire trunk was wrapped in polyethylene film. Patches and test material were removed after 24 h, with scoring for erythema and oedema at 24 h and 72 h. Erythema and oedema were present at each observation time. The primary irritation score was determined as 1.69 (out of a maximum of 8). Under the test conditions, atrazine was slightly irritating (intact skin) and mildly irritating (abraded skin). There was no sign of ulceration or necrosis.

Hydroxyatrazine (G 34048)

Kuhn (1991e): This study was conducted according to US EPA Guidelines no. 81-1. Young adult Sprague-Dawley HSD:(SD) strain rats from Harlan Sprague-Dawley Inc., Houston, Texas were fasted for at least 16 h then given **hydroxyatrazine** technical (FL-870869; Batch SL-910; purity unstated) at a 40% w/v concentration in 2.0% w/v aqueous CMC by gavage at a doses of 5050 mg/kg (5/sex). The animals were observed for at least 14 days. Results indicated an LD50 of >5050 mg/kg for both males and females. No animals died during the study and all animals appeared normal for the duration of the study. No abnormalities were observed at necropsy.

3.3 Acute Toxicity of Atrazine Formulations

3.3.1 Gesaprim 55FW

Note: The formulation used in the following study was 'Gesaprim 500FW' a 500 g/L suspension concentrate - see **Attachment 3-2** for a comparison of this A-3491S formulation used in the following studies with the Australian formulation.

Ciba-Geigy (1989b): This test was conducted according to OECD Guideline no. 401. Young adult Tif:RAIf(SPF) rats (5/sex) (7 to 8 weeks old; 176 to 201 g bodywt) were fasted overnight then given Gesaprim 500 FW [G30027 SC 500 (A-3491S)], a 500 g/L suspension concentrate formulation of atrazine, at a dose of 3000 mg/kg bw by gavage; the study vehicle was distilled water. The animals were observed for 14 days. No mortalities were observed, indicating an LD50 for the formulation of >3000 mg/kg in both sexes. Clinical symptoms included piloerection, hunched posture, dyspnoea, slightly reduced locomotor activity (females) and diarrhoea (males), with recovery within 5 to 8 days. No abnormalities were found at autopsy.

3.3.2 Marksman Herbicide

Note: The formulation used in these studies was 'Marksman Herbicide', a mixture containing 250 g/L atrazine and 130 g/L dicamba - see Attachment 3-3 for formulation.

median lethal dose studies ***LD 50/LC50***

Study	Species	Details	Outcome	Reference
po	rat (SD)	gavage (5/sex/gp)	5897 mg/kg	Naas 1985a
dermal	rat (SD)	shaved/intact/ 24 h occlusive (5/sex)	>2000 mg/kg	Nass 1985b
inhalation	rat (SD)	whole body/3.38 mg/L) (5/sex)	>3380 mg/m^3	Dudek 1985

Irritation studies

Irritation

Study	Species	Details	Outcome	Reference
eye	rabbit (NZ)	0.1 mL/conjunctival sac rinsed & unrinsed (6M, 3F)	slight irritant	Nass 1985c
skin	rabbit (NZ)	0.5 mL/intact/4 h occlusive/wiped (3/sex)	slight irritant	Nass 1985d

Skin sensitisation studies

Study	Species	Details	Outcome	Reference
skin sens.	guinea pig (D-H)	Buehler method/undiluted challenge-60% v/v (10/sex)	non-sensitiser	Nass 1985e
skin sens.	guinea pig	induction applications (10F)	non-sensitiser	Smith 1986

(D-H)

D-H = Dunkin Hartley; sens. = skin sensistisation

Results of the studies with this atrazine formulation are summarised below.

Naas (1985a): Death at doses of 4545, 5000, 5250 and 5550 mg/kg occurred in 2, 6, 3 and 4 (out of 5/sex) respectively after oral dosing. Deaths occurred on day 1 or 2, except for one animal at the high dose which died on day 8. Clinical signs consisted of lethargy (26/40), hypersensitivity to touch (16/40),

hypertonus (14/40), ataxia (13/40) and emaciation (5/40). Except for emaciation, which appeared only in the two highest groups, there was no pattern to the appearance of clinical signs. Signs were seen on the day of dosing or the day after, with surviving animals appearing normal by day 2. There were no significant treatment-related pathological signs in rats surviving until day 14. Meningeal haemorrhage, as well as congestion and haemorrhage of the gastrointestinal mucosa were seen in all rats that died. Pulmonary congestion was seen in 11 out of the 15 rats that died.

Naas (1985b): After dermal dosing, 1/5 male rabbits died on day 8 but was unrelated to the treatment. There were no test-related clinical signs. Three males and one female displayed slight erythema on day 1 only.

Dudek (1985): One male (of 5) died on day three and one female (of 5) on day 1 after inhalational exposure. Lethargy (2 animals), abnormal breathing (9), crusty muzzle (9), alopecia (2), and poor coat quality (9) were observed during the study. Most signs excluding abnormal breathing (only occurring up to day 4) occurred at times ranging from day 1 to 14. In animals found dead, there was pulmonary haemorrhage, black gastric contents, and in one animal, a dilated renal pelvis on necropsy examination.

Naas (1985c): No corneal lesions were evident in treated eyes of NZ White rabbits (6 males, 3 females). Conjunctival reactions were seen in all treated eyes (no significant difference between washed and unwashed) and consisted of slight to moderate redness and swelling, with slight discharge. Slight swelling and congestion of the iris were seen in 5 of 6 unwashed eyes and 2 of 3 washed eyes. Signs of irritation were apparent at 1 hour and had completely dissipated within 7 days. The maximum average score was 14.3 for the unwashed group and 11.7 for the washed group, this occurring at 1 hour. The substance is classed a slight eye irritant.

Naas (1985d): The skin irritation study in NZ White rabbits showed slight erythema (5/6) and oedema (2/6) at 4 hours, with erythema persisting in two rabbits until 48 hours. The primary irritation index was 0.1. Based on these results, the substance was classed a slight irritant.

Naas (1985e): In a skin sensitisation study in Dunkin-Hartley guinea pigs, one male (of 10) in the test group displayed slight patchy erythema 48 h after challenge. All animals in the positive control group (0.1% dinitrochlorobenzene in acetone) had slight patchy erythema at 24 h post-challenge, with only 2 of 10 showing erythema at 48 h. The irritation incidence indices were zero for the test and positive control groups. An irritation severity index of 0.025 was calculated for the test group at 48 h, with an index of 0.5 in the positive controls at 24 h. The test substance was classed a non-sensitiser based on these results.

Smith (1986): In this skin sensitisation study in guinea pigs using nine induction applications, no dermal reactions were noted in the test group. Brown stained fur was noted at the test site in all treated animals. All animals

in the positive control group (0.1% dinitrochlorobenzene in acetone) had slight to well defined erythema at 24 hours post-challenge. The test substance was classed a non-sensitiser based on these results.

4. SHORT-TERM REPEAT-DOSE STUDIES

4.1 Atrazine (G 30027)

4.1.1 4-Week rat dietary study

Amalgamated Chemicals (1984c) Preliminary 4 weeks Subacute Toxicity Study with Rat. Study No. Dr. S.D./Rm/Re 2-4-58-84. Pharmatox GmbH W. Germany 1984

Groups of 6 male SPF Wistar rats received dietary atrazine technical (0, 100, 500, 2000 and 4000 ppm) for 4 weeks. Mortality and clinical signs were observed daily. Bodyweights, food and water consumption were measured weekly. Organ weights and macroscopic pathology were recorded at autopsy for all animals.

There were no deaths, however food consumption was decreased in a dose dependent manner in all treated groups, weight gains were lower at 500 ppm and above, and water consumption and food utilisation were lower, and relative kidney and testes weights were increased at 2000 and 4000 ppm. Assuming the reduced food intake was a reflection of palatability of the diet, a NOEL for this study could be set at 100 ppm (estimated 5 mg/kg bw/d) based on these effects.

4.1.2 14-Day rat gavage study

Fitzgerald RE (1988) G30027 (Atrazine): 14-Day Oral Toxicity Study in Young Rats (Gavage). Ciba-GeigyLtd, Basle, Switzerland. GU Project no. 871290. Report date 28 Sept. 1988 (GLP; OECD)

This non-standard study was based on OECD Guideline no. 407. Its aim was to determine the effects on juvenile rats (because of the particular susceptibility of their blood forming, immune, and developing endocrine systems) and the reversibility of any effects which may be observed. For logistic reasons, the treatment period was actually 15 days.

Atrazine (Lot no. 210200; 98.2% purity) in 0.5% CMC and 0.1% Tween 80 was administered to Tif: RAIf(SPF)(hybrids of RII/1 x RII/2) rats from Ciba-Geigy Tierfarm, Stein, (15/sex/group) by gavage for 15 days at doses of 0, 25, 100, and 400 mg/kg/d (dose volume 10 mL/kg/day). Five animals/sex/group were kept for a recovery period of 14 days. Animals were 23-days old at the start of treatment (36-63 g weight range). Doses were selected on the basis of an acute oral toxicity range of 1869-3080 mg/kg, a NOEL of approximately 0.7 mg/kg/d in a two-year rat study, and dam mortality at 1000 mg/kg in a teratology study. Mortality and clinical signs were checked daily, bodyweights,

food and water consumption weekly, and laboratory investigations (haematology and blood chemistry) at the end of dosing and at the end of the recovery period. Haematology included RBC count, Hb, Hct, MCV, MCH, red cell morphology, WBC count, differential leucocyte count, and thrombocyte count. Blood chemistry included glucose, urea, total protein, albumin, globulins, A/G ratio, AST, ALT and AP. Brain, liver, adrenals, thymus, gonads and spleen were weighed, with histopathology conducted on spleen, lymph node (mesenteric), lymph node (axillary and popliteal), sternum with bone marrow, femur bone marrow, liver, large intestine, testis, ovary and thymus, brain and adrenals.

No treatment-related clinical signs were noted. There were two deaths at the high dose, one female (day 2) and one male (day 4).

Bodyweights were dose-dependently reduced in both sexes during the two dosing weeks, but only significantly reduced at 100 and 400 mg/kg; at the end of the dosing period, bodywts were 9.7-10.2% and 32-35% less than controls at the mid and high doses, respectively. At the end of the recovery period, the bodywt of males in both these groups was still lower than controls by 6.8% and 15% respectively (not statistically significant) but for females at the high dose, bodywt was still significantly lower than controls (16%).

There was a dose-related decrease in food consumption for both sexes at the mid- and high dose, which disappeared during the recovery period. Water consumption was not affected in females; in males it was decreased in week 1 at 200 and 400 mg/kg but in week 2 there was a dose-related increase in water consumption, still present in the two recovery weeks (although only significant at 400 mg/kg during week 1).

Haematology revealed a slight increase in RBC counts at the high dose, associated with a tendency towards erythrocyte microcytosis and hypochromasia, and a slightly increased occurrence of normoblasts. At this dose, WBC count was slightly lower, as a consequence of decreased lymphocyte counts. These findings were all reversible.

The following blood chemistry changes were reported:

- total plasma protein levels were unchanged at the high dose but minor fluctuations in fractions resulted in higher A/G ratios.
- AST and ALT were both increased at the high dose, ALT was slightly increased at 100 mg/kg and, in males only, at 25 mg/kg.

At the end of the recovery period, ALT was still slightly increased in treated males; otherwise, all other parameters were equivalent to controls.

In males there were dose-related decreases in body, thymus and spleen weights, with reduced brain, liver, adrenal and testes weights at the high dose only. In females, there were dose-related decreases in body, brain, liver, thymus, ovary and spleen weights, and a moderate decrease in adrenal weights at the high

dose. In recovery animals there were few statistically significant differences although at the high dose, absolute weights of adrenals (both sexes), thymus (males) and ovaries tended to be still lighter than control organ weights. The lack of data on relative organ weight changes with loss of body weight in rapidly-growing animals makes it difficult to determine whether any of these changes arise from specific target-organ effects or are more a reflection of gross bodywt loss due to more general toxic effects.

Macroscopic examination did not reveal any noteworthy findings. Microscopic pathology reported:

- slight/moderate fatty atrophy of bone marrow in 6 animals/sex at 400 mg/kg;
- decrease of extramedullary haemopoietic activity in the liver and spleen of high-dose rats and in the liver of 100 mg/kg rats;
- moderate necrosis of the thymic cortex in 1/10 high-dose females;
- lack of ovulation (mature tertiary follicles but no corpora lutea) in 1/10, 3/10, and 10/10 rats at 25, 100 and 400 mg/kg respectively.

At the end of the recovery period, all these effects were reversible, with signs of increased extramedullary haematopoiesis at the high dose (physiological rebound).

Histopathological examination of the two animals which died only revealed observations commonly found in animals found dead (congestion and autolysis of organs).

In conclusion, in immature Tif:RAIf(SPF) rats, atrazine at 400 mg/kg for 14 days produced substantial retardation in body and organ weight gain (spleen, thymus and, additionally, in females, brain, liver and ovary). Within two weeks, weights had recovered to almost normal. The lack of historical data for young, rapidly-maturing rats makes the observed changes in blood chemistry, haematology and histopathology more difficult to interpret than in adults but the fact that the effects were apparently reversible or almost completely so over the compound-free two-week recovery period indicates that atrazine delayed maturation but did not produce long-lasting effects, and no evidence of permanent effects on the immune system, haematopoietic tissues or endocrine organs. This non-standard study did not demonstrate a NOEL, although effects are considered minimal at 25 mg/kg bw/d [non-significant, very marginal lower bodywt than controls; ALT increased about 24% cf. controls; slightly reduced absolute organ wts (spleen and thymus in males, liver, thymus and ovary in females); no ovulation in 1/10 females].

4.1.3 21-day rabbit dermal study

Huber KR, Batastini G & Arthur AT (1989) Atrazine Technical: 21-Day Dermal Toxicity Study in Rabbits. Ciba-Geigy Corp., Summit, NJ. Report date 1 Dec. 1989. Toxicol/Pathol report 89044 (GLP; US EPA, OECD, Japan)

This study was conducted according to US EPA Guideline 82-2, OECD Guideline no. 410, and the Japanese MAFF. Atrazine technical (FL841820; 97.6% purity) was moistened with sterile water and applied daily to 120 to 240 cm^2 (approx. 5-10% of the total body surface) of the clipped skin (back and flank) of NZ White rabbits supplied by H.A.R.E. Inc., Hewitt, NJ; a gauze dressing was applied over the test site and secured with adhesive wrapping. An Elizabeth collar was applied to the animals and the atrazine allowed to stay in contact with the skin for approximately 6 h daily. Animals were 13-14 weeks old (2.34-3.06 kg) at study commencement. Dosing, which commenced on 29 March 1988 and was completed on 7 April 1988, was for a minimum of 25 days, at doses of 0, 10, 100 and 1000 mg/kg/d (5 animals/sex/group). Body weights, food consumption, clinical signs, physical, auditory and ophthalmoscopic examinations, dermal examinations, haematology, and clinical chemistry were recorded. At necropsy, 16 tissues were weighed and all animals underwent a very comprehensive histopathological assessment.

There were no deaths. Mucoid and/or few faeces were seen in 4/5 males and 5/5 females at the high dose. The sporadic nature of these findings (between days 4-21) was possibly secondary to reduced food consumption. Otherwise, there were no other clinical signs or findings arising from physical, auditory or ophthalmoscopic examinations.

There were no dermal findings in males. In females, there was slight erythema and scaling in one high-dose animal.

Body weight loss was seen in all high-dose animals on day 7, while a loss or only a slight increase occurred during the remainder of the study. The bodywt reductions were associated with a marked decrease in mean food consumption. One mid-dose female showed weight loss from day 7 for the reminder of the study and a transient slight decrease in mean percent bodywt gain was seen in mid-dose females on days 7 and 14, although food consumption was not affected.

Slight decreases in mean erythroid parameters (RBCs, Hb, Hct) were seen in high-dose animals, with a slight reduction in leucocytes in high-dose males. Concomitantly in these males there was a trend towards an increase in mean percent reticulocytes, but this was largely attributable to findings in 2/5 males. Statistically-significant clinical chemistry findings included decreased total serum proteins at the high dose, possibly due to reduced food consumption, a decrease in serum chloride (high-dose males), and increases in cholesterol (mid- and high-dose females) and triglycerides (high-dose females).

There were some statistically-significant changes in organ weights which were unlikely to be related to the reduced bodywts viz.

- an increase in mean absolute and relative (to body and brain weight) weights of spleen at the high dose, associated with decreased erythroid parameters and increased reticulocyte counts.

- increase in relative (to body) liver weight in high-dose females.

No compound-related gross pathological lesions were observed. Minimal or moderate acanthosis and focal subacute lymphocytic inflammation of the treated skin was noted in 3 high-dose females, whilst another had moderate hyperkertatosis.

In this rabbit dermal study, the NOEL was determined to be 10 mg/kg bw/d (females), based on small effects on bodywt gain and a possible increase in plasma cholesterol at the next highest dose of 100 mg/kg. The NOEL in males was 100 mg/kg bw/d, based on bodywt and food consumption effects, a small decrease in mean erythroid parameters and WBC counts, a small decrease in mean total serum protein, a decrease in mean serum chloride, and an increase in spleen weight (absolute and relative), all observed at the next highest dose of 1000 mg/kg.

4.1.4 14-Day female rat gavage - toxicity & hormone level study

Morseth SL (1990) 14-Day Repeated Dose Oral Toxicity/Hormone Study in Female Albino Rats with Atrazine and Diaminochlorotriazine. Ciba-Geigy, Greensboro, NC. Hazleton Labs, VA. HLA study No. 483-268 Study completion date 6 Mar. 1990 (no GLP statement)

Although this report was not accompanied by a protocol or GLP/QA statements, it appeared to be well conducted and reported. Atrazine (lot no. FL850612; 97.0% pure) or the atrazine metabolite, **diaminochlorotriazine** (DACT) (lot no. FL871776; 97.48% pure) was administered by oral gavage (corn starch and water vehicle) to female Sprague Dawley Crl:CD BR rats (15/group; approx.imately 12 weeks old, from Charles River Labs, Raleigh, NC) at doses of 0, 100, 200 or 400 mg/kg/d (reduced to 300 mg/kg/d on day 4) for a minimum of 2 weeks. Animals were monitored daily for clinical symptoms and bodyweight changes. After 2 weeks, animals in dioestrus were sacrificed; animals not in dioestrus were maintained on treatment until they entered dioestrus, and were then sacrificed. Serum prolactin, leutinizing hormone, follicle stimulating hormone, progesterone and oestrogen were measured in blood collected at sacrifice. The pituitary, uterus, ovaries, spleen and thymus were weighed.

One animal died during the first few days of treatment at doses of 400 mg/kg atrazine, and DACT. One animal in the high-dose atrazine group and 7 animals in the high-dose DACT group died after the doses had been reduced to 300 mg/kg/d. One animal also died at 200 mg/kg DACT.

Animals appeared thin and hunched at the highest doses, body weight gain was reduced, and absolute and relative thymus weights were reduced, in all atrazine and DACT-treated animals.

Absolute spleen weight was decreased in the 300 mg/kg DACT group, and relative spleen weights were significantly increased in the 100 and 200 mg/kg DACT treated groups.

Prolactin levels were significantly elevated and LH levels were slightly elevated in untreated animals after a single intraperitoneal injection of metoclopramide, used as a positive control to test the responsiveness of prolactin.

In atrazine treated rats, mean oestrogen levels, albeit variable, were lower at 200 mg/kg and above, LH was significantly lower at 300 mg/kg, and other hormones were normal. In DACT treated rats, oestrogen and progesterone levels were lower at doses of 200 mg/kg and above, LH was decreased in all treated groups, and prolactin was decreased at 100 and 200, but not 300 mg/kg (see Table).

Mean hormone levels in female SD rats treated with atrazine or DACT

Group (mg/kg)	Oestrogen (pg/mL)	Progesterone (ng/mL)	Prolactin (ng/mL)	LH (pg/mL)
Control	12.2 ± 9.3	9.7 ± 7.8	7.0 ± 6.2	579 ± 380
Control (PRL)*	14.7 ± 12.1	10.8 ± 3.7	559 ± 232	350 ± 232
Atrazine (100)	21.7 ± 15.6	9.4 ± 7.4	3.4 ± 1.4	396 ± 191
Atrazine (200)	3.3 ± 1.7	14.3 ± 8.1	3.7 ± 1.2	652 ± 457
Atrazine (300)	6.6 ± 3.5	8.8 ± 6.1	3.7 ± 1.7	223 ± 101
DACT (100)	11.4 ± 9.9	17.0 ± 14.3	2.8 ± 0.6	285 ± 01
DACT (200)	3.9± 1.6	2.9 ± 1.3	3.1 ± 0.8	235 ± 156
DACT (300)	3.7 ± 2.0	2.2 ± 0.7	7.6 ± 6.6	229 ± 110

* Control group intraperitoneally injected with metoclopramide 20 min before sacrifice to test prolactin (PRL) response. LH = luteinising hormone

Comment: The study demonstrated that in female SD rats, the herbicide atrazine perturbed estrogen, but not prolactin or FSH, levels at doses which were clearly toxic (200 mg/kg bw/d and above). LH was significantly lower at the high dose of 300 mg/kg/d. It was concluded that such effects could occur at lower doses in chronic studies, thus influencing mammary tumour development in a strain of rats already predisposed to developing them.

4.2 Desethylatrazine (G 30033)

No short-term repeat-dose studies submitted.

4.3 Desisopropylatrazine (G 28279)

No short-term repeat-dose studies submitted.

4.4 Diaminochlorotriazine (G 28273)

4.4.1 14-Day female rat gavage - toxicity & hormone level study

See Section 4.1.1 for assessment of a '14-Day Repeated Dose Oral Toxicity/Hormone Study in Female Albino Rats with Atrazine and Diaminochlorotriazine'.

4.4.2 Pilot 4-week rat dietary study

Thompson SS, Batastini GG & Arthur AT (1989) Diaminochlorotriazine (G 28273): Pilot 4-Week Oral Feeding Toxicity Study in Rats. Ciba-Geigy Corp., Summit, NJ. Lab. Study no. 872283. Toxicol/Pathol Report no.89074. Completion date 2 Oct. 1989

This study was conducted according to USA EPA Guideline 82-1. **Diaminochlorotriazine** (Lot no. FL-871243; purity not stated) was administered in the diet to Sprague Dawley rats from Charles River Labs, Kingston, NY (10/sex/group) at concentrations of 0, 10, 500, 1000 and 2000 ppm for at least 29 days. Tests revealed acceptable test compound stability and homogeneity and concentration in the diet (within $\pm$ 10%). Calculated mean daily compound intakes were 0, 0.89, 45.1, 85.5 and 153 mg/kg bw (males) and 0, 0.92, 48.7, 92.4 and 157.9 mg/kg bw (females). At study commencement, rats were approximately 6-weeks old. Dosing occurred over the period 31 July 1987 to 1 Sept. 1987. Observations of clinical condition and mortality were performed at least twice daily, physical/auditory examinations on days -16 and 26, bodyweight and food consumption weekly, and clinical laboratory determinations (haematology, clinical chemistry and urinalysis) at study termination. Haematology parameters included Hb, Hct, RBCs, WBCs and differentials, reticulocytes, Heinz bodies, platelets and prothrombin time. Clinical chemistry and urinalysis parameters measured were as in Appendix III. Organs weighed were thymus, heart and spleen. Organs were not examined histopathologically but gross examination paid particular attention to heart because this was a possible target organ of DACT in dogs (see Section 4.4.2).

There were 3 deaths after blood sampling on days 28 or 29, one control female and two high-dose animals (one/sex). No clinical signs related to treatment were noted, apart from few faeces during the first week of the study. Dose-related reductions in mean bodyweight and percent bodywt gain occurred at $\geq$ 500 ppm in males. Group mean female bodywts at 2000 ppm and percent gain in females at $\geq$ 1000 ppm were significantly decreased throughout the study. At term, the mean absolute bodywt of females fed 500 ppm was reduced; in males and females at 500 ppm, the mean bodywt gain was decreased 23% and 17% in males and females, respectively. Bodywt effects were considered secondary to reduced food consumption.

Haematology examination noted increases in mean platelet counts in males at $\geq$ 500 ppm and 2000 ppm females (dose-related) and the mean percentage of neutrophils was elevated in 2000 ppm males. Some decreases in red cell parameters were considered secondary to reduced food consumption. Some blood biochemical changes were considered incidental because they were

random, not dose-related, not supported by changes in related parameters, and/or within historical control values; decreases in mean total protein (high-dose females) were likely to be secondary to reduced food consumption. Urinalysis did not reveal any treatment-related changes.

Organ weight analysis indicated changes were related to the bodywt decreases, and thus did not reveal any noteworthy findings. There were no treatment-related gross pathological changes noted at autopsy.

The results show that, based on reductions in mean bodywt gain, concentrations of the atrazine metabolite, DACT, of $\leq$ 500 ppm in the diet of rats would be an adequate maximum feeding level. The NOEL in this study was 10 ppm (measured as 0.89-0.92 mg/kg bw/d), based on reductions in bodywt/bodywt gain, reduced food consumption, and changes in haematological parameters at the next highest dose of 500 ppm (45.1-48.7 mg/kg).

4.4.2 4-Week dog dietary study

Swallow JJ, Hazelette JR & Arthur AT (1989) Diaminochlorotriazine (G 28273): Pilot 4-Week Oral Toxicity Study in Dogs. Ciba-Geigy Corp., Summit, NJ. Lab. Study no. 872148. Toxicol/Pathol Report no. 89074. Completion date 10 May 1989

This pilot study was conducted to USA EPA Guideline 81-1. **Diaminochlorotriazine** (Lot no. FL-871040; purity not stated) was administered in the diet to beagle dogs from Marshall Farms, North Rose, NY (1/sex/group) at concentrations of 0, 500, 1000, 1500 and 2500 ppm for 29 days. Calculated mean daily compound intakes were 0, 14.1, 21.0, 39.5 and 36.8 mg/kg (males) and 0, 13.9, 27.2, 47.4 and 66.1 mg/kg (females); the lower intake in the high-dose male was due to reduced food intake. At study commencement, dogs were approx.12 to 18-months old. Dosing occurred over the period 15 April 1987 to 14 May 1987. Observations of clinical condition and mortality were performed at least twice daily, physical examinations on days -7 and 28, bodyweight and food consumption on days -8, 1, 8, 15, 22 and 29, clinical laboratory determinations (haematology and clinical chemistry) on days -10 and 28, and electrocardiography on days -14 and 27 (with cardiac rhythm evaluated on days 28 and 29 in the high-dose male). Haematology parameters included Hb, Hct, RBCs, WBCs and differentials. Clinical chemistry parameters measured were urea, AST, ALT, creatinine, CPK, LDH, sodium, potassium, chloride, calcium and inorganic phosphorus. Apart from the heart, organs were not examined histopathologically but only underwent gross examination.

Slight to severe treatment-related effects occurred at concentrations $\geq$ 1000 ppm and included:

- mild faecal changes (soft/mucoid/few faeces);
- dose-related reductions in bodywt, percent bodywt gain, and food consumption;

- periods of sino-atrial arrest in the male at 2500 ppm;
- gross and microscopic haemorrhagic focal lesions in the right atria at ≥ 1500 ppm.

The ECG changes (atrial mediated arrhythmia in the high-dose male) and cardiac pathological changes were considered to be primary treatment-related findings.

A suitable upper dose for testing was considered to be less than 1500 ppm. The NOEL was considered to be 500 ppm (14.1 mg/kg bw/d in males, 13.9 mg/kg bw/d in females), on the basis of the faecal, bodywt and food consumption at 1000 ppm (21 mg/kg bw/d in males, 27.2 mg/kg bw/d in females) and above. Cardiac effects were seen at ≥ 1500 ppm.

4.5 Atrazine Formulations

'Marksman Herbicide' (Sandoz)

Note: This liquid herbicide formulation contains 250 g/L atrazine and 130 g/L dicamba (see **Attachment 3-3** for formulation details).

4.5.1 21-Day rabbit dermal study

Morrow, LD (1986) Twenty-one Day Repeat-dose Dermal Toxicity Study in Rabbits using Marksman Herbicide Sandoz. Lab: American Biogenics, 1800 East Pershing Rd, Decatur, IL, USA. Study no. 410-2557 (GLP)

New Zealand White rabbits (5/sex/group) received 'Marksman Herbicide' by topical application to the skin under occlusive bandages for 6 h/day on 15 or 16 occasions over a 21-day period. Doses used were 0, 40, 200 or 1000 mg/kg/day. All animals were observed twice daily for clinical signs. Prior to treatment and at 24 hours after each treatment, the application sites of animals were evaluated for erythema and oedema according to the Draize scale. Body weights and feed consumption were measured weekly. Haematological and clinical chemistry analyses were performed on all rabbits just prior to sacrifice. Gross pathological examinations were performed on all animals, with the kidneys, liver, lungs, lymph nodes, skin and any abnormal tissues examined histologically.

No deaths or treatment-related clinical signs were observed. Body weight loss was seen in the high dose group during week one, but weight gain parallelled that of controls subsequently. Final body weight gain was reduced. No effects were noted on food consumption. Dermal reactions were confined to the two upper treatment groups. Erythema was seen at most intervals during weeks 2 and 3 in most high-dose and some mid-dose animals. The incidence of severe erythema was higher in the upper dose level (8/10 compared to 3/10 for the mid-dose). Slight oedema was noted in all high-dose animals during weeks 2 and 3. Desquamation was seen in 1 low-dose female, 8 mid-dose animals and all 10 high-dose animals during weeks 2 and 3. Fissuring was sporadically observed in high-dose rabbits during weeks 2 and 3. Clinical chemistry results

revealed an increase in glucose concentrations for both sexes at the high dose, apparently related to the integumentary lesions, which are known to cause such an effect. Other blood chemistry parameters were unaffected.

No significant treatment-related gross pathological findings were detected apart from those of skin. At the high dose, mild hyperkeratosis and acanthosis was seen. No other significant histological lesions were found. It is concluded that Marksman Herbicide causes slight to moderate dermal irritation, but no systemic toxicity when repeat dermal doses are given at up to 1000 mg/kg bw/d.

5. SUBCHRONIC STUDIES

5.1 Atrazine (G 30027)

5.1.1 90-Day rat dietary study

Tisdel M & Harrison DL (1977a) 90-Day Subacute Feeding Study of Atranex in Rats. Agan Chemical Manufacturers Ltd, Ashdod, Israel. Lab: Warf Institute Inc., Madison, Wisconsin. Study code T-636. Report date 12 July 1977 (pre-GLP study)

Weanling Sprague-Dawley rats from ARS/Sprague-Dawley Inc, Madison, Wisconsin (20/sex/group) were fed technical-grade atrazine (Agan, batch coded 7003; purity unstated) in the diet at 0, 200, 1000 and 5000 ppm, from weaning for 90 days. Initiation of the study was 27 Dec. 1976, with sacrifice in late March 1977. The following were recorded/measured: clinical signs (twice daily); body weights; before dosing commenced and thence weekly; food consumption (weekly); haematology and blood chemistry (5/sex/group at 30 and 90 days); gross postmortem examination (all animals which survived to term) and microscopic pathology (all high-dose animals and 10/sex from the mid-dose and control groups). Haematology parameters measured were those listed in Appendix III (but not including clotting parameters, platelet count or reticulocyte count, or blood smears), clinical chemistry included ALT, AST and AP, and urinalyses included ketones, bilirubin, glucose, protein, occult blood, specific gravity and pH. Histopathology was performed on the following organs: bone marrow, lymph nodes (including mesenteric), stomach, caecum, gonads, adrenals, thyroids, pituitary, spleen, pancreas, small intestine (3 levels), large intestine, prostate, uterus, kidneys, lungs, brain, skeletal muscle, liver, bladder, seminal vesicle, salivary glands, and heart.

Increased mortality and obvious signs of toxicity (emaciation, poor muscle tone, 'unthrifty' appearance) were observed in the 5000 ppm groups, as soon as 4 days after treatment. By six weeks on test, 13/21 males and 9/19 females in this group had died (with no further deaths until term) .

A dose-related reduction in food consumption was apparent in all groups, although this effect was quite small at the low-dose and became less apparent

as the study progressed. There were marked reductions in body weight parameters in the 1000 and 5000 ppm groups; in fact, at the high dose there was slight loss of bodywt over the first week, in both sexes. At term, bodyweights were 12% and 47% (males) and 12% and 36% lower than controls, in the mid-dose and high-dose groups respectively. Bodyweights were only very marginally depressed (2-2.5%) an the low dose.

Atrazine ingestion produced only minor changes in haematological parameters, blood chemistry values and urinalysis data. Statistically significant changes from control were observed for erythrocyte count (decrease), increases in neutrophil count and in alkaline phosphatase. However the values were within normal ranges and were confined to the high dose group. They probably reflect the poor health, stress, and slower growth rate within this group.

Organ weight changes in the 1000 and/or 5000 ppm groups for heart, liver, spleen, and kidneys reflect the lower growth rate in these groups since organ/body weight ratios were generally little changed. However, for gonads from high-dose animals, there was a mean 75% reduction in absolute wt, and a 47% reduction in relative weight; at the mid-dose there was no effect of atrazine on testes weights.

Gross pathological changes were confined to calcium-like deposits in the renal pelvis and small testes. These were found at a greater incidence than controls in all treatment groups:-

Observation*	Sex	Dose Group Control	200 ppm	1000 ppm	5000 ppm
calcium-like depositin renal pelvis	M	1/20 (5%)	4/20 (20%)	6/20 (30%)	2/8 (25%)
	F	1/20 (5%)	0/20 (0%)	0/20 (0%)	3/10 (30%)
small testes	M	0/20	0/20	0/20	7/8 (87.5%)

* Note: Gross pathology data only for animals surviving to term.

Microscopic pathology examinations were confined to the control, 1000 and 5000 ppm groups. Microscopic changes of significance were atrophic degenerate testes with no spermatogenesis, squamous metaplasia and/or hyperplasia of the renal epithelium, calcium deposits in the kidneys, extramedullary haematopoiesis in the spleen, squamous metaplasia of the urinary bladder epithelium, and erythroid hyperplasia in bone marrow. The incidences of such findings were as follows:-

Observation	Sex	Dose Group Control	1000 ppm	5000 ppm
Testes - no spermatogenesis	M	0/10 (0%)	0/10 (0%)	8/8 (100%)
Kidney - cell change	M	0/10 (0%)	0/10 (0%)	5/8 (62.5%)
	F	0/10 (0%)	0/10 (0%)	3/10 (30%)
Kidney - calcium deposit	M	0/10 (0%)	1/10 (10%)	5/8 (62.5%)
	F	0/10 (0%)	0/10 (0%)	4/10 (40%)
Spleen - haematopoiesis	M	0/10 (0%)	2/10 (20%)	4/8 (50%)
	F	0/10 (0%)	0/10 (0%)	7/10 (70%)
Bladder - cell change	M	0/10 (0%)	0/10 (0%)	6/8 (75%)
	F	0/10 (0%)	0/10 (0%)	0/10 (0%)
Bone marrow - hyperplasia	M	0/10 (0%)	0/10 (0%)	0/8 (0%)
	F	0/10 (0%)	0/10 (0%)	3/10 (30%)

Data for terminally-sacrificed animals (half the control and mid-dose animals examined). In the animals which died during the study, atrophic degenerate testes with no spermatogenesis were seen in 13/13.
Disregarding the very small effect of atrazine on bodywt gain during week 1, the NOEL in this study may be taken as 200 ppm (estimated as 20 mg/kg bw/d), on the basis of reduced food intake, body weights, calcium deposition in the renal pelvis and splenic haematopoiesis at the next higher dose of 1000 ppm (100 mg/kg).

Comment: Of particular note, given the concern about the possible endocrine disruptor effects of atrazine, was the finding of atrophic degenerate testes with no spermatogenesis at the high dose of 5000 ppm.

5.1.2 3-Month rat dietary study

Amalgamated Chemicals (1984) Three Months Subacute Toxicity (Feeding) Study in Rat. Report No. Dr Dickh/P/Re 2-4-58-84 Pharmatox GmbH, West Germany

Groups of Wistar rats (30/sex/group) received dietary atrazine technical (0, 20, 100 and 1000 ppm) for 90 days, and 10/sex received standard diet for an extra 30 days post-treatment. Clinical signs, weight, food consumption, feed efficacy, haematology, clinical chemistry and urinalysis were performed (days 50, 90, 120). Organ weights and macroscopic and microscopic pathology were recorded at termination.

Weight gains and food consumption were lower in males and females at 1000 ppm only, with slight reversal during the recovery period. All other parameters were normal. The NOEL was 100 ppm (estimated as 5 mg/kg bw/d).

5.1.3 3-Month rat dietary study

Bachmann M (1994) G 30027 Tech. (Atrazine): Three-Month Oral Toxicity Study in Rats (Administration in Food). Ciba-Geigy Ltd, Switzerland. Test no. 931063. Completion date 5 Aug. 1994 (GLP; OECD, USA, Japan, Switzerland)

This study was conducted according to OECD Guideline no. 408, USA EPA Guideline 82-, and the Japanese MAFF. It was conducted to determine NOELs for exposure to atrazine and reversibility of expected adverse effects. Atrazine (Batch FL-901747; 96.7% purity) was administered in the diet to Sprague Dawley-derived rats (Tif: RAIf (SPF) hybrids of RII/1 x RII/2) from Ciba-Geigy, Stein, Switzerland (10/sex/group) at concentrations of 0, 10, 50 and 500 ppm for 13 weeks; an additional 10/sex/group in the control and high-dose groups were kept for a one-month recovery period. Tests revealed acceptable test compound stability and homogeneity in the diet, with mean concentrations being between 93-100% of target. Calculated mean daily doses (corrected for analysed concentrations of compound in the diet) were 0, 0.60, 3.3 and 34.0 mg/kg bw (males) and 0, 0.66, 3.35, and 35.3 mg/kg (females). At study commencement, rats were approximately 5-6 weeks old (110-132 g bodywt at week -1). Dosing occurred over the period 14 July 1993 to 14-15 Oct. 1993. Observations of clinical condition and mortality were performed daily, ophthalmoscopy at the start and end of the study, bodyweight and food and water consumption weekly, and clinical laboratory determinations (haematology, clinical chemistry and urinalysis) at the end of the treatment period and at the end of the recovery period. Haematology parameters were those listed in Appendix III plus methaemoglobin, red cell volume and Hb concentration distribution width. Appendix III lists clinical chemistry parameters (but not including LDH or CPK)and urinalysis parameters measured. Organs weighed included body (exsanguinated), brain, liver, kidneys, adrenals and gonads. Organs examined histopathologically included: spleen, lymph node (mesenteric and axillary); trachea; lung; heart; aorta;

salivary glands (submandibular); liver; pancreas; oesophagus; stomach; intestine (small and large); kidneys; bladder; gonads; uterus; vagina; prostate; epididymes; pituitary; adrenals; thyroid/parathyroid; thymus; peripheral nerve; brain; femur with joint; spinal cord (3 levels); eye with optic nerve; mammary gland; and any organ with gross lesions.

Apart from one control female, there were no deaths and no clinical signs which were related to treatment; clonic convulsions occurred on two occasions in a high-dose female but this has been observed in untreated animals. A reduction in bodyweight gain occurred in high-dose animals such that mean male bodywts were about 15% (males) and 9% (females) below controls at the end of the treatment period. Males at the mid dose (50 ppm) were 8% lighter than controls at the end of treatment. A partial recovery of high-dose animals was noted, with mean bodywts 7% (males) and 5% (females) lower than controls at the end of the compound-free period. There was a corresponding 10% (males) and 5% (females) decrease in mean food consumption during weeks 1-13 at the high dose; during recovery, male food intake was comparable with controls whereas females consumed 14% more.

From week 5 on, high-dose females consumed consistently more water than controls; over the dosing period, overall water intake was up 16%. In high-dose males, water intake increased by up to 53% at the beginning of the recovery period but by the end of the study, normal water intakes were noted.

Ophthalmoscopic examinations did not reveal any treatment-related effects. Haematology, clinical biochemistry and urinalyses did not reveal any treatment-related changes.

Apart from a small (approx. 10%), reversible increase in relative liver weight (cf. bodywt) in high-dose females at the end of the dosing period, organ weight analysis did not revealed any findings which could not be related to the bodywt effects at the mid-and high doses.

There were no treatment-related gross pathological changes noted at autopsy. Microscopic examination revealed that the incidence and severity of haemosiderin deposition in the spleen was increased in high-dose animals sacrificed at the end of treatment; in males the incidence of minimal haemosiderosis was 0/10, 0/10, 1/10 and 3/10 in the control to high-dose groups, with possible recovery (3/10 in high-dose recovery males cf. 3/10 controls). In females, the incidence in the respective groups at the end of dosing was 4/10, 6/10, 6/10 and 8/10, with the animals affected at the control, low and mid doses reported as having minimal haemosiderosis but high-dose animals having moderate severity. At the end of the recovery period in females, the incidence was 4/10 controls vs. 8/10 at the high-dose (of which 8, 6 were reported to have only minimal haemosiderosis).

The NOEL for atrazine in this study was 10 ppm (0.60 mg/kg bw/d) for males, 50 ppm (3.35 mg/kg bw/d for females), on the basis of depressed bodywt gain at the next highest dose of 50 ppm (3.3 mg/kg) in males, and depressed bodywt

gain and food intake, and splenic haemosiderin deposition, at the next highest dose of 500 ppm (35.3 mg/kg) in females.

5.1.4 90-Day dog dietary study

Tisdel M & Harrison DL (1977b) 90-Day Subacute Feeding Study of Atranex in Dogs. Agan Chemical Manufacturers, Ashdod, Israel. Lab: Warf Institute Inc., Madison, Wisconsin. Study code T635. Report date 2 Aug. 1977 (pre-GLP study)

Purebred beagle dogs from Ridgeland Farm Inc., Mt Horeb, Wisconsin (4/sex/group) were fed 0, 200, 632 or 2000 ppm technical atrazine (Agan, batch coded 7003; purity unstated) in the diet for 90 days. No information was provided about stability, homogeneity or actual measured concentration of atrazine in the food admixture. The study was initiated on 12 Jan. 1977 and sacrifice of animals took place during the week of 18 April 1977. Body weights at commencement of the study ranged from approx. 6.8 to 8.1 kg (males) and 5.5 to 7.0 kg (females). Animals were observed daily for clinical signs, food consumption was measured daily (reported as weekly totals), bodweights were measured weekly, and haematology, blood chemistry and urinalysis were assessed at 0, 4, 8 and 13 weeks.

Haematology parameters measured were those listed in Appendix III (but not including clotting parameters, platelet count or reticulocyte count, or blood smears), clinical chemistry included those listed in Appendix III (but not including cholesterol, creatinine, GGT, LDH or CPK), and urinalyses included ketones, bilirubin, glucose, protein, occult blood, specific gravity and pH. Histopathology was performed on the following organs from all animals: bone, bone marrow, lymph nodes, salivary glands, stomach, caecum, gonads, adrenals, thyroids, pituitary, spleen, pancreas, trachea, jejunum, ileum, prostate, uterus, kidneys, urinary bladder, lungs, brain, spinal cord, liver, gall bladder, and heart, as well as any neoplasms. Heart, liver, spleen, kidneys, gonads and adrenals were weighed.

Clinical signs and behaviour, blood chemistry and electrolytes, urinalysis and organ/body weight ratios were unaffected by atrazine treatment.

Body weight gains were depressed at the high dose in both sexes. There was no effect on bodyweight in the mid- and low-dose females but in males there was a dose-related effect on mean bodyweights; after 13-weeks dosing, mean bodywts in the control to high-dose male groups respectively were 28.7%, 12.3%, 6.4% and -4.8% different from controls ie. at the high-dose, there was a mean bodywt loss. Looking at individual bodywts, all control males gained weight but in each of the dosed groups, 2/4 animals lost weight. Thus it would appear that even the low dose of atrazine (200 ppm) had an effect on bodywt gain.

Food consumption was depressed at the high-dose in both sexes throughout the study. At the mid-dose, food intake was depressed in both sexes on a number

of measured intervals whilst at the low dose, food intake was depressed in males at a number of intervals (of the order of 10 to 16%).

Anaemia (decreases in mean erythrocyte count, haematocrit and haemoglobin) was seen in mid- and high-dose animals. In low-dose males there was a marginal reduction in these parameters, albeit not statistically significant. There was a tendency toward elevated WBC counts at the high dose; it is possible this may be a response to small ulcerative areas on the skin of the head and/or neck region, although this finding was reported to be seen in both control and dosed animals.

Testicular weights were reduced in the high dose group (approx. 30% cf. controls); even when organ/body weight ratios were considered, there was a slight reduction in the testes/bodywt ratio, when this ratio should increase with bodywt loss if there is no specific toxicological effect on the testes.

It was reported that histological signs indicated a mild to total arrest of spermatogenesis in all (4/4) males of the 632 and 2000 ppm groups, and that some of the testes had abnormal cells or degenerate tubules. In low-dose males there was active spermatogenesis although 1/4 had abnormal cells in the seminiferous tubules. One control had one very small undescended testis with arrested spermatogenesis.

Correspondence concerning testicular findings were appended to the report, detailing re-investigation of the slides by another pathologist (JL Carter of Hazleton Labs America Inc, Madison, Wisconsin; reported on 30 Sept 1986). It was concluded that "no compound-related changes were observed in the testes examined histopathologically", a conclusion in opposition to the T-635 final report and consistent with findings in other Ciba-Geigy studies. Thus, there was minimal giant cell formation in all dose groups (a normal occurrence in beagles), no evidence of a dose-related increase in degeneration/atrophy of seminiferous tubules, and no evidence of arrested spermatogenesis. Clearly the two conclusions based on the same histopathology slides are contradictory and there is insufficient further information or comment to establish which assessment is correct. However, it must be borne in mind that there was no indications of testicular toxicity in a chronic study with atrazine in dogs (see Section 6.1.18).

On the basis of effects on bodywt and food intake seen at the lowest dose used in this 90-day dog study (200 ppm), a NOEL was not established. Discounting the food intake and bodywt effects at the low dose, a NOEL of 200 ppm (estimated to be about 5 mg/kg bw/d) could be set, based on reduced testicular weights (other signs of testicular toxicity are in question in this study) and anaemia at the next higher dose tested of 632 ppm.

5.2 Desethylatrazine (G 30033)

5.2.1 13-Week rat dietary study

Drake JC (1971a) G 30033 (Desethylatrazine): Thirteen-Week Dietary Toxicity Study in Rats. Ciba-Geigy Ltd, Switzerland. Lab: Geigy Pharmaceuticals, Wilmslow, UK. Study no. 2/71/S.L. Date 2 Feb. 1971

Desethylatrazine (ref. G30 033. P. 2. Krist.; 97.5% purity) was administered in the diet to Sprague-Dawley derived rats (20/sex/group) at concentrations of 0, 500 and 1000 ppm for 13 weeks. At study commencement they were approx. 6 weeks old (154-186 g bodywt). Tests on the diet for acceptable test compound stability, homogeneity and concentration were not performed. On the basis of measured food intake, the average daily intake of test material was 0, 32 and 66.8 mg/kg bw/d (males) and 0, 35.5 and 69.8 mg/kg bw/d (females). Observations of clinical condition and mortality were performed daily, ophthalmoscopy at the end of weeks 4, 8 and 13, bodyweight and food and water consumption weekly, and clinical laboratory determinations (haematology, clinical chemistry and urinalysis) during weeks 5, 9 and 13. Haematology parameters measured were those listed in Appendix III plus erythrocyte sedimentation rate and methaemoglobin but not including MCHC, MCV or MCH. Clinical chemistry parameters measured were Na, K, Cl, glucose, urea, ALT, AST, AP, serum proteins (total and electrophoretic) and cholinesterase. Urinalysis parameters were those listed in Appendix III (not including urobilinogen). Organs weighed included heart, lungs, adrenal, brain, thymus, thyroids, testes, pituitary, liver, spleen, ovaries, kidney and uterus/prostate. Organs examined histopathologically included the above plus lymph node (mesenteric); rib junction, bone marrow; trachea; aorta; salivary gland; pancreas; stomach; colon; eye; mammary gland; muscle; oesophagus; gall bladder; skin; tongue; intestine (small); bladder; peripheral nerve; and any organ with gross lesions.

There were no compound-related deaths and no clinical signs reported. A reduction in bodyweight gain occurred at both doses such that throughout treatment, animals were up to 17% (low dose) and 23% (high dose) lighter than controls, associated with decreases in mean food consumption.

Ophthalmoscopic examinations did not reveal any treatment-related effects. Haematology data indicated mild anaemia at the high dose. Clinical biochemistry results and urinalyses did not reveal any obvious treatment-related changes. Organ weight changes reflected the reduced bodywt of dosed animals.

There were no treatment-related gross or microscopic pathological changes noted at autopsy.

The results show that the atrazine metabolite, desethylatrazine resulted in limited adverse effects at both doses, viz. reduced bodywt gain, with mild anaemia at 1000 ppm. A NOEL was not established in this study.

5.2.2 3-Month rat dietary study

Gersprach R (1991) G 30033 (Desethylatrazine): Three-Month Oral Toxicity Study in Rats (Administration in Food). Ciba-Geigy Ltd, Switzerland. Study no. 901264. Completion date 22 October 1991 (GLP; OECD, USA, Japan, Switzerland)

This study was conducted according to OECD Guideline no. 408 and USA EPA Guideline 82-1. **Desethylatrazine** (Batch FL-901515; 95.7% purity) was administered in the diet to rats (Tif: RAIf (SPF) hybrids of RII/1 x RII/2) from Ciba-Geigy, Stein, Switzerland (10/sex/group) at concentrations of 0, 10, 50 and 500 ppm for 13 weeks; calculated mean daily doses (corrected for analysed concentrations of compound in the diet) were 0, 0.681, 3.20 and 35.2 mg/kg bw (males) and 0, 0.727, 3.35, and 38.7 mg/kg (females). Tests revealed acceptable test compound stability and homogeneity in the diet, with mean actual concentrations being between 91-97% of target. At study commencement they were approximately 5-6 weeks old (100-140 g bodywt). Dosing occurred over the period 11 Dec. 1990 to 13-14 Mar. 1991. Observations of clinical condition and mortality were performed daily, ophthalmoscopy at the start and end of the study, bodyweight and food and water consumption weekly, and clinical laboratory determinations (haematology, clinical chemistry and urinalysis) at week 14. Haematology parameters were those listed in Appendix III plus investigation of red cell morphometric parameters. Clinical chemistry parameters measured were as in Appendix III (but not including LDH or CPK). Urinalysis parameters were those listed in Appendix III. Organs weighed are listed at Appendix IV (not including thyroid but including thymus). Organs examined histopathologically included: spleen, lymph node (mesenteric, axillary); sternum with marrow; trachea; lung; heart; aorta; salivary glands (submandibular); liver; pancreas; oesophagus; stomach; intestine (small and large); kidneys; bladder; gonads; uterus; vagina; pituitary; adrenals; thyroid/parathyroid;; thymus; peripheral nerve; brain; and any organ with gross lesions.

Doses used were chosen on the basis of a 28-day study conducted by the Research & Consulting Co., Itingen, Switzerland (Project no. 252088) at dietary doses of 50, 500 and 1500 ppm.

There were no deaths and no clinical signs which were related to treatment. A reduction in bodyweight gain occurred at the high dose such that throughout treatment, males were up to 15% and females 12% lighter than controls, associated with corresponding 10% and 6% decreases in mean food consumption.

Ophthalmoscopic examinations did not reveal any treatment-related effects. Haematology data indicated slight reductions in Hct and MCV and higher MCHC in females at the high dose. Clinical biochemistry results noted minimally lower plasma glucose levels in high-dose males, and a minor increase in serum AP in high-dose females. Urinalysis did not reveal any treatment-related changes.

Organ weight analysis revealed an increase in relative (to body wt) weights of liver in high-dose females (12%); otherwise, organ weight changes reflected the reduced bodywt of high-dose animals.

There were no treatment-related gross or microscopic pathological changes noted at autopsy.

The results show that the atrazine metabolite, desethylatrazine resulted in only minor adverse effects, restricted to the highest dose. The NOEL for desethylatrazine in this study was 50 ppm (3.2 mg/kg bw/d in males, 3.35 mg/kg/d in females), based on reduced bodywt and food intake (both sexes), minimal changes in haematology and SAP activity (females), and a small increase in relative liver weight (females) at the next dose of 500 ppm.

5.2.3 13-Week dog dietary study

Rudzki MW, Batastini G & Arthur AT (1992) G-30033 (Desethylatrazine): 13-Week Feeding Study in Dogs. Ciba-Geigy Corporation, Division of Toxicology/Pathology, Summit, NJ. Study no. 902187. Toxicology/Pathology report 91073 (MIN 902187). Study completion date 16 April 1992 (GLP; US EPA)

This study was conducted according to US EPA Guidelines 82-1. **Desethylatrazine** (FL-901515; 95.7% pure) was administered in the diet to purebred beagle dogs from Marshall Farms, NY (4/sex/group), at concentrations of 0, 15, 100 and 1000 ppm for 13 weeks. Concentrations, stability and homogeneity of admixtures were confirmed by analyses to be acceptable; all samples analysed were between 89-110% of the target concentration. Mean daily compound intakes were 0, 0.56, 3.71 and 28.85 mg/kg (males) and 0, 0.51, 3.88 and 32.18 mg/kg (females) in the control - high-dose groups respectively. Observations of clinical condition and mortality were carried out daily; bodyweight and food consumption weekly; physical auditory and ophthalmoscopic examinations were carried out pre-dosing and at week 13; electrocardiographic examinations were conducted pre-dosing and during weeks 4, 8 and 12; haematology, clinical chemistry and urinalysis were all performed pre-dosing and during weeks 5/6 and 12/13. Parameters measured are listed in Appendix III (haematology included examination of RBC morphology and Heinz body counts, but did not include MCV, MCHC or MCH calculations).

Dogs were fasted for 12 hours prior to a full autopsy. Organs weighed included: lung; heart; aorta; salivary glands (mandibular); liver; spleen; kidneys; gonads; prostate; uterus; pituitary; adrenals; thyroid/parathyroid; thymus; brain; epididymes. Organs examined histopathologically included: spleen, lymph node (medial retropharyngeal, axillary); sternum with marrow; trachea; lung; heart; aorta; salivary glands (mandibular); liver; pancreas; femur (with marrow and articular surface); gallbladder; lacrimal glands; oesophagus; stomach; small intestine (duodenum, jejunum, ileum), large intestine (caecum, colon, rectum); kidneys; urinary bladder; eyes (with optic nerve); female

genital organs (ovaries, uterus, vagina); male genital organs (testes, epididymes, prostate); mammary gland; pituitary; adrenals; thyroid/parathyroid; thymus; peripheral (sciatic) nerve; brain (3 levels); spinal cord (three levels); stomach (cardia, fundus, pylorus); thymus (when present); skeletal (thigh) muscle; skin; tongue; and any organ with gross lesions.

There were no deaths and no clinical signs which were related to treatment. A treatment-related effect on bodyweight parameters occurred in 1000 ppm animals. In males an initial small bodywt loss occurred during week one, with maintenance of this weight for 6 weeks then they gained weight at almost the same rate as controls. In high-dose females an initial small mean bodywt loss occurred during week one, with maintenance of this weight, or only a very slight gain, for the remainder of the study. At the end of the study, high-dose males had gained 6.9% of their starting bodywt (cf. controls, 27.2%), and high-dose females 1.7% (cf. controls; 30.9%). Some decreases in food consumption were noted in high-dose animals, associated with concomitant reductions in mean bodywt parameters and possibly partly related to food palatability.

Physical, auditory, and ophthalmoscopic examinations did not reveal any treatment-related effects. One high-dose dog had a paroxysmal atrial fibrillation, evident on day 50 and 92 but not on day 78, possibly related to treatment since it was an arrhythmia not associated with an underlying disease; there were no treatment-related changes in heart weight or in histopathological findings.

Haematology, clinical biochemistry and urinalysis results noted some reductions in RBCs, Hb and Hct, and decreases in mean serum total cholesterol and triglycerides in high-dose animals. Some statistically significant changes in clinical chemistry parameters were reported but none were dose-related, minimal relative to individual baselines, and/or did not persist over time.

Organ weight changes at the high dose were considered to be incidental to the lower bodywts at this dose.

There were no treatment-related gross pathology findings at necropsy. Treatment-related histopathological changes were noted in the kidneys, with mild renal tubular epithelial hyperplasia/basophilia in 3 males and 2 females at the high dose. A single high-dose male had haemorrhagic inflammation with angiomatous hyperplasia in the right atrial wall of the heart. Thymic atrophy and anovulatory uterine atrophy were observed in 3 and 4 high-dose females, respectively, considered to be secondary to reduced food consumption and body wt gain at this dose.

The results suggest that the target organs for the atrazine metabolite, desethylatrazine, in dogs were the heart and kidney. Electrocardiographic findings of fibrillation in one high-dose female and haemorrhagic inflammation in the right atrial wall of the heart of one high-dose male indicated possible cardiac effects, whilst mild renal tubular epithelial hyperplasia/basophilia was reported in 3 males and 2 females at the high dose. On the basis of these

effects at 1000 ppm (28.9-32.2 mg/kg), the NOEL for this study was 100 ppm [3.7 (males) and 3.9 (females) mg/kg bw/d].

5.3 Desisopropylatrazine (G 28279)

5.3.1 90-Day dietary rat study

Smith PS (1996) 90-Day Subacute Oral Toxicity Study with G 28279 (Desisopropylatrazine) in Albino Rats. Ciba-Geigy Ltd, Switzerland. Lab. Industrial Bio-Test Labs Inc., Northbrook, Ill., USA. Study no. IBT B9244. Report date 17 Sept. 1971

Technical **desisopropylatrazine** (ARS No. 1846/70; purity unstated) was administered in the diet to albino rats from Charles River Breeding Labs, North Wilmington, Mass. (20/sex/group) at concentrations of 0, 500 and 1000 ppm for 90 days; there did not appear to be any tests of compound stability or homogeneity in the diet. At study commencement, rats were approximately 136-155 g bodywt. Observations of clinical condition and mortality were performed daily, bodyweight and food consumption weekly, and clinical laboratory determinations (haematology, clinical chemistry and urinalysis) after 45 and 84 days (10/sex from control and high-dose groups only). Haematology parameters measured were Hct, RBC counts, Hb, total leucocyte counts and leucocyte differential count. Clinical chemistry parameters measured were BUN, AP, AST and fasted glucose. Urinalysis parameters examined were glucose, albumin, pH, specific gravity and microscopic examination. Organs weighed were liver, kidneys, spleen, gonads, heart and brain. Organs examined histopathologically included: spleen, lymph node (cervical and mesenteric); bone (femur) and marrow; trachea; lung; heart; aorta; salivary glands; liver; pancreas; oesophagus; stomach; small intestine (duodenum, jejunum and ileum); caecum; colon; kidneys; bladder; gonads; prostate; seminal vesicle; uterus; vagina; pituitary; adrenals; thyroid/parathyroid; thymus; peripheral nerve; eye and optic nerve; brain (3 levels); spinal cord; and any organ with gross lesions.

Apart from one death ascribed to acute respiratory infection (and two due to trauma during blood collection), there were no deaths and no clinical signs which were related to treatment. A reduction in bodyweight gain occurred at the high dose in males; mean male bodywts were about 11.5% lighter than controls at termination; low-dose males were about 6% lighter, although this was not statistically significant; this figure did not take into account the fact that the starting weight of low-dose animals was 6.8% heavier than controls. Mean food consumption was not affected.

Haematology, clinical biochemistry and urinalyses did not reveal any noteworthy treatment-related changes.

Organ weight analysis revealed an increase in mean relative (to bodywt and brain wt) weight of liver in high-dose females; other small changes were those expected as a consequence of reduced bodywt.

There were no treatment-related gross pathological or microscopic changes noted at autopsy.

The results show that the atrazine metabolite, desisopropylatrazine, at 1000 ppm in the diet of rats led to reduced weight gain in males and a slight increase in relative weight of the liver in high-dose females. Since there was a small effect on bodywt gain at the lowest dose tested of 500 ppm, a NOEL for desisopropylatrazine in this study was not established.

Note: There was no validation report attached to this IBT study, nor any indication from Ciba-Geigy as to whether it had been reviewed or not; on superficial examination, the study appeared to be quite well reported

5.3.2 3-Month rat dietary study

Schneider M (1992) G 28279 (Desisopropylatrazine): Three-Month Oral Toxicity Study in Rats (Administration in Food). Ciba-Geigy Ltd, Switzerland. Study no. 901261. Completion date 8 May 1992 (GLP; OECD, USA, Japan, Switzerland)

This study was conducted according to OECD Guideline no. 408 and USEPA Guideline 82-1. **Desisopropylatrazine** (Batch FL-901747; 96.7% purity) was administered in the diet to rats (Tif: RAIf (SPF) hybrids of RII/1 x RII/2) from Ciba-Geigy, Stein, Switzerland (10/sex/group) at concentrations of 0, 10, 50 and 500 ppm for 13 weeks; tests revealed acceptable test compound stability and homogeneity in the diet, with mean actual concentrations being between 83-91% of target. Calculated mean daily doses (corrected for analysed concentrations of compound in the diet) were 0, 0.60, 3.2 and 34.9 mg/kg bw (males) and 0, 0.64, 3.3, and 37.5 mg/kg (females). At study commencement, rats were approx. 5-6 weeks old (100-130 g bodywt at week -1). Dosing occurred over the period 16 Jan. 1991 to 18 April 1991. Observations of clinical condition and mortality were performed daily, ophthalmoscopy at the start and end of the study, bodyweight and food and water consumption weekly, and clinical laboratory determinations (haematology, clinical chemistry and urinalysis) at week 14. Haematology parameters were those listed in Appendix III plus investigation of red cell morphometric parameters. Clinical chemistry parameters measured were as in Appendix III (but not including LDH or CPK). Urinalysis parameters were those listed in Appendix III. Organs weighed are listed at Appendix IV (not including thyroid but including thymus). Organs examined histopathologically included: spleen, lymph node (mesenteric, axillary); sternum with marrow; trachea; lung; heart; aorta; salivary glands (submandibular); liver; pancreas; oesophagus; stomach; intestine (small and large); kidneys; bladder; gonads; uterus; vagina; pituitary; adrenals; thyroid/parathyroid; thymus; peripheral nerve; brain; and any organ with gross lesions.

Doses used were chosen on the basis of a 28-day study conducted by the Research & Consulting Co., Itingen, Switzerland (Project no. 252090) at dietary doses of 50, 500 and 2000 ppm.

There were no deaths and no clinical signs which were related to treatment. A reduction in bodyweight gain occurred at the mid dose (males) and high dose (both sexes) such that mean male bodywts were about 3-4% (50 ppm) and 6-9% (500 ppm) lighter than controls, and mean female bodywt about 9-13% (500 ppm) lighter than controls. In 500 ppm females there was a corresponding 7% decrease in mean food consumption during weeks 1-7, with minimal, if any effect on food consumption in males. Average water consumption was increased at the high dose (11% in males, 31% in females).

Ophthalmoscopic examinations did not reveal any treatment-related effects. Haematology, clinical biochemistry and urinalysis did not reveal any treatment-related changes.

Organ weight analysis revealed an increase in mean relative (to body wt) weight of liver in high-dose animals.

There were no treatment-related gross pathological changes noted at autopsy. Microscopic examination revealed minimal fatty change of the adrenal cortex in high-dose males (7/10 *vs* 1/10 controls); hypertrophy of thyroid follicular epithelium in high dose males (8/10 *vs* 2/10 incidence in all other groups); hypertrophy of pituitary cells in the adenohypophysis (TSH-producing cells) of high-dose males (5/10 *vs* 0/10 controls); extramedullary haematopoiesis in the spleen of high-dose females (10/10 *vs* 4/10 controls); and extramedullary haematopoiesis in the liver of high-dose females (3/10 *vs* 0/10 controls).

The results show that the atrazine metabolite, desisopropylatrazine, at 500 ppm in the diet of rats resulted in activation of the thyroid gland and hypertrophy of TSH-producing cells in the pituitary gland of males, and a degree of extramedullary haematopoiesis in the spleen and liver of females, together with a slight increase in relative weight of the liver in high-dose females. The NOEL for desisopropylatrazine in this study was 50 ppm (3.2 mg/kg bw/d in males, 3.3 mg/kg bw/d in females), based on these findings.

5.3.3 13-Week dog dietary study

Thompson SS, Batastini G & Arthur AT (1992) G-28279 (desisopropylatrazine): 13-Week Feeding Study in Dogs. Ciba-Geigy Corporation, Division of Toxicology/Pathology, Summit, NJ. Study no. 912021. Toxicology/Pathology report 91073 (MIN 902187). Study completion date 22 April 1992 (GLP; US EPA)

This study was conducted according to US EPA Guidelines 82-1. **Desisopropylatrazine** (FL-901747; 96.7% pure) was administered in the diet to purebred beagle dogs from Marshall Farms, NY (4/sex/group), at concentrations of 0, 15, 100, 500 and 1000 ppm for at least 14 weeks. Concentrations, stability and homogeneity of admixtures were confirmed by analyses to be acceptable; all samples analysed were between $\pm$7% of the target concentration. Mean daily compound intakes were 0, 0.6, 3.8, 18.9 and 33.4 mg/kg (males), and 0, 0.6, 3.8, 18.0 and 33.3 mg/kg (females) in the control - high-dose groups respectively. Observations of clinical condition and mortality were carried out daily; bodyweight and food consumption weekly; physical auditory and ophthalmoscopic examinations were carried out pre-dosing and at week 14; electrocardiographic examinations were conducted pre-dosing and during weeks 4, 8 and 13/14; haematology, clinical chemistry and urinalysis were all performed pre-dosing and during weeks 6 and 11-12 (urinalysis) and 13 (haematology and biochemistry). Parameters measured are listed in Appendix III (haematology included examination of RBC morphology and Heinz body counts, but did not include MCV, MCHC or MCH calculations).

Dogs were fasted for 12 hours prior to a full autopsy. Organs weighed included: lung; heart; aorta; salivary glands (mandibular); liver; spleen; kidneys; gonads; prostate; uterus; pituitary; adrenals; thyroid/parathyroid; thymus; brain; epididymes. Organs examined histopathologically included: spleen, lymph node (medial retropharyngeal, axillary); sternum with marrow; trachea; lung; heart; aorta; salivary glands (mandibular); liver; pancreas; femur (with marrow and articular surface); gallbladder; lacrimal glands; oesophagus; stomach; small intestine (duodenum, jejunum, ileum), large intestine (caecum, colon, rectum); kidneys; urinary bladder; eyes (with optic nerve); female genital organs (ovaries, uterus, vagina); male genital organs (testes, epididymes, prostate); mammary gland; pituitary; adrenals; thyroid/parathyroid; thymus; peripheral (sciatic) nerve; brain (3 levels); spinal cord (three levels); stomach (cardia, fundus, pylorus); thymus (when present); skeletal (thigh) muscle; skin; tongue; and any organ with gross lesions.

There were no deaths and no clinical signs which were related to treatment. A treatment-related effect on bodyweight parameters occurred in 500 and 1000 ppm animals. In 1000 ppm males and 500 and 1000 ppm females, an initial small bodywt loss occurred during week one, with, essentially, maintenance of this weight for the duration of the study (unlike dogs in other groups which gained weight). The mean bodywt of females at 500 ppm increased from study commencement but lagged slightly behind controls. At the end of the study, 500 and 1000 ppm males had gained/lost 13.5% and -2.7% of their starting

bodywt, respectively (cf. controls, 21.4%), whilst 500- and 1000 ppm females lost 0.4% and 6.3% of their starting bodywt, respectively (cf. controls; 17.3% gain). Some decreases in food consumption were noted in 500 and 1000 ppm animals, associated with concomitant reductions in mean bodywt parameters and possibly partly related to food palatability.

Physical, auditory, ophthalmoscopic and electrocardiographic examinations did not reveal any treatment-related effects.

Haematology, clinical biochemistry and urinalysis results noted some isolated, random, statistically significant changes at some time points but none which could be clearly related to compound administration. Two dogs (littermates), one 500 ppm male and one 1000 ppm female became anaemic by the end of the study; the male had moderate anaemia with increased polychromasia, and leukopaenia with severe neutropaenia by day 86, whilst the female (the one with the severest weight reduction) developed a severe anaemia with a marked reticulocyte response. This correlated with splenic and hepatic extramedullary haematopoiesis and bone marrow erythroid hyperplasia, and splenic enlargement in both dogs. The reporting laboratory considered these findings to be unrelated to treatment.

Organ weight changes at 500 and 1000 ppm included a decrease in absolute and relative (to brain) weights of heart; absolute heart weights were 86 and 79% of control (males) and 83% and 78% (females), respectively; the changes in female heart weights were not statistically significant. This was not accompanied by any electrocardiographic or histopathological findings and may be related, at least in part, to the bodyweight changes.

There were no treatment-related gross or microscopic pathology findings at necropsy.

The results suggest that effects related to the atrazine metabolite, desisopropylatrazine, in dogs were seen at dietary concentrations $\geq$ 500 ppm and were limited to reductions in bodywt parameters, food consumption and possible heart wt decrease. On the basis of these effects, the NOEL for this study was 100 ppm (3.8 mg/kg bw/d).

Anaemia was seen to develop in two dosed dogs, although this was reasoned to be unrelated to treatment. In view of the small number of animals per group, it is not possible to unequivocally conclude whether or not this is the case.

5.4 Diaminochlorotriazine (G 28273)

5.4.1 13-Week dietary rat study

Drake JC (1971b) G 28273 (Diaminochlorotriazine): Thirteen-Week Dietary Toxicity Study in Rats. Ciba-Geigy Ltd, Switzerland. Lab: Geigy Pharmaceuticals, Wilmslow, UK. Study no. 8/71/S.L. Date 27 July 1971

Diaminochlorotriazine (Batch 5, manufactured 16.10.70; 99.9% purity) was administered in the diet to Sprague-Dawley derived rats (20/sex/group) at concentrations of 0, 500 and 1000 ppm for 13 weeks. At study commencement they were approx. 6 weeks old (154-186 g bodywt). Tests on the diet for acceptable test compound stability, homogeneity and concentration were not performed but diets were prepared fresh weekly. On the basis of measured food intake, the average daily intake of test material was 0, 34.3 and 70.6 mg/kg bw/d (males) and 0, 34.7 and 74.2 mg/kg bw/d (females). Observations of clinical condition and mortality were performed daily, ophthalmoscopy at the end of weeks 4, 8 and 13, bodyweight and food and water consumption weekly, and clinical laboratory determinations (haematology, clinical chemistry and urinalysis) during weeks 5, 9 and 13 on 5/sex/gp. Haematology parameters measured were those listed in Appendix III plus erythrocyte sedimentation rate and methaemoglobin but not including MCHC, MCV or MCH. Clinical chemistry parameters measured were Na, K, Cl, glucose, urea, AST, ALT, AP, serum proteins (total and electrophoretic) and cholinesterase. Urinalysis parameters were those listed in Appendix III (not including urobilinogen). Organs weighed included heart, lungs, adrenal, brain, thyroid, testes, pituitary, liver, spleen, ovaries, and kidney. Organs examined histopathologically included the above plus lymph node; rib junction, bone marrow; trachea; salivary gland; pancreas; stomach; colon; eye; muscle; oesophagus; skin; intestine (small); bladder; spinal cord; and any organ with gross lesions.

There were no compound-related deaths and no clinical signs reported. A reduction in bodyweight gain occurred at both doses such that throughout treatment, animals were 10-11% (low dose) and 18-20% (high dose) lighter than controls, associated with decreases in mean food consumption at both doses.

Ophthalmoscopic and auditory examinations did not reveal any treatment-related effects. Haematology data indicated mild anaemia at the high dose from week 5, with a reduction in one or more of PCV, RBCs, Hb, as well as WBC counts. Prothrombin times were shorter in weeks 5 and 13. At 500 ppm, there was a fall in PCV by week 13. Clinical biochemistry results and urinalyses revealed isolated changes, probably of limited toxicological significance. Organ weight changes which possibly were more than a reflection of the reduced bodywt of dosed animals included increase of the wt (absolute, and relative to brain) of the thyroid glands in high dose males and all dosed females, and of the lungs (all dosed animals); the increase in absolute wt of the thyroids at the high dose was between 12-23% and for lungs at the high dose, 8-9%.

There were no treatment-related gross or microscopic pathological changes noted at autopsy.

The results show that diaminochlorotriazine resulted in some adverse effects at both doses viz. reduced bodywt gain and mild anaemia. Increased wts of thyroids (high dose males and dosed females) and lungs (both doses) were noted, although no pathological findings were reported for any of these organs. A NOEL was not established in this study.

5.4.2 90-Day dietary rat study

Pettersen JC, Richter AD & Gilles PA (1991) Diaminochlorotriazine (G 28273): 90-Day Subchronic Dietary Toxicity Study in Rats. Ciba-Geigy Corp., Farmington, CT. Study no. F-00006. Completion date 5 Nov. 1991 (GLP; OECD, USA, Japan)

This study was conducted according to OECD Guideline no. 408 and USEPA Guideline 82-1. **Diaminochlorotriazine** (Lot no. FL-871776; 98.2% purity) was administered in the diet to Crl:CD (SD) BR rats from Charles river Labs, Kingston, NY (10/sex/group) at concentrations of 0, 10, 100, 250 and 500 ppm for 90 days; doses were chosen on the basis of a 4-week dose range-finding study in which large bodywt reductions were seen at 1000 ppm. Tests revealed acceptable test compound stability and homogeneity and concentration in the diet. Calculated mean daily compound intake doses were 0, 0.7, 6.7, 16.7 and 34.1 mg/kg bw/d (males) and 0, 0.7, 7.6, 19.7 and 40.2 mg/kg bw/d (females). At study commencement, rats were approximately 6-weeks old. Dosing occurred over the period 29 Nov. 1988 to 3 March 1989. Observations of clinical condition and mortality were performed at least twice daily, ophthalmoscopy at the start and end of the study, physical examination, bodyweight and food and water consumption weekly, and clinical laboratory determinations (haematology, clinical chemistry and urinalysis) at study termination. Haematology parameters were those listed in Appendix I. Clinical chemistry parameters measured were as in Appendix I (but not including LDH). Urinalysis parameters were those listed in Appendix III. For oestrus cycle determination, vaginal smears were examined on days 14-28 (control and high dose) and days 42-56 and 70-85 for all females. For hormone analysis, blood samples (from up to 10 females/dose level) were taken from animals believed to be in proestrus on the day of collection. Organs weighed are listed at Appendix IV (not including thyroid but including thymus and lungs). Organs examined histopathologically included those listed in Appendix IV (not including zymbal's gland or 3 head sections but a nasal passage section [level 2]).

There were no deaths and no clinical signs which were related to treatment. A reduction in bodyweight gain occurred at the high dose (males) such that mean male bodywts were 87% of controls (week 12). Group mean female bodywts were not statistically different from controls although differences in weight gain were seen in females at 250 ppm (85% of control weight gain) and 500

ppm (83% of control weight gain) females. Food consumption was not affected.

Ophthalmoscopic examinations did not reveal any treatment-related effects. Haematology and urinalysis did not reveal any treatment-related changes. Clinical biochemistry changes of statistical significance included slight decreases in calcium (high-dose animals); total protein (mid-dose and high-dose males); and globulin (high-dose males).
Oestrous cycle data are reported in the study addendum (see following assessment). Analysis of serum for oestradiol, progesterone, prolactin and corticosterone did not reveal any treatment-related effects; because of the effects of the compound on the oestrus cycle, there were only a limited number of animals determined to be in proestrus at the time of blood collection. Thus, there were no animals from the 250 ppm group and only 4 from the 500 ppm group.

Organ weight analysis did not reveal any noteworthy findings.

There were no treatment-related gross or microscopic pathological changes noted at autopsy.

The results show that the atrazine metabolite, DACT, administered in the diet of rats resulted in reduced bodywt gain in females at 250 ppm and both sexes at 500 ppm. At 100 ppm and above, the compound affected oestrus cycling (see below; Section 5.4.3). Analysis of serum oestradiol, progesterone, prolactin and corticosterone did not show any treatment-related effects but there were only a limited number of animals sampled. The NOEL for DACT in this study was 10 ppm (0.7 mg/kg bw/d), based on the oestrus-cycle effects at 100 ppm.

5.4.3 90-Day dietary rat study - addendum

Terranova P (1991) 90-Day Subchronic Dietary Toxicity Study with G-28273 (diaminochlorotriazine) in Rats: Report Addendum - Effects of G-28273 Technical Administration on Estrous Cycle Parameters in Females Sprague-Dawley Rats. Ciba-Geigy Corporation, Greensboro, NC. Laboratory: Department of Physiology, University of Kansas Medical Center, Kansas City, USA Study no:F-00006, report addendum. Report date 27 March 1991 (GLP: Not applicable USA/"acceptable scientific practices" statement provided)

This addendum to a 90-day subchronic dietary study with the atrazine metabolite, **diaminochlorotriazine** (DACT; G-28273) (Study no. F-00006) was prepared for the Ciba-Geigy Corporation by a consultant at the Department of Physiology, University of Kansas. Its aim was to determine the effect of DACT treatment on the oestrous cycle of female rats. The laboratory data evaluated in this addendum was collected at the Ciba-Geigy Corporation, Environmental Health Center, Farmington, CT, USA, between Nov. 1988 and March 1989.

The original study involved administration of DACT to Sprague-Dawley rats (5/sex/group) for 90 days at dietary concentrations of 0, 10, 100, 250 or 500 ppm. Vaginal smears were prepared on study days 42-56 and 70-85 for all females. Smears were classified into proestrus, oestrus, metoestrus or dioestrus. The duration of the oestrous cycle and the number of cycles in the observation periods were calculated. The incidence of persistent oestrus and persistent dioestrus also were calculated.

Statistical analysis showed that the proportion of animals exhibiting normal 4-5 days oestrous cycles was slightly reduced in treated groups at doses of $\geq$ 100 ppm, with statistical significance at doses of 250 and 500 ppm on days 70-85. The incidence of persistent oestrus also increased at doses of $\geq$ 100 ppm but was only statistically significant in the 250 ppm group on days 70-85. Persistent dioestrus was not seen in any group. There was considerable individual variability in all the data, particularly for the treated groups and this prevented more exact determination of oestrous cycle effects.

Finding:-	**4-5 day cycles**		**Persistent oestrus**		**Persistent dioestrus**	
Days:-	**42-56**	**70-85**	**42-56**	**70-85**	**42-56**	**70-85**
Dose (ppm)						
0	14/15	15/15	0/15	0/15	2/15	0/15
10	12/15	13/15	1/15	1/15	2/15	3/15
100	9/15	14/15	3/15	2/15	3/15	1/15
250	8/15	7/15*	5/15	8/15*	4/15	3/15
500	8/15	6/15*	4/15	4/15	1/15	5/15

* significantly different from control ($p<0.05$) using Fisher's Exact test
Note: Totals in a particular dose group at a particular examination time may not equal 15 since one animal could display a regular 4-5 day cycle followed by persistent oestrus or diestus.

The results indicated that the atrazine metabolite DACT affected the oestrous cycle of rats by increasing the length of the cycle and a tendency to increasing the incidence of persistent oestrus at doses of 100 ppm or over. The NOEL in this investigation was 10 ppm (0.7 mg/kg bw/d).

5.5 Hydroxyatrazine (G 34048)

5.5.1 90-Day rat dietary study

Rudzki MW, McCormick GC & Arthur AT (1989) 90 Day Oral toxicity Study in Rats - Hyroxyatrazine. Ciba-Geigy Corporation, Summit, NJ, USA. Study no. 822146. Report date 25 October 1989. (GLP; USA) (A3162/17 B3; data submission date 8 March 1994)

This study was conducted according to US EPA Guideline 82-1**.** **Hydroxyatrazine** (FL 870869; 97.1% purity) was administered in the diet to Crl:CD Br Sprague-Dawley rats from Charles River Laboratories, Kingston, NY (15/sex/group) at concentrations of 0, 10, 100, 300 and 600 ppm for 13 weeks; calculated mean daily doses were 0, 0.64, 6.30, 18.89 and 37.47 mg/kg (males) and 0, 0.75, 7.35, 22.73 and 45.64 mg/kg (females). Rats were approximately 6 weeks old at the start of dosing, with bodywts in the range 167-234 g (males) and 77-188 g (females). Dosing commenced 18 May 1988 and finished on 22 Aug. 1988. Observations of clinical condition and mortality were performed daily, bodyweight and food consumption weekly, water consumption, urine volume, physical auditory and ophthalmoscopic examinations and clinical laboratory determinations (haematology, clinical chemistry and urinalysis), pre-dose and at week 13. Appendix III indicates haematology parameters measured (not including MCH, MCHC, MCV), urinalysis parameters (not including nitrate) and clinical chemistry (not including CPK or cholinesterase activity). Organs weighed were those listed in Appendix IV (plus epididymes, prostate, salivasry glands, thymus and uterus). Organs examined histologically included adrenals; any organ with gross lesions; aorta; bladder; brain (3 levels); eye with optic nerve; female genital organs (ovaries, uterus, vagina); femur with joint and marrow; gall bladder; Harderian glands, heart; large intestine (caecum, colon, rectum); kidneys; lacrimal (exorbital gland); liver; lung; lymph node (mesenteric and submaxillary); male genital organs (epididymes, prostate, seminal vesicles, testes); mammary gland; oesophagus; pancreas; peripheral nerve; pituitary; salivary glands (submaxillary); skeletal muscle (thigh); skin; small intestine (duodenum, ileum, jejunum), spinal cord (3 levels); spleen; sternum with marrow; stomach (glandular, non-glandular); thymic region tissue, thyroid/parathyroid; tongue; and trachea.

There were no deaths and no clinical signs which were related to treatment. A small, treatment-related reduction in cumulative bodyweight gain occurred at the high dose, associated with a decrease in mean food consumption in males. At the end of the study, cumulative weight gain at the high dose was about 88% of the controls; this was not associated with statistically reduced mean absolute bodyweight. Water consumption was increased by approx. 127% and 93% in males and females respectively at the high dose (correlated with increased urine volumes, as well as elevated kidney weights and renal histological changes - see below).

Physical, auditory, ophthalmoscopic and electrocardiographic examinations did not reveal any treatment-related effects. Haematology data indicated reductions in mean RBC counts, Hb and Hct at the high dose. Clinical biochemistry results noted increased BUN, creatinine, sodium and chloride at the high dose, probably related to the other renal effects found in pathology (see below). Urinalysis revealed treatment-related decreases in mean specific gravity in high dose females, combined with increased urine volumes in 300 and 600 ppm males and 600 ppm females of 38%, 228% and 147% respectively.

Organ weight analysis revealed an increase in absolute and relative (to body and brain) kidney weights at the high dose (33-34% increase in absolute weights).

Treatment-related gross pathological changes noted at autopsy were pitted or rough kidneys (with or without pale or tan discolouration) in 29/30 high dose animals, 4/15 300 ppm males and 2/15 300 ppm females (at the 300 ppm level, pitted kidneys sometimes had a greenish discolouration). No other gross changes were noted at necropsy.

Microscopic pathology revealed kidney changes in 600 ppm animals, consisting of marked tubular dilation and tubular basophilia, extensive chronic hyperplastic inflammation in the interstices, and cellular casts; this lesion was coded as toxic nephrosis. In a large proportion of high dose animals, there were anisotropic crystals in the papillary tubules. In 7/15 males and 11/15 females at 300 ppm, minimal tubular dilation and tubular basophilia, with minimal subacute interstitial inflammation were observed, often occurring unilaterally.

All the gross and microscopic renal findings were described as being similar to those found in the 'companion' 13-week dog study with hydroxyatrazine (study no. 892076; see Section 5.5.2 below).

The results show that the target organ for the toxicological effect of the atrazine metabolite, hydroxyatrazine, is the kidney and the other clinical and functional symptoms were related to the renal effects which occurred at dietary concentrations of $\geq$ 300 ppm. The NOEL for hydroxyatrazine in this study was 100 ppm (6.30 mg/kg bw/d in males, 7.35 mg/kg bw/d in females), based on nephrotoxicity observed at the next highest dose of 18.9-22.7 mg/kg bw/d.

5.5.2 13-Week dietary dog study

Chau RY, McCormick GC & Arthur AT (1990) Hydroxyatrazine: 13-Week Feeding Study in Dogs. Laboratory: Division of Toxicology/Pathology, Ciba-Geigy Corporation, Summit, NJ. Study no. 892076. Study completion date 20 Mar. 1990 (GLP; US EPA)

Hydroxyatrazine (FL-870869) was administered in the diet to beagle dogs (4/sex/group) at concentrations of 0, 15, 150, 1500 and 6000 ppm for 13 weeks. Concentrations, stability and homogeneity of admixtures were confirmed by analyses to be acceptable. Observations of clinical condition, mortality, bodyweight and food consumption were carried out daily; physical auditory and ophthalmoscopic examinations were carried out pre-dosing and at week 13; electrocardiographic examinations were conducted pre-dosing and at week 12; haematology, clinical chemistry and urinalysis were all performed pre-dosing and at week 13. Dogs were fasted for 12 hours prior to a full autopsy. Appendix III indicates haematology parameters measured (not including MCH, MCHC, MCV or blood smear but including Heinz body determinations), urinalysis parameters (not including nitrate) and clinical chemistry (not including CPK or cholinesterase activity). Organs weighed were those listed in Appendix IV (plus epididymes). Organs examined histologically included adrenals; any organ with gross lesions; aorta; bladder; brain (3 levels); epididymes; eye with optic nerve; femur with joint and marrow; gall bladder; gonads; heart; intestine (small and large); kidneys; liver; lung; lymph node (mesenteric and retropharyngeal); mammary gland; oesophagus; pancreas; peripheral nerve; pituitary; prostate; salivary glands (mandibular); skin; spinal cord (3 levels); spleen; sternum with marrow; stomach (cardia, fundus, pylorus); thymic region tissue, thyroid/parathyroid; tongue; trachea; uterus; and vagina.

Mean daily compound intakes were 0, 0.6, 5.8, 59.6 and 247.7 mg/kg (males) and 0, 0.6, 6.2, 63.9 and 222.1 mg/kg (females) in the control - high-dose groups respectively.

There were no deaths and no clinical signs which were related to treatment. A treatment-related reduction in cumulative bodyweight gain occurred in females at 1500 ppm and males at 6000 ppm but this was not associated with statistically reduced mean absolute bodyweight or weight gain. There was a transient reduction in food consumption in 1500 and 6000 ppm females on days 7 and 14 and in 6000 ppm males on day 7 which may have been associated with an initial impalatability of the dietary mixtures.

Physical, auditory, ophthalmoscopic and electrocardiographic examinations did not reveal any treatment-related effects. Haematology and clinical biochemistry results were unremarkable although an increased level of BUN in one, and creatine in two, high-dose females was probably related to the other renal effects found in pathology (see below).

Urinalysis revealed treatment-related decreases in mean specific gravity in both 1500 ppm and 6000 ppm males and females on day 85, combined with increased urine volumes (two-fold increase compared to controls) of a lighter colour than controls.

There was no effect on organ weights and the only treatment-related gross and histopathological changes noted at autopsy were in the kidneys which were pitted or rough in 3/4 1500 ppm males, 1/4 1500 ppm females, 4/4 6000 ppm males and 2/4 6000 ppm females, respectively. Microscopic pathology revealed kidney changes in all of the 1500 and 6000 ppm males and females, consisting of multifocal chronic nephropathy of minimal to marked severity with tubal dilation, tubular atrophy and tubular basophilia, often with prominent chronic interstitial fibrosis and lymphocytic infiltrations. Both sexes were equally affected and lesions were usually in the cortex, although the medulla was also sometimes involved. Intratubular crystalline casts were found in most 1500 and 6000 ppm animals but the nature of the crystals was not determined. All the gross and microscopic renal findings are described as being similar to those found in the 'companion' 13-week rat study with hydroxyatrazine (study no. 882146; Section 5.5.1).

The results show that the target organ for the toxicological effect of the atrazine metabolite hydroxyatrazine in dogs is the kidney, and the other clinical and functional symptoms were related to the renal effects which occurred at dietary concentrations of $\geq$ 1500 ppm. On the basis of these effects, the NOEL for hydroxyatrazine in this study was 150 ppm (5.8 - 6.2 mg/kg bw/day) for both male and female beagle dogs. This toxic appears common to rats and dogs and appears to occur at similar doses.

6. CHRONIC STUDIES

6.1 Atrazine (G 30027)

IARC (1991) reviewed three mouse studies (ip and sc dosing) by Donna et al (1981, 1986), aimed at assessing carcinogenicity, but concluded that they were inadequate studies in terms of length of dosing, incomplete reporting, intercurrent disease, the use of only one dose level, and the short duration of observation and reporting; thus they are not re-assessed here.

6.1.1 18-Month mouse dietary study

Innes JRM, Ulland BM, Valerio MG, Petrucelli L, Fishbein L, Hart ER, Pallotta AJ, Bates RR, Falk HL, Gart JJ, Klein M, Mitchell I & Peters J (1969) Bioassay of Pesticides and Industrial Chemicals for Tumorigenicity in Mice: A Preliminary Note. J Natl Cancer Inst 42: 1101-1114)

This study, involving almost 20,000 mice, was conducted by Bionetics Research labs, Litton Industries, Maryland, and the National Cancer Institute, Maryland; 120 pesticides and industrial chemicals were investigated. Two

strains of mice [(C57BL/6 x C3H/Anf)F1 and C57BL6 x AKR)F1] (18/sex/strain; ie. 72 animals per compound) were given atrazine (source and purity unstated) in 0.5% gelatine by gavage from 7 days of age at an initial dose of 21.5 mg/kg (this dose was not adjusted with increasing bodyweight) up until 4 weeks of age, then the compound was administered in the diet at 82 ppm until necropsy at approximately 18 months. Other groups were treated under the same schedule with simazine, at an initial gavage dose of 215 mg/kg, then at 603 ppm in the diet. Postmortem procedures included external examination, gross examination of thoracic and abdominal cavities, and histological examination of major organs and all grossly visible lesions; the cranium was not dissected.

Atrazine and simazine were included in a table of compounds which did not cause a significant increase in tumours after oral administration, at a dose which was claimed to be an maximum tolerated dose (MTD). Otherwise, no further information was given about any findings after the administration of these two compounds.

6.1.2 21/22-Month mouse dietary study

Sumner DD (1981a) Carcinogenicity Study with Atrazine Technical in Albino Mice. Ciba-Geigy Corp. Industrial Bio-Test Laboratories Inc., Wedge's Creek Research Farm, & Globe Animal Laboratory Facility, Wisconsin. IBT no. 8580-8906. 30 June 1981 (Validated IBT Study)

This report was prepared by Ciba-Geigy personnel, based upon validated data generated by IBT. Charles River CD-1 Swiss white mice (60 animals/sex/group; 5-7 weeks old at study commencement) were given atrazine (FL-761261; 96.4% purity) at 0, 10, 300 and 1000 ppm in the diet. Dietary analysis at regular intervals revealed levels generally close to target levels, apart from the low-dose level during the last month of the study (5.3-6.3 ppm); the time-weighted average concentrations were 9.7, 309.3 and 1017.4 ppm in the low- to high-dose groups respectively. The study was terminated at 21 months (males) and 22 months (females) when survival levels were around 20%. Approximately 9 months after "initiation" (study commencement?), mice were transferred from the Wedge's Creek Research Farm of IBT and transferred to the Globe animal laboratory facility, 5.6 miles away. Female mice were housed in groups of ten per cage for the first 17 months of the study and thereafter individually. Male mice were housed individually throughout.

Observations included clinical signs and mortality (daily), body weight (monthly), and histopathology (all animals which survived to term, as well as animals which died or were killed *in extremis*). Pathological examinations were conducted by Experimental Pathology Labs Inc., Raleigh, NC (pathology report dated 28 Jan. 1980, with quality assurance certification); the organs and tissues examined are listed in Appendix IV (but did not include blood smears, bone, epididymes, gall bladder, Harderian gland, head (3 levels), lacrimal gland, smooth muscle, rectum, seminal vesicle, sternum, thymus, vagina, and Zymbal's gland).

Body weight depression was noted in males (approx. 5 -7% lower than controls) and females (approx. 6%) at the 1000 ppm level. It was noted that whilst raw, mean and graphical data on bodyweights were supplied, some entries in the summary tables were clearly in error, being inconsistent with bodyweights taken at times before and after. Survival was reduced in high-dose females (median survival time of 616 days cf. 657 days in controls) but not in males 560 days cf. 551 days in controls).

The only reported compound-related effect on gross pathology was a possible increase, at the 1000 ppm dose level, in the incidence of a granular/irregular surface on kidneys (37 high-dose animals cf. 11 controls), or white/pale kidneys (39 high-dose animals cf. 23 controls).

The only tumour type highlighted as possibly increased in atrazine-treated animals was alveolar cell tumours, with an incidence as follows:-

Alveolar cell	sex	control	10 ppm	300 ppm	1000 ppm
No. animals examined	M	60	57	56	55
	F	55	58	60	59
carcinoma	M	4	8	6	3
	F	2	2	6	5
adenoma	M	2	4	3	0
	F	0	1	2	0
Combined	M	6	12	9	3
	F	2	3	8	5
Total	M/F	8 (7.0%)	15 (13.0%)	17 (14.7%)	8 (7.0%)

Experimental Pathology Labs Inc. stated in their histology report that "the total number in each group may represent a variation that could be expected in a comparable group of mice". It would have been useful if specific information on the incidences of these pathological features in other IBT studies using Charles River CD-1 strain mice had been provided. Since there was no obvious dose-related increase in incidence and the historical control incidence of these neoplasms is quite high in CD-1 mice [historical control data from 1450 mice, collected over a period of 7 years to 1992, showed a combined incidence of carcinomas and adenomas of 21.8% in males and 13.8% in females (Chandra & Frith, 1992)], it is unlikely that these reported incidences reflect a tumorigenic affect of atrazine.

There were no other compound-related microscopic histopathological findings.

The NOEL may be taken as 300 ppm (estimated as 45 mg/kg bw/d), based on increased mortality (females only), reduced body weight (both sexes), and macroscopic kidney pathology (granular/irregular surface, or pale/white appearance) at the next dose of 1000 ppm.

Sumner DD (1981b) Validation Report of the above IBT study. Sept. 1981

In accordance with US EPA review criteria specified in correspondence to Ciba-Geigy dated 27 July 1977 and 22 March 1978, a validation review was conducted by the Agricultural Division of Ciba-Geigy. Since the study was initiated prior to the introduction of GLP, this review was conducted using "state of the art" guidelines and standards. The review indicated that the study was performed in accordance with its protocol, with exceptions as noted in the review. Deficiencies in study were generally of a minor nature and would not have affected the final conclusions. At several points in the study report (page 5 of the Ciba-Geigy 'Exhibit C' document and page 38 of the report summary), the test compound mentioned is simazine rather than atrazine! It is assumed these are typographical errors. Other studies on Ciba-Geigy compounds were being conducted by IBT, including one on simazine (Study No. 8580-08907), a study which shared common controls with the atrazine study.

6.1.3 91-Week mouse dietary study

Hazelette JR & Green JD (1987a) Atrazine Technical: 91-Week Oral Carcinogenicity Study in Mice. Ciba-Geigy Corp., Summit NJ. 1987. Project no. MIN 842120; Ref. no. 2-001-26; Toxicology/pathology report no. 87069. Study completion date 30 Oct. 1987 (GLP; US EPA)

The study conformed with OECD Test Guideline no. 451 as well as specific US and Japanese guidelines for the conduct of such studies. Atrazine technical (Batch no. FL #841802) was administered to CD-1[Crl:CD-1(ICR) BR] mice from Charles River Labs, Kingston, NY (60/sex/group) at dietary concentrations of 0, 10, 300, 1500 or 3000 ppm for at least 91 weeks. Treatment started on 31 Oct. 1984 and ended 22 Aug. 1986. Bodywts just prior to commencement of dosing were 21.9-32.8 g (males) and 16.0-25.2 g (females). Admixtures were stable for at least 40 days at room temperature. Concentration/homogeneity analyses were done approximately every 4 (year one) to eight (year 2) weeks; actual levels were always within 10% of target concentrations. Mortality, clinical signs, bodyweight, food consumption, water consumption, auditory and ophthalmological examinations, palpable masses were recorded in all animals. Haematology parameters (Hb, Hct, RBCs, WBCs and differential count) were measured during weeks 92-95 and blood smears were performed during weeks 52 and 78. All animals were necropsied during weeks 92-95. Organ weights (liver, brain plus brainstem, kidneys, adrenals, testes plus epididymes), gross and microscopic pathology were recorded at necropsy for all animals. Organs taken for pathological examination included those listed in Appendix IV (with the addition of tongue but not including the head, lacrimal glands, or Zymbals gland).
Mean calculated daily compound intakes in the 10 to 3000 ppm groups respectively were 1.2, 38.4, 194.0 and 385.7 mg/kg bw/d (males) and 1.6, 47.9, 246.9 and 482.7 mg/kg bw/d (females).

Treatment-related effects included:

- decreased survival for females only, in the high dose group (leading to early study termination); at term, survival was 43% (controls), 39% (10 ppm), 43% (300 ppm), 45% (1500 ppm) and 25% (3000 ppm).
- dose-related reductions in mean body weight and percent body weight gain at 300 ppm and above for both sexes; at one year, bodywt at the high dose was of the order of 13% (males) and 11% (females) lower than controls and in females at term this difference had increased to approx. 20%.
- reduced mean food consumption and water intake at 1500 and 3000 ppm (correlated with bodywt reductions);
- reduced mean haemoglobin, RBC count and haematocrit at 1500 ppm (males) and 3000 ppm (both sexes); these may relate to decreased water and/or food consumption, and decreased bodywt.
- an increased incidence of atrial thrombi at 1500 and 3000 ppm; these were predominantly found in dead or moribund animals.

No treatment related effects were noted with regard to incidences of neoplasms. Of the 301 animals (control and treatment groups) dying before scheduled sacrifice, the majority were attributed to spontaneously occurring renal amyloidosis. The numbers of mice with benign and malignant neoplasms were as follows:

No of mice with observ'n		**Dosage Group**				
	Sex	**0 ppm**	**10 ppm**	**300 ppm**	**1500 ppm**	**3000 ppm**
necropsied	M	59	60	60	60	58
	F	60	59	60	60	60
with benign neoplasms	M	14	17	13	12	12
	F	9	9	8	14	5
with malig. neoplasms	M	14	16	21	9	11
	F	29	18	16	18	13
with all neoplasms	M	26	28	28	20	23
	F	33	22	22	28	18

In conclusion, there was no evidence of carcinogenicity of atrazine in mice at doses which significantly compromised the bodywt of both sexes and the survival of females. The NOEL was at least 10 ppm (1.2 mg/kg bw/d in males, 1.6 mg/kg bw/d in females) based on small decreases in bodywt/bodywt gain at the next higher dose of 300 ppm.

6.1.4 2-Year rat dietary study (50W formulation)

Keller JG (1961) Atrazine 50W. Two-year Dietary Administration - Rats. Geigy Agricultural Chemicals. Hazleton Labs, Falls Church, VA. Report Date 10 March 1961 (A3162/2 B26)

Albino rats (30/sex/group; males were 68-89 g, females 63-89 g at study commencement) were fed at 0, 1, 10 and 100 ppm of atrazine in diet until 65 weeks when the 1 ppm group was switched to 1000 ppm. The study was terminated at 104 weeks. Atrazine 50W (lot no. AP No. 9584) was a fine, wettable, light beige powder "reportedly" containing 48.25% active ingredient; all doses were calculated in terms of this analysis. Five animals/sex/group were sacrificed at 26 and 52 weeks. From the period from 68 to 104 weeks, compound intake in the 10, 100 and 1000 ppm groups was 0.40-0.41, 3.8-3.5, and 36.0-33.9 mg/kg bw/d (males) and 0.51-0.41, 5.3-4.8, and 45.9-42.0 mg/kg bw/d (females), respectively.

Haematological examinations (microhaematocrits and differential leucocyte counts) were conducted on 5 animals/sex at 26 and 52 weeks and on all survivors at term. Urinalyses (sugar, protein, bile pigments, specific gravity, gross and microscopic appearance) were performed on 3 animals/sex at 26 and 52 weeks and on all survivors at term (pooled samples within each sex). The following organs (from up to 5 animals/sex/group) were weighed at 26 and 52 weeks: liver, kidneys, adrenals and testes. At term, pituitary, thyroid, spleen weights were included. Histopathological examination of 26 animals at term included brain, pituitary, thyroid, heart, lung, spleen, liver, kidney, stomach, small and large intestines, bladder, bone marrow, pancreas, adrenals, skeletal muscle, peripheral nerve, testes, and all unusual lesions. At 52 weeks, tissues from 5 animals/sex underwent histopathological examination viz. thyroid, liver, kidney, stomach, small and large intestines, bladder, adrenals, and gonads.

Atrazine in this formulation did not significantly affect general appearance and behaviour (apart from transient coat roughness and piloerection after 20 weeks at 10 and 100 ppm), survival, haematology, urinalysis, organ weights, macroscopic and microscopic neoplastic and non-neoplastic pathology. A small erratic trend towards decreased bodyweights was noted at 100 ppm between 52-78 weeks and in animals switched to 1000 ppm at week 65. However, statistical analysis was not feasible due to the high overall mortality during the final three months of the study; at 104 weeks, overall survival in all groups, including controls, was very low (2-4 males/group, 1-6 females/group). Food consumption in all groups was generally comparable, with slightly lower intake in females which were switched from 1 ppm to 1000 ppm. No blood chemistry tests were performed.

An NOEL of 100 ppm (approx. 4 to 6 mg/kg bw/d) would appear to be appropriate for the effects of atrazine in depressing body weight and food consumption. However, this was a relatively minor effect and its significance is clouded by the fact that the 1000 ppm dose was not started until week 65 of the test. The study was of limited value because of the low survival rates and

the fact that a limited number of "representative animals" were taken for a limited range of assays.

6.1.5 2-Year rat dietary study

Spindler M & Sumner DD (1981) Two-Year Chronic Oral Toxicity Study with Technical Atrazine in Albino Rats. Ciba-Geigy Corp. Industrial Biotest Labs Inc., Northbrook, Illinois, IBT no. 622-06769. Report Date 15 Jan. 1981

This IBT study was reported and validated in part by Ciba-Geigy, using IBT records and a draft report.

Charles River strain albino rats (60/sex/group) were fed atrazine (FL 750337; 98% purity) at 0, 10, 100 and 1000 ppm in diet for two years. A further 5 animals/sex in a high-dose recovery group (as well as 5 extra control animals/sex) were dosed for 12 months followed by a 4-week recovery period. Animals were housed individually; feeding commenced at 28 days of age. Diets were prepared fresh on a weekly basis, with analysis for content at 0, 3 and 6 months; they were within $\pm$ 15% of the expected amount. In the low-dose, mid-dose and high-dose groups respectively, test material intake was calculated to vary between 0.3 to 0.7,3.2-6.9 and 35.9-74.6 mg/kg bw/d (males), and 0.4-0.8, 4.7-8.2, and 58.6-89.5 mg/kg bw/d (females) (higher intakes measured at week 6, with compound intake/body weight decreasing with age).

Survival was not significantly affected by dosing and no appreciable differences were reported in gross observations or behaviour. Reduced body weights and bodyweight gains (measured at the start of dosing, weekly for 13 weeks, then monthly thereafter) were noted at 1000 ppm and females at 100 ppm showed occasional statistically-significant reductions in bodyweights. Food consumption (measured for the first 13 weeks, then for months 6, 14 and 24) was slightly reduced at 1000 ppm but not at 10 or 100 ppm.

Organ weights and organ weight ratios were determined in 5 animals/sex in the control group and in high-dose recovery group which was dosed for 12 months followed by a 4-week recovery period; no significant differences were observed.

Compound-related changes in haematology at the high dose included decreased RBC counts (females; 6 and 12 months), Hb (females; 6 and 12 months), Hct (both sexes at 6 months, females at 12 months) and MCV (males; 6 months) and increased mean corpuscular Hb (female rats; 3 months) and MCHC both sexes; 3 months). Compound-related changes in blood chemistry at the high dose included increases in BUN (males at 6 months; females at 3 and 6 months), AST (females; 3 and 12 months), ALT (both sexes at 3 months; females at 6 months) and cholesterol (females; 3 and 6 months) and decreases in SAP (males; 18 months). No results for haematology or clinical chemistry were obtained for the lower treatment rates (10 and 100 ppm) and therefore it

was not possible to establish no-effect levels for any of these parameters. Urinalysis parameters measured in control and high-dose animals (glucose, albumin, specific gravity, pH, microscopic elements including leukocytes, erythrocytes and crystals) measured at 3, 6, 12, 13 (recovery animals), 18 and 24 months did not reveal any compound-related findings.

Gross pathological examination indicated that the incidences of small or cyanotic testes and dermal/subdermal masses in females (suggestive of mammary tumours?) were possibly more often observed in high-dose animals than controls, although neither increase was statistically significant. These possible findings could not be confirmed because of inadequate microscopic assessment.

Mixing of samples and errors in pathology records meant that re-evaluation of the microscopic pathology could not be completed. In the words of the validating staff, "In addition to some omissions and discrepancies in the study records, the pathology materials were not conducive to verification of the IBTL diagnosis, nor could the slides be re-evaluated in a meaningful manner. Therefore, the study is not considered adequate to assess the chronic toxicity or oncogenicity of atrazine".

The deficiencies in the study do not allow for the establishment of an overall NOEL. Nor is the study useful in assessing the chronic toxicity or oncogenicity of atrazine.

Sumner DD (1981c) Validation Report of the above IBT study. 30 Jan. 1981

In accordance with US EPA review criteria specified in correspondence to Ciba-Geigy dated 27 July 1977 and 22 March 1978, a validation review was conducted by the Agricultural Division of Ciba-Geigy Corporation. Since the study was initiated prior to the introduction of GLP, this review was conducted using "state of the art" guidelines and standards. The review, based on visitation records, audit reports, memos, telephone conversations, and personnel interviews, indicated that the study was performed in accordance with its protocol, with exceptions as noted in the review, and that the report prepared by Ciba-Geigy, using IBT records and a draft report, was an accurate documentation of the study records and findings.

6.1.6 2-Year SD rat dietary study

Mayhew DA (1986) Twenty Four Month Combined Chronic Oral Toxicity and Oncogenicity Study in Rats utilizing Atrazine Technical. Ciba-Geigy Corp., Greensboro, NC. Lab: American Biogenics Corporation, Decatur, IL. (called ToxiGenics Inc. prior to 12 Feb. 1985). Study No. 410-1102. EPA MRID no. 00141874. Report date 29 April 1986 (GLP, US EPA)

Sprague-Dawley Crl:COBS CD(SD)BR rats from Charles River Breeding Labs, Portage, MI, (90/sex; control and high dose groups, 70/sex; intermediate doses) were fed technical atrazine (lot no. FL0821575) at 0, 10, 70, 500 and 1000 ppm in the diet. The extra rats in the control and high dose groups were used for an interim sacrifice (12 months) and for a 31-32 day recovery experiment (between the 12th and 13th month of the study). Diets were prepared weekly although dietary admixtures were stable for up to 14 days at ambient temperature; monthly analyses of diet revealed that all samples were within 8% (and most within 5%) of the target and that the compound was homogeneously admixed. Haematology (parameters measured included those listed in Appendix III plus Heinz bodies), blood chemistry (parameters measured listed in Appendix III, except LDH) and urinalysis tests (Appendix III) were performed at 3, 6, 12 and 18 months and at final sacrifice. Organs weighed were adrenals, brain (plus brainstem), gonads (testes with epididymes), kidneys, liver and thyroid. Organs examined histopathologically included those listed in Appendix IV, not including aorta, optic nerve, gall bladder, head (3 sections), lacrimal gland, rectum, sternum, and Zymbal's gland.

Mean test article intake in the low- to high-dose groups respectively ranged from 0.5-0.4, 3.3-2.4, 24.2-17.9, and 49.2-41.2 mg/kg bw/d in males and from 0.6-0.4, 4.5-2.8, 33.3-26.0, and 67.7-51.8 mg/kg bw/d in females (ranges given cover the 4- and 23-month mean data).

12-Month Interim Kill and 1-Month Recovery Animal Data

In control and high-dose animals sacrificed at 12 months (plus animals dying to 12 months), compound-related microscopic alterations were noted in kidney (both sexes) and liver (females). Pelvic calculi (but not microcalculi) were noted with increased incidence in the high-dose animals, as was centrilobular hepatocellular necrosis. In recovery animals (10 control and 10 high-dose animals per sex) sacrificed at 13 months after 12-months dosing, these findings (kidney calculi and hepatocellular necrosis) were marginally increased in dosed animals.

The incidences of neoplastic findings were as follows:-

Effect	0 ppm	10 ppm	70 ppm	500 ppm	1000 ppm
12-Mnth Sacrifice & Deaths					
No. animals examined	12	5	1	5	15
Mammary gland:-					
• adenocarcinoma	0	1	1	0	3
• fibroadenoma	0	0	1	1	1
• Pituitary adenoma	1	0	0	0	4
1-Mnth Recovery (13-mnth kill)					
No. animals examined	10	-	-	-	10
Mammary gland:-					
• adenocarcinoma	0				5
• fibroadenoma	0				2
• Pituitary adenoma	0				5*

*The incidence of lactation and galactoceles was also increased in high-dose females

Terminal Kill Data

Survival to study termination was increased with respect to controls in males of the 1000 ppm group (67% vs 44%) but was decreased in 1000 ppm females (26% vs 50%). Although there were no obvious atrazine-induced effects on food conversion, both food consumption, body weights and body weight gains were reduced (NOEL = 70 ppm). The two latter parameters tended towards control in the interim recovery group that had previously been fed at the 1000 ppm level for 12 months. At the high-dose (1000 ppm), males were about 19%, and females 27% lighter than controls at 24 months whilst at the 500 ppm dose, reductions were 8% (males) and 19% (females).

Behavioural changes appeared to be restricted to males, in which increased irritability was noted in the high dose groups (500 and 1000 ppm). Atrazine appeared to produce no adverse effects on ophthalmological examination or on urinalysis parameters. Haematological changes were restricted to females of the high dose group, with decreases in erythrocyte count, haemoglobin and haematocrit. Sporadic changes occurred in a number of blood chemistry parameters, however, the only changes of note were decreases in blood glucose and triglyceride levels, and only at the high dose.

A number of changes in organ weight parameters were noted in the high dose group, but these largely reflect the marked body weight reductions found at this dose level. Antemortem assessment of palpable tissue masses indicated possible atrazine-induced effects, with increased incidences in the perianal region (500 and 1000 ppm females), back (1000 ppm females), neck (70 and 500 ppm females), inguinal region (500 ppm females) and sides (500 and 1000 ppm males).

Non-neoplastic changes

The Table shows the number of animals displaying an effect/the number of animals examined for degenerative changes and hyperplasia in various tissues. Although the incidence of the various effects was greater in the 1000 ppm groups, dose-relationships were either poor or absent, and in broad terms the incidence in the high dose animals was only about 2-3 times that found in the controls or in the lower dose animals. The marrow and spleen changes may well reflect the ulcerated and necrotic lesions found in conjunction with the neoplastic findings in the high dose females; myeloid hyperplasia of the bone marrow and splenic enlargement with extramedullary haematopoiesis are observed frequently in female rats with mammary fibroadenomas (see below). The higher incidence of mammary acinar hyperplasia may be an age-related phenomenon in males of the 1000 ppm group whose survival was increased with respect to controls. The retinal findings were reported as identical to those occurring after exposure to high intensity light for extended periods while the skeletal muscle degenerative findings were those observed in aged animals.

Incidences of Non-neoplastic Lesions in SD Rats	**Dietary Concentration (ppm)**				
Effect (Sex)	**0**	**10**	**70**	**500**	**1000**
Retinal degeneration (M)	2/65	2/65	5/67	5/67	7/67
(F)	12/68	9/65	13/69	16/65	22/65
Skeletal muscle viz. (M)	6/64	7/65	7/67	10/66	28/67
rectus femoris degen. (F)	5/67	4/65	9/69	8/64	13/64
Pelvic calculi and microcalculi (M)	18/65	21/65	14/67	24/67	39/67
Liver centrilobular degeneration (F)	3/68	3/65	2/69	4/65	12/65
Testes, interstitial cell hyperplasia	1/65	0/65	0/67	1/67	2/67
Prostate, epithelial hyperplasia	12/65	16/63	11/66	17/67	29/66
Mammary gland acinar hyperplasia (M)	7/58	1/59	5/61	7/64	21/65
Bone marrow, myeloid hyperplasia (sternum & femur) (F)	46/136	44/130	44/138	71/130	98/129
Spleen, extramedullary haematopoiesis (F)	12/67	14/65	18/69	22/65	28/65
Kidney, hyperplasia of transitional epithelium (F)	17/68	10/65	5/69	19/65	31/65
Bladder, hyperplasia of transitional epithelium (F)	4/67	0/65	1/69	3/65	10/64

Discounting hyperplastic or neoplastic changes, a NOEL of 500 ppm was appropriate for a number of pathological effects, some of which were of

doubtful significance; these included retinal degeneration, increases in kidney calculi and microcalculi in males and centrilobular degenerative changes in the livers of female rats. Likewise, hyperplasia was found in a number of tissues from rats in the high dose (1000 ppm) groups.

Neoplastic changes

Neoplastic changes were restricted to the testes of male rats and the mammary glands of females. The incidence of the testicular changes (interstitial cell tumours) was as follows: 1/65 (control), 3/65 (10 ppm), 2/67 (70 ppm), 2/67 (100 ppm) and 7/67 (1000 ppm), i.e. there was no clear dose-response relationship and the increased incidence in the 1000 ppm group was not marked. As well, the increased survival at the high dose is likely to have contributed to the higher incidence of this age-related tumour at this dose. These interstitial or Leydig cell tumours were nodular masses composed of regular cells with uniform rounded nuclei and eosinophilic cytoplasm resembling normal interstitial cells, and they compressed adjacent seminiferous tubules. The hyperplasia (see Table above) was usually diffuse and infiltrated between seminiferous tubules without causing compression.

The most notable neoplastic effects were those found in the mammary glands of female rats. For the most part these were fibroadenomas and adenocarcinomas. The percentages of female rats with mammary tumours (all types, single or multiple) were as follows: control, 53.0%; 10 ppm, 60.9%; 70 ppm, 69.1%; 500 ppm, 72.3%; 1000 ppm, 87.5% and the total number of tumours/rats examined was control, 55/66; 10 ppm, 68/64; 70 ppm, 91/68; 100 ppm, 130/65; 1000 ppm, 138/64.

In four 24-month studies conducted by ToxiGenics Inc. during 1984, the control incidence of female rats (same strain and source) bearing mammary tumours ranged from 40% to 51.1%.

Mammary Gland Tumours in Females SDs by No. of Rats and No. of Tumours

Mammary gland	**Control**		**10 ppm**		**70 ppm**		**500 ppm**		**1000 ppm**	
Number of rats with the diagnosed tumour										
	T	**D**	**T**	**D**	**T**	**D**	**T**	**D**	**T**	**D**
(no. rats examined)	33	33	31	33	30	38	26	39	18	46
fibroadenoma	13	16	15	14	18	17	16	22	12	30
adenocarcinoma	6	9	7	8	10	16	11	16	8	27
adenoma	-	1	-	-	1	-	-	1	-	2
carcinosarcoma	-	-	-	-	-	-	-	-	-	2
Rats with tumours	16	19	19	20	21	26	21	26	14	42

Total number of primary mammary tumours

Mammary gland	Control		10 ppm		70 ppm		500 ppm		1000 ppm	
fibroadenoma	19	18	26	20	28	20	43	38	25	44
adenocarcinoma	6	11	10	12	19	23	18	30	16	48
adenoma	-	1	-	-	1	-	-	1	-	3
carcinosarcoma	-	-	-	-	-	-	-	-	-	2
Total no's	25	30	36	32	48	43	61	69	41	97

T = rats killed at study term; D = rats dying or killed moribund during the study

The majority of fibroadenomas were composed of lobules of acinar epithelium (in a few, the predominant feature was branching ducts) separated by varying amounts of dense amounts of collagenous tissue; they were frequently large and occasionally had areas of necrosis.

Adenocarcinomas occurred with varying histological patterns; in some, acinar or tubular patterns were seen, in others, papillary or solid areas. Some appeared to have arisen from anaplastic and atypical epithelial elements in a tumour which otherwise resembled a fibroadenoma. In all adenocarcinomas, the nuclei were hyperchromatic with numerous mitoses. Epithelial cells were arranged in multiple layers in glandular patterns with little stroma. These tumours were frequently ulcerated or necrotic.

Adenomas occurred infrequently and were characterised by fairly well differentiated epithelial elements arranged in acinar or tubular patterns with little or no stroma.

Carcinosarcomas consisted of proliferating and malignant infiltrative growth of both glandular and sarcomatous elements.

Mammary tumours in females were significantly increased in the 500 ppm and 1000 ppm groups and trend analysis indicated 10 ppm as a NOEL (approx. 0.4-0.6 mg/kgbw/d). In male rats a NOEL may be set at 70 ppm (approx. 2.4-3.3 mg/kg bw/d), on the basis of decreased body weight, behavioural effects, palpable tissue masses (sides) and skeletal muscle (rectus femoris) degeneration at the next highest dose level of 500 ppm.

Data from 12-month sacrifice animals plus a group sacrificed at 13 months after a 1-month compound-free period indicated that in dosed animals (only the high-dose investigated at these times) there was an increased incidence of mammary tumours cf. controls at 12-13 months into the study.

No effects of atrazine on heart were noted after dosing rats at 1000 ppm for 24 months; this may be compared with the cardiac effects, in dogs, of atrazine (chronic study; Section 6.1.19) and its metabolites, desethylatrazine (subchronic study; Section 5.2.3) and diaminochloro-triazine (short-term repeat-dose study; Section 4.4.2: and chronic study; Section 6.4.1).

6.1.7 2-Year F344 rat dietary study

Pinter A, Torok G, Borzsonyi M, Surjan A, Csik M, Kelecsenyi Z and Kocsis Z (1990) Long-Term Carcinogenicity Bioassay of the Herbicide Atrazine in F344 Rats. National Institute of Hygiene, Dept of Morphology, Budapest, Hungary. Neoplasma 37, 533-544

This study was performed by the Hungarian National Institute of Hygiene within the framework of an agreement with the International Agency for Research on Cancer (IARC). Atrazine was administered in the diet to inbred Fischer F344/LATI rats (50-56/sex/dose; 150-180 g at start) at 0, 375 and 750 ppm for 126 weeks. This strain, originally obtained from IFFA Credo (Ico) in 1981, has been bred in Hungary since then; while IFFA Credo appears to be an animal supply company of established international reputation, there is little information about the Hungarian colony eg. it was stated that "information on the spontaneous tumour rate for this strain is not available", although it was claimed that the spectrum of most frequent tumours corresponded to that of the US NCI/NTP Fischer rat strain. Atrazine was obtained from Ciba-Geigy (98.9% pure; Batch no. 0041124, Code no. G30027). Initial doses were 500 and 1000 ppm but were lowered after 8 weeks because of decreased bodywt gain and increased water consumption at the high dose.

Only 2/55 low-dose males, 2/53 high-dose males and 6/53 low-dose females survived to 123-126 weeks, albeit in a moribund condition. Numbers dying in the respective groups were: controls (22 M, 16 F); 375 ppm (20M, 14F); 750 ppm (15M, 11F); the others were killed during the course of the study. Cumulative survival curves indicated enhanced survival of atrazine-treated males (both doses), with a non-significant trend towards slightly increased survival in dosed females.

Bodywt gain was lower in treated animals, ostensibly dose-related, leading to an approximate 10-12% reduction in mean bodywts at 100 weeks at the high dose. Food intake was not significantly affected nor was water intake (after the dose was lowered; see above).

Some tumour incidence data is given in the accompanying Tables.

Males

Organ/Tumour	Control	Low Dose	High Dose
Mammary gland			
fibroma			3/53
fibroadenoma	1/48	1/51	2/53
adenoma			4/53
adenocarcinoma			1/53
no. animals with tumours	1/48	1/51	8/53
no. benign tumours	1/48 (2.1%)	1/512.0%)	9/53 (17.0%)*+
Organ/Tumour	**Control**	**Low Dose**	**High Dose**

Pituitary gland			
adenoma	9/42	21/41	17/43
Haematopoietic System			
leukaemia	13/47	21/47	21/48
lymphoma	9/47	5/47	11/48
combined	22/44 (50%)	26/47 (55.3%)	32/48 (66.7%)

Females

Organ/Tumour	Control	Low Dose	High Dose
Mammary gland			
fibroma	1/47	1/53	-
fibroadenoma	12/47	13/53	19/53
adenoma	2/47	7/53	5/54
adenocarcinoma	2/47	3/53	2/54
no. rats with tumours	16/47 (34.0%)	19/53 (35.8%)	19/54 (35.2%)
Uterus			
adenomatous polyp	9/45	9/52	3/45
adenocarcinoma	6/45	8/52	13/45*
squamous cell carcinoma	1/45	-	-
malig. mesenchymal tumour	-	2/52	1/45
no. rats with tumours	16/45	19/52	17/45
no. malignant tumours	7/45 (15.6%)	10/52 (19.2%)	14/45 (31.1%)#
Pituitary gland			
adenoma	32/41	23/43	35/50
Haematopoietic System			
leukaemia	8/44	10/52	15/51
lymphoma	4/44	6/52	7/51
combined	12/44 (27.3%)	16/52 (30.8%)	22/51 (43.1%)#

*p<0.05 Peto's incidental tumour test;
+ p<0.01 Fisher exact test, highdose vs low dose
# p<0.05 Cochran-Armitage trend test

In males, the only statistically-significant change was in the incidence of benign mammary tumours at the high dose, with mean latencies of 111 weeks (controls), 119 weeks (low dose) and 121.3 weeks (high dose). It may be noted that in NTP studies with Fischer F344/N rats, the control incidence of male benign mammary tumours was 13.4%, close to the incidence observed in the high-dose animals in this study (although note that F344/LATI strain rats were used in this study, for which there was no historical control data available). Apart from the benign mammary tumours, the only other indication of a possible increase in tumours in males was in pituitary gland adenomas, claimed not to be statistically significant (and with a combined incidence in males and

females of 49.4%, 52.4% and 55.9% in the control to high-dose groups respectively).

In females, there was a small increase in uterine adenocarcinomas at the high dose, with respective latencies (control, low dose and high dose groups) of 104.5, 110.6 and 108.2 weeks ie. there was a non-significant trend to increased latency with atrazine dose. The number of adenomatous polyps showed a negative trend, with no changes in latency.

The number of leukaemias and lymphomas increased significantly in high-dose females, with respective latencies (control, low-dose and high-dose respectively) being 96.3, 101.7 and 103.7 weeks ie. there was no evidence of any earlier onset of these tumours. In males there was no significant increase in the numbers of these haematopoietic tumours. It may be noted that the background incidence of combined leukaemias/lymphomas in these studies (50% in males, 27.3% in females) was significantly higher than in NTP studies with Fischer F344/N rats (30.1% for males, 18.9% for females; Solleveld, Haseman & McConnell, 1984).

In conclusion, there was an increase in benign mammary tumours in males (although with a possible slight increase in latency cf. those tumours in control animals), an increase in malignant uterine tumours in females, and an increase in haematopoietic system tumours. It is possible that atrazine treatment may have affected hormonal balance since the mammary gland and uterine tumours may be hormone-dependent tumours. However, it must be noted that there was no increase in mammary tumours in females. In this study, the dosed males actually had increased longevity cf. control animals. Unfortunately, historical control data for the specific strain used was not available for comparison with the results obtained in this study. The increases in male mammary tumours is likely to be largely attributable to the significantly longer lifespan of the high-dose animals[2]. Furthermore, the incidence is well within the historical control incidence for Fischer rats in NTP studies. However, neither tumour to age adjustment nor comparison to background control data of the laboratory were performed in this study. In two other studies conducted by Hazleton (Osheroff, 1990b; Thakur, 1991b, Eldridge et al, 1993: see Section 6.1.13, 6.1.14 & 6.1.15, & Thakur, 1992b: see Section 6.1.16), no increase in mammary tumours was noted in male Fischer 344 rats, although it should be noted that the increase in this study was only seen at the high dose of 750 ppm, not at the low dose of 375 ppm, whereas in the two Hazleton studies, the highest dose used was 400 ppm. The lack of any increase in breast cancer incidence in the females does not lend support to the hypothesis that atrazine is acting as a xenoestrogen.

[2] A laboratory visit by Novartis scientists noted that, of 18/55 high-dose males surviving at the time the last control animal died, 5/18 had mammary tumours (Novartis comment on the draft of the ECRP report, submitted 29 April 1997).

Since there was a dose-related depression of bodywt gain, no NOEL was established in this study. However, a NOEL for significant increases in several tumour types at the highest dose tested (37.5 mg/kg bw/d) was 375 ppm (estimated 18.75 mg/kg bw/d).

6.1.8 2-Year SD rat dietary study (*in utero* exposure)

Rudzki MW, McCormick GC & Arthur AT (1991) Chronic Toxicity Study in Rats. Ciba-Geigy Corp., Summit, New Jersey, USA. Division of Toxicology/Pathology, Safety Eval'n Facility. Lab. study no. 852214. Study completion date 28 January 1991 (not full GLP)

The pathology component of this study is reported separately below (Section 6.1.9) and the tumour incidence data (especially pituitary tumours) is discussed. This report includes information on bodyweight, haematology, clinical biochemistry, urinalyses, clinical observations, oestrous cycle and organ weights. Because it used rats from a previously-conducted study, it was not conducted in full compliance with US EPA GLP.

The study was conducted according to US EPA Guidelines no. 83-1. Groups of Crl:VAF/Plus CD(SD)BR Sprague-Dawley rats (50/sex), taken from the F1 generation of a 2-generation reproduction study (no. 852063) were dosed with technical grade atrazine (FL 841802; 97.6% purity) at 0, 10, 50 or 500 ppm for 8, 35, 52 or 104 weeks. The rats had been previously exposed to the same dose levels *in utero*. From weeks 65-104, 10 females of the 500 ppm group were placed on a control diet (0 ppm) and 10 of the control females were fed 500 ppm. Diets were homogeneous and compound concentrations in the diet were within 7% of the target concentration at all times, with excellent stability. Interim sacrifice (10/sex/group) were carried out at 8 and 32 weeks. All surviving males were killed at 52 weeks and surviving females at 105 weeks. Haematology, clinical chemistry, and urinalysis parameters measured are listed in Appendix III. Organs weighed included those in Appendix IIVplus epididymes, pituitaries, seminal vesicles, submaxillary salivary glands, thymus, and uteri.

Mean compound intakes for the control to high-dose groups (calculated to the end of week 52 for males, week 64 for females) were calculated to be 0, 0.5, 2.3 and 23.6 mg/kg bw/d (males) and 0, 0.7, 3.5 and 37.6 mg/kgbw/d (females).

There were no treatment-related effects on survival and the most frequently observed clinical signs were cachexia, paleness, perineal staining and stains on fur, which were all typical for animals of this strain and age. No ophthalmoscopic changes were noted as a result of treatment.

Decreased mean body weights were seen in the 500 ppm animals throughout the study, accompanied by decreased food consumption. These reductions were statistically significant for a large proportion of the study days and the

cumulative bodyweight gain for males (83% of controls) and females (83% of control) were both statistically significant.

Haematology findings were unremarkable with the only findings possibly associated with treatment being a reduction in red cell parameters (RBCs, haemoglobin and haematocrit) in 500 ppm females on days 268 and 364. These reductions did not correlate with any other findings and may have been related to the depressed bodyweight in the high-dose animals. No other treatment-related effects on haematology were seen.
Serum biochemistry and urinalysis results were both unremarkable, apart from a possible treatment-related increase in mean serum cholesterol, probably of little toxicological significance; in females, cholesterol (measured on days 87, 177, 268 and 364) was increased at the high dose (approx. 30%, statistically significant at all times apart from the day 364 assay), at the mid-dose (20-25% range, statistically significant only at day 87), and the low-dose (10-22% range, not statistically significant at any time point).

No differences in oestrous cycle between control and treated groups were noted. The stage of the oestrous cycle was determined from vaginal washes of females at weeks 35 and 104. All animals sacrificed at week 35 were in oestrus and the majority of animals in all groups killed at the end of the study (week 104) were in dioestrus. No differences between control and treatment groups were noted. At autopsy there were no apparent treatment-related changes in organ weights in any of the treatment groups.

The findings described above indicate a minimal toxicological effect of technical grade atrazine. The carcinogenic effect of treatment is fully discussed in the pathology evaluation (see Section 6.1.9). In summary, the incidences of pituitary tumours and mammary gland tumours at 104 weeks were as follows:-

Atrazine	Pituitary Tumors	Mammary Gland Tumours	Adenomas	Carcinomas	Aden.+ Carc.
0 ppm	45%	20%	40%	55%	
10 ppm	67%	20%	13%	33%	
50 ppm	55%	33%	17%	43%	
500 ppm	85%	37%	32%	58%	

Considering non-neoplastic changes, treatment-related changes at 500 ppm included reductions in mean body weight and food consumption (both sexes); reduction in mean erythroid parameters (females) and increases in mean serum cholesterol (females). The latter finding was also seen at 50 ppm. On the basis of these LOELs, the NOEL in males was 50 ppm (2.3 mg/kg bw/d) and 10 ppm (0.7 mg/kg bw/d) in females.

6.1.9 2-Year SD rat dietary study - pathology report

Ackerman LJ (1991) Atrazine Technical: Chronic Toxicity Study in Rats. Pathology Report. Ciba-Geigy Corp., Summit, NJ. Lab: Experimental

Pathology Laboratories Inc., USA. Study No. MIN 852214. Pathology report no. 88117. Report date 7 May 1988. Amended Report dated 16 Jan. 1991 (with separate summary written by Iversen WO)

Groups of Crl:VAF/Plus CD(SD)BR Sprague-Dawley rats (50/sex), taken from the F1 generation of a 2-generation reproduction study (no. 852063; Mainero et al, 1987: see Section 7.2) were dosed with technical grade atrazine (FL 841802; 97.6% purity) at 0, 10, 50 or 500 ppm for 8, 35, 52 or 104 weeks. The rats had been previously exposed to the same dose levels *in utero*. From weeks 65-104, 10 females of the 500 ppm group were placed on a control diet (0 ppm) and 10 of the control females were fed 500 ppm. Male rats from the laboratory stock (untreated) were assigned to similar treatment groups for 8 and 52 weeks.
Microscopic examinations were performed on female pituitary and mammary glands, on any palpable or gross lesions, and on all male mammary glands, testes, and any gross lesions. Female pituitary glands were stained for prolactin, FSH and LH at weeks 8, 35 and 104.

Male rats fed 10, 50 or 500 ppm atrazine for 8 and 52 weeks showed no gross lesions or treatment related changes in the mammary gland or testes. The testes showed incidental changes, primarily unilateral atrophy of the spermatogenic tubules, with or without sperm granulomas. Female rats fed 10, 50 or 500 ppm atrazine for 8 and 35 weeks showed no treatment related lesions or palpable masses, and no treatment-related changes in the pituitaries and mammary glands.

There was an increased incidence of pituitary tumours (all adenomas of the anterior pituitary) in female rats exposed to 10, 50, and 500 ppm atrazine for 104 weeks, compared to controls (male pituitaries not sampled). There was also an increased incidence of pituitary tumours in female rats fed 500 ppm atrazine from week 65 to week 104, whilst rats returned to normal diet from 500 ppm atrazine after 65 weeks had a slightly lower incidence of pituitary tumours than rats fed 500 ppm for the entire 104 weeks.

The incidences of pituitary tumours (adenomas) in female animals were: -

atrazine (ppm)	104 weeks exposure	exposure after 65 weeks
0	9/20 (45%)	6/8 (75%)
10	20/30 (67%)	-
50	16/29 (55%)	-
500	16/19 (85%)	8/9 (89%)
*Historical controls**		
adenoma		80 - 89%
carcinoma		0 - 4%

* From seven 104-week studies conducted between Jan. 1983 to June 1985 (start dates)

Concurrent with the increased incidence of pituitary tumours, there was an increased incidence of angiectasis and pigment deposition in the pituitary.

There was a high incidence of mammary gland tumours in both the control and high dose female rats after 104 weeks of exposure:

Atrazine (ppm)	Fibroadenomas	Carcinomas	Combined
0	4/20 (20%)	8/20 (40%)	11/20 (55%)
10	6/30 (20%)	4/30 (13%)	10/30 (33%)
50	10/30 (33%)	5/30 (17%)	13/30 (43%)
50	7/19 (37%)	6/19 (32%)	11/190 (58%)
Historical mean & range	29%	17% (26-31%)	(14-20%)

There was also a high incidence of carcinomas in the 10 control females placed on 500 ppm after 65 weeks, and a proportionately lower incidence of mammary gland tumours in 500 ppm females removed from treatment at 65 weeks. Additional microscopic changes present in the mammary glands consisted of duct ectasia, cysts, and atypical epithelial proliferations. No pituitary tumours or mammary gland neoplasms were observed in any rats prior to 104 weeks.

Immunohistocytochemical stains for prolactin, FSH and LH showed a high incidence of prolactin staining adenomas of the pituitaries in all groups, with very few tumours staining for FSH or LH. The incidence of prolactin staining cells in the pituitaries of mid-dose, but not high-dose females was slightly higher. The overall staining of pituitary adenomas at 104 weeks for prolactin, FSH and LH was, respectively, 70.7% 5.3% and 8.0%. Most, but not all rats with mammary tumours had prolactin-staining pituitary adenomas.

Comments: The study findings were obscured by a lack of statistical tests on tumour incidences. Chi-square tests of pituitary tumour incidences showed that the numbers of tumours were only significantly increased in high dose animals (weeks 0-104, 65-104), and there was no overall association between dose and pituitary tumour incidence ($P<0.05$). Historical control data on pituitary tumour incidences indicate an unusually low control incidence.

Mammary tumours were not significantly increased in treated female rats in the present study. A previous study in the same strain showed significant increases in mammary tumours at 500 and 1000 ppm (72% and 88% incidence respectively). This difference cannot be accounted for on the basis of differences in control incidences, which were almost identical (55%, 53%).

In the present study, increased incidences of pituitary tumours were not accompanied by increases in the percentages of prolactin-staining tumours, or mammary gland tumours.

In conclusion, no treatment-related changes were observed in male rats exposed to atrazine at dietary concentrations up to 500 ppm. The apparent increase in pituitary tumours in females at the high dose (males not affected) at the high dose must be considered in relation to the historical control incidence; it

appears that the incidence in control, low- and mid-dose animals was unusually low and that the incidence at the high-dose was within the normal range of incidence for this strain and laboratory. Longevity can be eliminated as a factor in incidence differences in this study since the mortality rates were similar across groups.

Of the mammary tumour-bearing animals with pituitary neoplasms, 75% of the pituitaries stained positive for prolactin. Thus, results from this complex protocol indicate some hormonal influence of the pituitary on the mammary gland but the exact significance is equivocal. Mammary tumours were not significantly increased in treated female rats in the present study.

6.1.10 2-Year SD rat dietary study - interim report: hormone levels

Osheroff MR (1990a) Determination of Hormone Levels in Sprague-Dawley Rats Treated with Atrazine Technical: 12-Month Interim Report. Ciba-Geigy Corp., Greensboro, NC, USA. Hazleton Labs America Inc., Vienna, Virginia, USA Study no. HLA Study No. 483-278; Interval completion date 21 Mar. 1990 (GLP; US EPA, Japanese MAFF)

and

6.1.11 2-Year SD rat dietary study - final report: hormone levels

Thakur AK (1991a) Determination of Hormone Levels in Sprague-Dawley Rats Treated with Atrazine Technical. Ciba-Geigy Corp., Greensboro, NC, USA. Lab: Hazleton Washington Inc., Vienna, Virginia, USA. Study no. HWA 483-278. Completion date 17 October 1991 (GLP; US EPA, Japanese MAFF)

This study, conducted in accordance with OECD Guideline no. 451, was designed to investigate the toxicity/oncogenicity of technical grade atrazine and to attempt to correlate serum hormone levels with the onset of mammary tumours in female Sprague-Dawley (SD) rats.

Female Crl:CD BR Sprague-Dawley rats from Charles River Labs, Raleigh, NC (70/group) were given 0, 70 or 400 ppm atrazine technical (FL-850612; Batch no. D3413J10; purity 97%) in the diet for at least 2 years. Dosing commenced on 22 March 1989 when rats were approx. 6-weeks old and necropsy was completed on 27 March 1991. Analyses of diet for stability, homogeneity and compound concentration (on 26 randomly-selected weekly mixes) revealed acceptable mixing, with concentrations within 2.5% of target (not corrected for purity). Weekly mean (but not overall mean) intakes were calculated; at week 52, intakes were 0, 3.7 and 23.1 mg/kg bw/d and at week 100, intakes were 0, 3.3 and 27.4 mg/kg bw/d. Observations were made for mortality and moribundity (twice daily), clinical signs (daily), thorough physical examination (weekly), and body weights and food consumption (weekly for 16 weeks then every 4 weeks). Interim kills were performed on 10 rats per group at 1, 3, 9, 12 15 and 18 months; at 24 months the remaining rats were killed. To prevent stress-related increases in circulating plasma hormone

levels, animals were killed by decapitation without anaesthesia. Trunk blood was collected and analysed by the sponsor (Ciba-Geigy) for hormone levels. A full autopsy was performed on all animals after both scheduled and unscheduled deaths. Microscopic pathology examinations were performed on pituitary, mammary glands, uterus, and ovaries.

Prior to each scheduled interim kill, the oestrous cycles of the designated animals were determined using a daily vaginal smear test. After a minimum of 14 or maximum of 21 days of vaginal smears, each animal was killed at the proestrous phase.

The number of unscheduled deaths which occurred during the study was 5, 6 and 8 in the 0, 70 and 400 ppm groups, respectively and five of the deaths in the 400 ppm group occurred prior to the earliest death in the control or 70 ppm groups. Statistical analysis therefore indicated a significant negative trend in survival at 400 ppm. Clinical examinations revealed rough haircoat, alopecia,, sores, swollen body areas and an earlier onset of palpable masses (confirmed histologically as tumours) at the high dose. Approximately 64% of the total masses in the 400 ppm group were first noted in the first year of the study compared to 38% and 19% in the control and 70 ppm groups, respectively.

As described in previous chronic studies with this substance, mean body weight and bodyweight gain were statistically reduced for the 400 ppm group at all time intervals and sporadically for the 70 ppm group. This was accompanied by slight but statistically significant reductions in food 2consumption, particularly in the early part of the study.

Gross pathology The incidence of fluid-filled uteri increased in atrazine treated groups (5, 15 and 15 for 0, 70, 400 ppm, respectively), with most cases being recorded in the first year. The effect was not accompanied by histopathological changes.

Organ weights Statistically significant changes observed were sporadic and unrelated to treatment.

Histopathology Microscopic evaluations were performed on stained sections of pituitary, ovaries, uterus and mammary gland at each of the interim scheduled sacrifice intervals and remaining animals killed at the end of dosing period, and on the unscheduled deaths during the study.

Statistical analysis of tumour incidence revealed no significant effect of treatment on pituitary tumour incidences and overall, both treated groups showed slightly lower prevalence rates than the control. There was no significant difference in lifetime mammary tumour incidences (carcinoma/fibroadenomas) but because of the slightly earlier onset time seen in the 400 ppm group (5 rats with mammary tumours at 9 months cf. the first control group mammary tumour at 12 months), there was a significant positive trend for combined carcinomas/fibroadenomas. The incidence of the non-neoplastic lesion, mammary galactocele, was increased in atrazine-treated

animals during the first year of the study. Overall incidences of pituitary and mammary lesions are shown in the Table (see below).

Incidence of pituitary and mammary gland lesions in female SD rats (70/group)

	Group		
Lesion	**0 ppm**	**70 ppm**	**400 ppm**
Pituitary adenoma			
1-12 months	2	2	8
13-24 months	20	4	12
Total	22	16	20
Mammary galactocele			
1-12 months	6	9	20
13-24 months	23	21	21
Total	29	30	41
Mammary fibroadenoma			
1-12 months	1	0	4
13-24 months	7	12	9
Total	8	12	13
Mammary carcinoma			
1-12 months	0	0	6
13-24 months	9	1	5
Total	9	4	11

No correlation between animals with mammary tumours and those with pituitary tumours was readily apparent. Of the 0, 70 and 400 ppm animals with mammary tumours, 67%, 53% and 57% respectively had pituitary tumours; conversely, 45%, 47% and 60% of those with pituitary tumours had mammary tumours.

Oestrous cycles and hormone levels Oestrous cycle and hormone level data (oestradiol and progesterone) are presented in Appendix VI, VII and VIII. Overall, atrazine-treated SD rats tended to spend more days in oestrus than did age-matched untreated controls; this effect was statistically significant at 400 ppm after 9 and 18 months and in the 70 ppm group after 1 and 9 months. The proportion of time SD controls spent in oestrus increased with age (Appendix VI). Oestradiol concentrations were significantly elevated at 3 months at 400 ppm (non-significantly at 70 ppm), marginally elevated at 9 months at 400 ppm. Progesterone levels were unaffected.

Based on the data presented in this report, the toxicological effects of atrazine are: slightly reduced lifespan (Appendix VIII); reduced mean bodyweight/gain; an increased incidence of fluid-filled uteri; an increased incidence of mammary galactocele; a somewhat earlier onset of mammary and pituitary tumours, without any increase in their overall lifetime incidence. All of the above

effects were seen at 400 ppm and reduced bodyweight and fluid-filled uteri (indicative of oestrus?) also occurred at 70 ppm so a NOEL was not able to be determined.

6.1.12 2-Year SD rat dietary study

Thakur AK (1992a) Oncogenicity Study in Sprague-Dawley Rats with Atrazine Technical. Lab: Hazleton Washington Inc., Vienna, Virginia, USA. (name changed during May 1990 to Hazleton Washington, Inc.) Study no. HLA/HWA-483-275. Report date 27 Jan. 1992. Ciba-Geigy Corp., Greensboro, NC. (GLP; US, Japan)

This study was conducted according to US EPA guideline no. 83-2. Atrazine technical (FL-860612; Batch no. D3413J10; purity 97%) was administered to female Crl:CD BR Sprague-Dawley rats from Charles River Labs, Raleigh, NC (60/group) at dietary levels of 0, 70 and 400 ppm for at least 104 weeks. Dosing commenced on 23 Feb. 1989 when rats were approximately 6-weeks old and necropsy was completed on 22 March 1991. Analyses of diet for stability, homogeneity and compound concentration on 26 randomly-selected weekly mixes revealed acceptable mixing, with concentrations within 2.5% of target (not corrected for purity). Weekly mean (but not overall mean) intakes were calculated; at week 52, intakes were 0, 3.5 and 21.6 mg/kg bw/d and at week 104, intakes were 0, 3.0 and 20.7 mg/kg bw/d. Observations were made for mortality and moribundity (twice daily), clinical signs (daily), thorough physical examination (weekly), body weights and food consumption (weekly for 16 weeks then every 4 weeks), and haematology (leucocyte differential and cell morphology only, at weeks 52, 78 and 104). All surviving animals were killed after 24 months; gross pathology, organ weights (liver, kidneys, brain/brainstem, spleen, thyroids/parathyroids and uterus) and microscopic pathology (uterus, ovary, pituitary and mammary glands) investigations were performed.

The numbers of unscheduled deaths which occurred during the treatment period were 29, 35 and 38 at 0, 70 and 400 ppm, giving 52, 41 and 37% survival rates, respectively; there was a statistically significant negative trend for survival. Weekly physical examinations of the animals revealed a large number of palpable tissue masses in each group, typical of this strain of rats.

There were treatment-related decreases in mean bodyweight gain in the high dose group throughout the study; the mean bodywt change at week 104 was -4.3% cf. controls. The mid-dose group had an increased mean body weight compared to controls for the whole interval from 13-104 weeks of the study; at term, the mean bodywt was 15.7% greater than controls. A decrease in food consumption was noted for the high-dose group during the first 13 weeks of the study.

Haematology at week 52 indicated a significant increase in the nucleated red cell count; it was suggested that this was not consistent with findings in other studies and was unlikely to be of biological significance. However, mild

anaemia was sufficiently commonly observed in other studies with triazines to suggest that inhibition of haematopoiesis may be a toxic effect of the class and this finding could be a compensatory response to that effect.

There were no unusual gross findings associated with atrazine treatment. When palpable masses were confirmed histopathologically as tumours, the first palpation of the mass was used to establish an onset time of the tumour. Absolute and relative organ weights did not reveal any noteworthy findings.

Microscopic examination was carried out on stained sections of pituitary, mammary glands, uterus and ovaries. Almost all animals in all groups exhibited pituitary and/or mammary tumours (see Table below) but there was no overall dose-related increase in the incidence of these tumours. However, there were a greater number of mammary tumour-bearing animals in the high-dose group during the first 52 weeks of the study (see Table below; also Appendix VIII), indicating that 400 ppm atrazine had shortened the time to onset of the tumours. No such effect was seen at 400 ppm for pituitary tumours or at 70 ppm for mammary tumours. There was a significant decrease in endometrial stromal polyps in the uterus of dosed animals; incidences were 8/60, 0/60 and 1/60 in the control, low-dose and high-dose groups.

Summary of pituitary and mammary tumour incidence in female SD rats (60/gp)

Dose (ppm)	No. of animals with pituitary tumours	No. of animals with mammary tumours	No. of animals with both tumours
0	44	45	34
70	46	34	26
400	47	49	39

Onset time of palpable mammary masses (fibroadenomas and/or carcinomas)

Dose (ppm)	No. of rats with mammary palpable mass	
	weeks 1-52	weeks 53-106
0	2 (46,46)	44
70	3 (34,35,50)	31
400	9 (20,25,27,27,35, 37,39,47,50)	40

Note: No's. in brackets indicate the week in which palpable mass was first detected

The above findings indicate a NOEL of 70 ppm (approximately 3.5 mg/kg bw/d), based on reduced bodyweight, slightly increased mortality rate and an earlier onset of mammary tumours at the next dose. There was no statistically-significant effect at the high-dose for lifetime mammary fibroadenoma and/or carcinoma incidence; in fact, at the 70 ppm dose, the lifetime incidence of this was significantly lower than controls. There was no effect at either dose on the onset or incidence of pituitary tumours.

6.1.13 2-Year female F344 rat dietary study - interim report

Osheroff MR (1990b) Determination of Hormone Levels in Fischer-344 Rats Treated with Atrazine Technical: 52-Week Interim Report. Ciba-Geigy Corp., Greensboro, NC, USA. Hazleton Labs America Inc., Vienna, Virginia, USA Study no. HLA Study No. 483-279; Interval completion date 2 April 1990 (GLP; US EPA, Japanese MAFF)

and

6.1.14 2-Year female F344 rat dietary study - toxicology report

Thakur AK (1991b) Determination of Hormone Levels in Fischer-344 Rats Treated with Atrazine Technical. Ciba-Geigy Corp., Greensboro, NC, USA. Lab: Hazleton Washington Inc., Vienna, Virginia, USA. Study no. HWA 483-279. Completion date 8 Nov. 1991 (GLP; US EPA, Japanese MAFF)

and

6.1.15 2-Year female F344 rat dietary study - endocrine report

Eldridge JC, Wetzel LT, Tisdel MO & Luempert LG (1993) Determination of Hormone Levels in Fischer-344 Rats Treated with Atrazine Technical. Ciba-Geigy Corp., Greensboro, NC, USA. Lab: Bowman Gray School of Medicine, Winston-Salem, North Carolina, USA. Study no. HWA 483-279. Completion date 8 April 1993 (GLP; US EPA, Japanese MAFF)

This study, conducted in accordance with OECD Guideline no. 451, was designed to investigate the toxicity/oncogenicity of technical grade atrazine and its effects, if any, on the oestrous cycle and on selected hormone levels in female Fischer-344 rats. The toxicology portion of this study (including histopathology) was presented in the report dated 8 Nov. 1991, whilst the report dated 8 April 1993 presented the methods, results, discussion and conclusions of the endocrine portion of the study. It was set up and conducted in an identical manner to study HWA 483-278 above (Osheroff, 1990; Thakur, 1991a: Section 6.1.10 & 6.1.11), but using female CDF (Fischer-344)/Crl BR strain rats instead of female Sprague Dawley rats. Additional doses of 10 ppm and 200 ppm were included in the dosing schedule, giving a total of five groups: 0, 10, 70, 200 and 400 ppm (70 female rats/group). As for the previous study, the results of the oestrous cycle and hormone level analyses were reported.

Atrazine technical (FL-850612; Batch no. D3413J10; purity 97%) was used. Analyses of diet for stability, homogeneity and compound concentration (on 26 randomly-selected weekly mixes) revealed acceptable mixing, with concentrations within 3.7% of target. Rats were from Charles River Labs, Raleigh, NC, with dosing in the diet for up to 104 weeks; dosing commenced on 27 March 1989 when rats were approximately 7-weeks old and terminal necropsies were completed on 1 April 1991. Weekly mean (but not overall mean) intakes were calculated; at week 52, intakes at 0, 10, 70, 200 and 400 ppm in the diet were 0, 0.60, 4.3, 12.4 and 24.5 mg/kg bw/d and at week 104, intakes were 0, 0.60, 3.95, 11.9 and 24.15 mg/kg bw/d. Observations were made for mortality and moribundity (twice daily), clinical signs (daily), thorough physical examination (weekly), and body weights and food consumption (weekly for 16 weeks then every 4 weeks). Interim kills were performed on 10 rats/group at 1, 3, 9, 12 15 and 18 months; at 24 months the remaining rats were killed. To prevent stress-related increases in circulating plasma hormone levels, animals were killed by decapitation without anaesthesia. Trunk blood was collected and analysed by the sponsor (Ciba-Geigy) for hormone level determinations. A full autopsy was performed on all animals after both scheduled and unscheduled deaths. Microscopic pathology examinations were performed on pituitary, mammary glands, uterus, and ovaries.

Vaginal smears (for vaginal cytology and oestrous cycle staging) were taken from 10 animals/group beginning two weeks prior to the start of each scheduled sacrifice. Hormone assays on serum samples from rats sacrificed at

1, 3, 9, 12, 15, 18 and 24 months included RIA assays for oestradiol, progesterone, prolactin and corticosterone.

In this study there was no effect of treatment on survival or on the incidence of clinical observations. Mean bodyweight and bodyweight gain were generally lower in the 200 and 400 ppm groups and these were statistically significant at some points over about the first year for the 200 ppm group and throughout the study for the high-dose group; at 52 weeks, bodywt gains (as a percentage of control bodywt gain) were 110.7%, 98.5%, 94.3% and 89.2% in the low- to high-dose groups respectively. In terms of overall mean bodyweights, effects were relatively small, with 5.2% and 6.6% reductions at the 200 and 400 ppm doses at study term. As with other studies, the reduced bodyweights were accompanied by slight reductions in food consumption by the treated animals, especially in the early stages of the study.

Gross pathology/organ weights There were no treatment-related gross pathological or organ weight findings.

Histopathology Microscopic evaluation was carried out on stained sections of pituitary, ovaries, uterus and mammary gland. None of the lesions seen showed dose-related incidence in either number of animals affected or the time of onset.

Oestrous cycles and hormone levels Oestrous cycle and hormone level data (oestradiol and progesterone) are presented in Appendix V, VI and VII. No effects of atrazine on the percentage of days female Fischer rats spent in oestrus were seen. Normal age-related changes in control and treated animals included a shift in oestrous cycle patterns towards an enhancement of the percent of total days spent in proestrus at the expense of days in oestrus. Whereas the time Sprague-Dawley controls spent in oestrus tended to increase with age, that in Fischer rats tended to decrease, especially after 15 months. Serum progesterone levels rose continuously throughout the study whereas oestradiol levels increased over approx. the first year then declined. Oestradiol and progesterone concentrations were not significantly affected by atrazine dosing.

The NOEL may be taken as 70 ppm (between 3.95-4.3 mg/kg bw/d), based on reduced bodyweight gain at the higher doses of 200 and 400 ppm.

6.1.16 2-Year F344 rat dietary study

Thakur AJ (1992b) Two-Year Dietary Oncogenicity Study in Fischer-344 Rats with Atrazine Technical. Lab: Hazleton Washington Inc., Vienna, Virginia, USA. Study no. HWA-483-277. Report date 18 Feb. 1992. Ciba-Geigy Corp., Greensboro, NC. (GLP; US, Japan)

This study was conducted according to US EPA guideline no. 83-2. Cr1:CDF (Fischer-344) rats from Charles River Labs, Raleigh, NC (60/sex/group) were given atrazine technical (FL-850612; Batch no. D3413J10; purity 97%) at dietary levels of 0, 10, 70, 200 and 400 ppm for at least 2 years. Dosing commenced on 28 March 1989 when rats were approx. 8-weeks old and

necropsy was completed on 8 April 1991. Analyses of diet for stability, homogeneity and compound concentration on 26 randomly-selected weekly mixes revealed acceptable mixing, with concentrations within 3.7% of target (not corrected for purity). Weekly mean (but not overall mean) intakes were calculated; at week 52, respective intakes (control - high-dose groups) were 0, 0.42, 2.9, 8.2 and 17.5 (males) and 0, 0.54, 3.9, 11.5 and 24.6 (females) mg/kg bw/d and at week 104, were 0, 0.48, 3.40, 9.7 and 20.5 (males) and 0, 0.55, 3.8, 11.2 and 24.2 (females) mg/kg bw/d. Animals were observed for bodyweight, mortality, morbidity and clinical signs. Blood samples were taken for haematology examination (leukocyte differential and cell morphology only) at weeks 52, 78 and 104 of treatment from control, high-dose and all moribund animals. After 24 months, all surviving animals were autopsied. Organ wts from all animals killed at scheduled sacrifice were recorded (organs listed at Appendix IV plus pituitary, uterus and seminal vesicles). Histopathology was carried out on all tissues from the control and high-dose groups and from those which died during the study; organs examined included those listed in Appendix IV (except gall bladder, Harderian, lacrimal and zymbal's glands). The pituitary, lung, liver, kidney, mammary gland and any gross lesions from the other dosage groups were also examined.

There was no increased mortality in any of the treatment groups and statistical analysis did not show any significant differences between treated and control groups. Clinical observations were only those expected for this strain of rat and were not treatment related.

A small dose-related decrease in bodyweight gain was noted for the 200 ppm (3% less weight than controls at 104 weeks) and 400 ppm (approx. 6% less weight than controls) groups and this was associated with decreased mean food consumption throughout the study for 400 ppm males and during the first 13 weeks for 400 ppm females.

At gross pathological examination, no differences were found between treated and control groups. No dose-related effects on organ weight or organ weight ratios were found; there was no evidence of any decrease in absolute or relative testicle weights.

The most common cause of unscheduled deaths was mononuclear cell leukaemia but the incidence was not related to dose and was within historical limits. Pituitary and mammary tumours were quite common in all groups but again were not related to dose. Mammary tumours were less common than for SD rats (see studies at Sections 6.1.10, 6.1.11 and 6.1.12 above) and there were no significant differences between the groups. The incidences of pituitary and mammary tumours are shown in the Table below. The number of high-dose rats with pituitary neoplasia was somewhat less than in the control or lower dose groups.

Pituitary and mammary tumours in Fischer-344 rats (60/group)

Dose (ppm)	Sex	Adenoma	Pituitary Carcinoma	Mammary Fibroadenoma	Carcinoma	Total
	Male					
0		15	0	2	0	2
10		14	0	0	1	1
70		15	0	0	0	0
200		11	0	0	0	0
400		8	0	1	0	1
	Female					
0		22	1	2	2	4
10		26	2	5	0	5
70		20	0	5	2	7
200		19	1	7	3	10
400		13	2	6	2	8

Hazleton Washington historical tumour incidence - Fischer rats.

pituitary tumours:	Range (male)	16-36%
	Range (female)	32-60%
mammary tumours:	Range (female)	0-20%

A significant increase in the non-neoplastic lesion, kidney transitional cell hyperplasia, was noted by the pathologist for males in the high dose group (with a significant increased trend at the lower doses). No significant effect was reported for females. However, data relating to this lesion could not be found in any of the summary tables or in the individual animal data. In the liver of females, but not males, there was an apparent small increase in hepatopathy (described as "leukaemia-associated hepatopathy") in dosed groups but there was no dose-relationship (5/60, 16/60, 11/60, 10/60 and 13/60 in the control - high-dose groups respectively). The incidence of the neoplastic finding, mononuclear cell leukaemia (haematoneoplasia), was 8/60, 18/60, 14/60, 11/60 and 15/60 in females ie. there was no dose related trend and the increase was only significant at the low dose, although not significant with correction for multiple comparisons. The historical control incidence (Hazleton Washington) was 10-40% ie. the above rates were well within the historical control range. There was no increase in this finding in dosed males.

Overall, the results of this study did not indicate any carcinogenic effect of atrazine technical for Fischer-344 rats at dietary doses up to 400 ppm and the only toxic effect of the treatment was slightly decreased bodyweight gain. The NOEL for this effect was 70 ppm (approximately 3.5 mg/kg bw/d).

6.1.17 One-Year female SD rat dietary study

Pettersen JC & Turner JC (1995) 1-Year Chronic Toxicity Study with Atrazine Technical in Rats. Ciba-Geigy Corp., Farmington, CT. Study no. F-00171. Completion date 8 Dec. 1995 (GLP; OECD, USA, Japan)

This study was conducted according to OECD Guideline no. 453 ,USA EPA Guideline 83-5 and MAFF Guideline 1985, and was designed to determine the effect of atrazine dosing for 12 months on the mammary and pituitary glands of female SD rats, the oestrous cycle, and plasma levels of oestradiol, luteinising hormone, progesterone and prolactin. Atrazine (Lot no. FL-881692; 97.1% purity) was administered in the diet to female Crl:CD (SD) BR rats from Charles River Labs, Raleigh, NC (55/group) at concentrations of 0, 15, 30, 50, 70 and 400 ppm for up to 12 months; doses were chosen on the basis of HLA Project no's. 483-275 and 483-278, in which CD rats were given atrazine in the diet at 70 and 400 ppm for up to two years. Ten females/group were sacrificed at 3, 6 and 9 months, and the remainder (25/group) at 12 months. At each of these time points, oestrous cycle determinations, plasma hormone levels, and pathological examinations were recorded. At study commencement, rats were approx. 6 weeks old. Dosing occurred over the period 29 Nov. 1988 to 3 March 1989. Observations of clinical condition and mortality were performed at least twice daily, physical examination (including palpation), bodyweight and food consumption weekly. For oestrous cycle determination, vaginal smears were taken from 21 days before scheduled sacrifice for between 14-22 consecutive days. For hormone analysis, blood samples (from 15 females/dose) were taken from the intra-orbital plexus from animals during the first proestrus seen after a minimum of 14-days oestrous cycle sampling. Other sacrifice animals were killed during proestus (as outlined above) and trunk blood collected; animals were individually isolated before decapitation to prevent stress-related changes in hormone levels. Organs weighed, only in scheduled sacrifice animals, included brain, ovaries, uterus and pituitary gland. Tissues examined histopathologically included pituitary gland, ovaries, uterus, vagina, inguinal skin with mammary gland, and any other masses or gross lesions.

Tests revealed acceptable test compound stability and homogeneity and concentration in the diet; concentrations were within 3.3% of the target at all doses. Calculated mean daily compound intakes were 0, 0.8, 1.7, 2.8, 4.1 and 23.9 mg/kg bw/d, respectively.

There were no clinical signs which were related to treatment and unscheduled deaths were 1, 2, 0, 1, 2 and 2 in the control to high-dose groups respectively ie. there was no compound-related increase in mortality. Statistically-significant decreases in bodyweight and bodyweight gain occurred at 400 ppm; over the course of the study, bodywt gains ranged from 82-89% of controls. Occasionally, slight statistically-significant reductions in bodywts and bodywt gains were seen at 70 ppm. Food consumption was slightly affected at 400 ppm during the first 11 weeks of the study (88-95% of control intake).

Raw oestrous cycle data were included in the report without comment but there did not appear to be any information on blood hormone levels; it is assumed that these will be reported in a study addendum.

Organ weight analysis was stated not to reveal any statistically-significant findings in mean organ weights at any sacrifice interval. However, absolute and relative pituitary weight was increased at the 12-month sacrifice in high-dose animals (values 148% and 170% of controls, respectively). Uterine weights at the high-dose were also heavier than controls at 6-, 9- and 12-month intervals. At 12 months, for example, absolute and relative weights were 113% and 125% of controls, respectively; with bodywt loss due to food restriction, there is a usually a decrease in relative uterine weight. Also, at the 9-month sacrifice interval there looked to be a decrease in absolute and relative (to bodywt) weights of ovaries in dosed animals, but there appeared to be an error in the calculations.

Treatment-related gross necropsy findings were confined to the high dose (400 ppm) and included an increased incidence of animals with skin masses (9/55 vs 4/55 controls), mammary gland hypertrophy (11/55 vs 6/55 controls) and pituitary gland enlargement (9/55 vs 5/55 controls).

The results of histopathological examinations are presented in the following Table.

Finding **Dose Group (ppm)**	**0**	**15**	**30**	**50**	**70**	**400**
mammary gland: no. examined	55	55	55	55	55	55
adenocarcinoma (malig.)	1	2	-	1	1	6
adenoma (benign)	-	-	1	-	1	1
fibroadenoma (benign)	2	2	2	1	4	4
pituitary gland: no. examined	54	55	53	54	55	54
adenoma (benign)	2	5	6	4	1	5
hyperplasia	2	-	4	3	1	2

It was noted that:

- there was no increase in non-neoplastic hyperplasias, galactoceles (milk cysts), or xanthogranulomatous inflammation in mammary gland sections;
- there was no increase in incidence of mammary gland adenomas or fibroadenomas;
- mammary gland adenocarcinomas were increased at the high dose (6/55 vs 1/55 controls);
- there was a slight increase in pituitary adenomas in some dosed groups but incidences were not statistically-significant and not in any way dose-related, so were considered spurious.

There were no statistically significant differences in the incidence of mammary gland adenomas fibroadenomas or adenocarcinomas at feeding levels up to 400 ppm, when analysed individually, although when combined, there was a

significant increase at 400 ppm. A significant positive trend for onset time existed when adenomas and fibroadenomas were combined or when all three tumour types were combined. No trend was evident if 400 ppm results were excluded. The NOEL for mammary tumour incidence and onset time was 70 ppm (4.1 mg/kg/d).

These pathology findings were confirmed by an independent pathologist, Robert F McConnell, of Consulting Pathology Services, Flemington, NJ. Findings of this study were completely consistent with previous studies (study no's 483-275 and 483-278; Sections 6.1.10 & 11, and 6.1.12) which indicated that, although the number of Sprague Dawley female rats with tumours increased at 12 months, this increase resulted from an earlier onset, without an overall increase in mammary tumour incidence following lifetime (2-year) exposure.

6.1.18 One-year dog dietary study (80W formulation)

Woodard MW, Cockrell KO, Lobdell BJ & Woodard G (1964) Atrazine - Safety Evaluation by Dietary Feeding to Dogs for 105-Weeks. Geigy Agricultural Chemicals. Woodard Research Corp. Laboratory & Consulting Service, Herndon, VA. Report date 27 Oct. 1964

Purebred beagles (3/sex/group) were fed atrazine in the diet at 0, 15, 150 and 1500 ppm (calculated as the active ingredient) for 105 weeks. The material, labelled as atrazine 80W (one lot no. 79769, the other unidentified), was a putty-coloured powder of unstated purity; it is a wettable powder containing 80% atrazine.

There were no deaths. Low-dose animals appeared to gain more weight than controls whilst high-dose animals lost weight (approx. 5% of starting weight) over the course of the study. Food intake (measured over short intervals at the start of each year of the study) appeared to be slightly reduced at the high dose and in mid-dose females. After approx. 6 months, muscular tremors of the rear limbs and sacral areas was noted in 5/6 high-dose dogs, and one mid-dose male had a convulsive seizure in the 20th month.

Haematological examination (Hb, Hct, sedimentation rate, total and differential WBC count) at 10 time points during the study and at term appeared to indicate a transient reduction in Hb and Hct at the high-dose early in the study. Clinical chemistry assays (BUN, AP, AST) did not reveal any significant compound-related effects. Urinalyses (appearance, specific gravity, pH, protein, sugar, microscopy) were negative for revealing any compound-related effects.

Slightly increased weights of adrenals were noted at the high dose (20% absolute increase, 36% in relative organ weight). Although highly variable, weights of ovaries and uteri of high-dose females were also reported as slightly increased. No significant histopathological changes were recorded.

The value of this study is limited because of the limited number of animals used. A NOEL of 150 ppm (equivalent to 3.75 mg/kg bw/d) could be established, based on decreased body weight and food consumption, and increased weights of adrenal glands (and possibly ovaries and uteri) at the next highest dose level of 1500 ppm. At the high dose of 1500 ppm, there were no apparent effects on mortality, on the limited haematology, clinical chemistry and urinalysis parameters determined, or on macroscopic and microscopic pathology.

6.1.19 One-year dog dietary study

O'Connor DJ, McCormick GC & Green JD (1987) Atrazine Technical: Chronic Toxicity Study in Dogs. Ciba-Geigy Corp. Agricultural Division, Greensboro, NC. Lab: Ciba-Geigy Pharmaceuticals Division, Summit, NJ. Study no. 852008. Toxicology/Pathology report 87048. Study completion date 27 Oct. 1987. (GLP; US EPA)

This study was conducted according to US EPA Guidelines no. 83-1. Purebred beagle dogs from Marshall Farms, North Rose, NY (4 to 6/sex/group) received dietary atrazine technical (Batch no. FL 850612; purity not stated) of 0, 15, 150 or 1000 ppm for at least 52 consecutive weeks. Treatment commenced on 12 Sept. 1985 and concluded on 16 Sept. 1986. Achieved concentrations were within 10% of target and homogeneity and stability were acceptable. Clinical signs, bodyweights, food consumption, physical/auditory (weeks-3, 13, 26, 39, 52), ophthalmoscopic (weeks-2, 10, 14, 26, 39, 52) and electro-cardiograph (weeks-2, 13, 26, 39, 53) examinations, haematology, serum chemistry and urinalysis (weeks -4, 13, 26, 39, 52) were recorded. Clinical chemistry and urinalysis parameters measured were those listed in Appendix III; haematology parameters included Hb, Hct, RBCs, WBCs, differential cell count, platelets, prothrombin time, reticulocytes and Heinz bodies. Animals were necropsied during week 53. Organs weighed were those listed in Appendix IV (plus pituitary and epididymes). Organs examined histopathologically were those listed in Appendix IV (plus tongue, but not including head sections, Harderian and Zymbal's glands).

Mean compound intakes were 0.48, 4.97 and 33.65 mg/kg bw/d (males) and 0.48, 4.97 and 33.8 mg/kg bw/d (females) in the 15, 150 and 1000 ppm groups respectively.

The following animals were sacrificed moribund: one 150 ppm male (day 75); one 1000 ppm male (day 250) and one 1000 ppm female (day 113). The death at the 150 ppm dose was suggested to be disseminated arteritis, a recognised spontaneous disorder of beagles. The high-dose female had compound-related cardiac insufficiency (abnormal ECG and degeneration of atrial myocardium), leading to ascites and cachexia, as well as depressed erythroid parameters, low serum protein values, liver adhesions and liver pathology (centrilobular necrosis). The high-dose male had compound-related myocardial lesions as well as skin lesions associated with *Demodex folliculorum* mites, both with consequent effects on clinical laboratory parameters.

At the high dose of 1000 ppm, toxicity was evident in terms of moribundity (2 animals), cachexia and ascites, reductions in bodyweight (approx. 20% for males, 125 for females) and bodyweight gain, reduced food consumption, irregular heartbeats and increased heart rate (mainly males), ECG alterations such as moderately decreased P-II values in both sexes, slightly decreased QT values, moderate decreases in PR values, atrial premature complexes in one female, and atrial fibrillation in both sexes, increased platelet counts, slightly decreased serum protein and albumin, slightly decreased absolute heart weight in females, slightly increased relative liver weights in males, moderate to severe gross or microscopic cardiac lesions consisting primarily of dilatation of right and left atria and myocardial degeneration (atrophy, myolysis) of the atria. Urinalyses were normal.

Statistically significant, but probably incidental, ECG changes noted at lower dose groups were: decreased mean electrical axis at day 175 and increased P-II and PR at day 267 in 15 ppm females, increased R waves at day 361 in 150 ppm males, and decreased P-II at day 175 in 150 ppm females. Moderate dilatation of right atrium, minimal dilatation of the left atrium, and a pale lesion on the epicardium of the left ventricle were seen in one 150 ppm male but these were attributed to polyarteritis (characterised by necrosis with focal sub-acute lymphocytic infiltration) which has been spontaneously seen in dogs; this polyarteritis was evident in arteries in the mesentery and thymus, and focal necrosis in the testes.

Although atrial dilatation occurred in one male, and decreased P-II waves occurred on one day in several females at 150 ppm, these changes were not associated with the pathology evident in high-dose animals and were therefore considered to be unrelated to treatment. The reporting cardiologist described them as “clearly” incidental.

The study clearly demonstrated cardiac toxicity in dogs for atrazine; however, the study authors did not comment on the possible causes of this. There were some slight cardiac effects at 150 ppm but it was suggested that these did not appear to be directly related to the compound (see below). On the basis of this, the NOEL may be set at 150 ppm (4.97 mg/kg bw/d).

6.1.20 One-year dog dietary study - supplement to 6.1.19

Wetzel LT (1989) Supplemental Information for the Chronic Toxicity Study in Dogs. (EPA MRID No. 40431301). Ciba-Geigy, Summit, NJ. Study No. 852008. Study completion date 6 Nov. 1989 (GLP - refer previous report)

The company responded to US EPA concerns regarding cardiac toxicity in the l-year dog study (Section 6.1.19).

The company considered that the decreased P-II amplitude in 150 ppm females was not treatment related because-

(i) the decrease (relative to values measured at the earlier and later intervals viz. days 85 and 276) occurred in only one animal on one day (day 175) ie. it was reversed at the next measurement (day 267);
(ii) the change was of small magnitude (0.1 mV) and was significant only due to a decrease in the standard error about the mean, rather than to a change in the mean, and ECGs were measured only in 0.1 mV increments;
(iii) P waves in dogs vary spontaneously with foreleg position change, and as a result of respiratory arrhythmia;
(iv) P wave amplitudes distributions are usually skewed, making parametric statistical comparisons unreliable;
(v) spontaneous variations of 0.1 mV are common;

The company considered that the atrial dilatation in one 150 ppm male dog was not related to treatment because-

(i) atrial size was estimated qualitatively, after formalin fixation. Several control animals exhibited mild dilatation, but were considered within the normal range;
(ii) there was no evidence of microscopic liver or heart lesions in the same animal, and ECGs and all other parameters were normal;
(iii) a review of the data by an expert in canine electrocardiography concurred with a diagnosis of polyarteritis.

In view of the limited group size, it is not possible to conclude with absolute certainty that the effects observed at 150 ppm are or are not related to atrazine dosing. However, it appears that observations at 15 and 150 ppm were spontaneous findings.

6.2 Desethylatrazine (G 30033)

No chronic studies cited.

6.3 Desisopropylatrazine (G 28279)

No chronic studies cited.

6.4 Diaminochlorotriazine (G 28273)

6.4.1 One-year dog dietary study

Thompson SS, Batastini GG & Arthur AT (1990) Diaminochlorotriazine -13/52-Week Oral Toxicity Study in Dogs, Ciba-Geigy, Summit, NJ, USA. Study No. 872151 Study completion date 17 Jan. 1990 (GLP; USA, Japan)

This study was conducted according to EPA Guidelines no. 82-1. **Diaminochlorotriazine** (G 28273; Batch no. FL 871423; 98.7% purity), an atrazine metabolite, was administered in the diet to groups of 8-10 male and female beagles for 13 or 52 weeks. Diets were prepared weekly and were determined to be homogeneous and stable for at least 31 days at room temperature; concentrations were all within $\pm$13% of targets. Dose levels were chosen on the basis of a 4-week study in which gross and microscopic haemorrhagic changes in the right atrium were noted in dogs at dietary doses $\geq$ 1500 ppm. Dose levels were 0, 5 or 100 ppm for 13 or 52 weeks (low- and mid-dose groups). High-dose animals were given 1500 ppm for 6 weeks, reducing to 750 ppm from week 7 because of toxic signs; females remained on this dose until termination (weeks 13 or 52) or through week 13 with a 39-week recovery period (2 animals), whilst males remained on 750 ppm through week 8 then put on control diet (0 ppm) for weeks 9-13 because their condition at the lower dose did not improve. Two males underwent scheduled sacrifice at week 13 and the remainder were re-established on 750 ppm until necropsy at 52 weeks.

Compound intakes (mg/kg bw/d) ranged from 0.1-0.2 (5 ppm), 3.2-3.9 (100 ppm), 41.0-48.9 (1500 ppm) and 22.0-26.8 (750 ppm) in males and 0.1-0.2 (5 ppm), 2.7-3.8 (100 ppm), 41.6-48.3 (1500 ppm) and 24.3-31.7 (750 ppm) in females.

Clinical signs, bodyweight, food consumption, ophthalmology, 10-lead ECG, haematology (parameters as at Appendix III), serum biochemistry (parameters as at Appendix III but not including GGT or cholinesterase activity) and urinalysis (parameters as at Appendix III) were recorded (pre-dose and at a number of times post-dose), gross and microscopic pathology was performed at termination. Organ weights (only on dogs killed at scheduled sacrifice) were performed (see list at Appendix IV plus epididymes, pituitary and thymus).

Treatment-related effects occurred only in high-dose (750, 1500 ppm) animals, and included the death (sacrificed moribund) of 5 males and 2 females, and faecal effects (mucoid, bloody, few, or bloody stools or diarrhoea). Inappetence, hypothermia, laboured breathing, hunched posture, abnormal gait, lethargy, recumbency and vocalisation were seen, only in dogs sacrificed moribund. Clinical signs which were seen in all high-dose-group dogs were inactivity, paleness, cachexia, abdominal distension and emaciation, signs considered secondary to impaired heart function. Also in the high dose group only, impaired cardiac function (tachyarrhythmia, atrial fibrillation, precordial thrill), bodyweight loss and reduced weight gain and food consumption, anaemia with a reticulocyte response, decreased mean serum albumin, calcium

and cholesterol levels and elevated LDH values, increased mean spleen, liver and kidney weights, gross and microscopic cardiac lesions primarily in the right atrium and secondary changes (fluid accumulation and lesions in the liver, testes, bone marrow, and thymus) were noted; pathological changes are detailed below. Animals showed some reversibility of symptoms (increased cholesterol and erythroid parameters, no cardiac abnormalities as determined by clinical, electrocardiography and pathology) during the recovery period.

Treatment-related gross pathological lesions were limited to the high-dose group and included soft enlarged heart with thickened valves, right and left atria distended, enlarged, dark, soft and/or thrombotic, and tan lesions in papillary muscle; at least one of these lesions was seen in all seven dogs sacrificed moribund during the dosing period, 2/5 dogs (1 M, 1F) sacrificed at 13 weeks, and 2/6 dogs (1M, 1F) sacrificed at term. No lesions were seen in the two recovery animals (females). Microscopic pathology of the heart lesions, primarily chronic myocarditis, were closely associated with the gross cardiac changes; the right atrium was most often affected although the ventricles and papillary muscles were involved in several individual animals.

Also in the high-dose group, liver lesions included passive congestion associated with centrilobular fibrosis/atrophy, bile stasis, necrosis, haemorrhage, haemosiderosis and inflammation, were reported as were lesions in bone marrow (hyperplasia), thymus (atrophy) and testes (hypospermatogenesis with associated hypospermia in the epididymes); these findings were stated to occur secondarily to the heart lesions although the reasons for this conclusion are were not stated.

The NOEL for the atrazine metabolite, **diaminochlorotriazine** was 100 ppm (3.2-3.9 mg/kg bw/d in males, 2.7-3.8 mg/kg bw/d in females) on the basis of a range of effects at the next highest dose of 750 ppm (increased mortality, cardiovascular, haematological and blood biochemical effects, organ weight changes, fluid accumulation, and liver, testes, bone marrow and thymus pathology).

Comment: The authors reported gross and microscopic changes in the right atria of dogs orally dosed at $\geq$ 1500 ppm in a 4-week pilot study, indicating an early onset of the cardiac inflammation produced by diaminochlorotriazine (Swallow, Hazelette & Arthur, 1989; see Section 4.4.2).

6.5 Hydroxyatrazine (G 34048)

6.5.1 2-Year SD rat dietary study

Chow E & Emeigh Hart SG (1995) 2-Year Dietary Chronic Toxicity/Oncogenicity Study with G-34048 (Hydroxyatrazine) Technical in Rats. Ciba-Geigy Corp., Farmington, CT. Lab study no. F-00125. Report date 27 Jan. 1995 (GLP; OECD, US EPA, Japan)

Note: Interim reports dated 26 Jan. 1993 (Chow & Emeigh Hart, 1995) and 22 June 1994 Chow & Emeigh Hart, 1995) were also submitted.

This study was conducted according to EPA Guidelines no. 83-5, OECD Test Guideline 453, and MAFF Testing Guidelines 1985. **Hydroxyatrazine** (lot no. FL 870869; 97.1% purity), an atrazine metabolite, was administered in the diet to groups of Crl:CD(SD)BR Sprague-Dawley derived rats (70/sex/group, with an extra 10/sex or the control and high-dose groups) for up to two years; doses were 0, 10, 25, 200 and 400 ppm. Ten/sex (plus an extra 10/sex for control and high dose) were scheduled for sacrifice at one year. Diets were determined to be adequately homogeneous and stable. Clinical signs, bodyweight, food consumption, water intake, haematology, serum biochemistry and urinalysis were recorded. Organ weights were recorded and all animals were necropsied for gross and histopathological evaluation; at the time of this interim report, microscopic evaluation was still in progress.

The weighted average compound consumption was 0, 0.388, 0.962, 7.75 and 17.4 mg/kg bw/d (males) and 0, 0.475, 1.17, 9.53 and 22.3 mg/kg bw/d (females) in the respective groups.

All high-dose animals were killed at 18 months because of excessive mortality; 20% of males and 18% of females were surviving at this time. Adjusted percentage survival rates at 24 months in the 0, 10, 25 and 200-ppm groups were 20%, 25%, 25% and 28% (males) and 23%, 30%, 31% and 39% (females) ie. survival was not compromised by hydroxyatrazine at these doses.

Decreases in cumulative bodywt gains and bodywts were observed throughout the study at 400 ppm; at 18 months, mean body wts were 28% (males) and 41% (females) lower than controls

Food consumption was reduced in both sexes at 400 ppm; at week 75, intake was 23% lower than controls. Increases in water consumption were observed at $\geq$ 200 ppm, only during the first year at 200 ppm, not measured beyond 52 weeks at the high dose. At 400 ppm, weekly water intake was 3.3-3.6x and 2-3x control intake at week 28 and 52, respectively. At 200 ppm, intake was increased 1.2-1.4x at the 28 and 52-week time points, with no differences from control at 78 and 105 weeks.

Treatment-related haematological changes were seen at 400 ppm, including decreases in RBC counts, Hb, Hct and MCHC in both sexes and MCH in

females, and increases in MCV in males. In addition, increases in leucocyte counts, platelet counts and segmented neutrophil counts were observed in both sexes at more than one interval. Several other statistically-significant haematology changes were not assessed as being treatment-related.

Statistically-significant treatment-related changes in blood chemistry at 400 ppm included (1) increased calcium, phosphorus, BUN and creatinine (both sexes); (2) decreased glucose, total protein, and albumin (both sexes); (3) decreased creatine kinase activity (males), globulin level (males) and albumin/globulin ratio (females); (4) increased potassium and cholesterol (females); (5) slight elevations in GGT (males).

GGT was also slightly elevated in females at $\geq$ 25 ppm.

Treatment-related changes in urinalysis parameters at 400 ppm included, in overnight samples, (1) decreased pH and specific gravity, and increased urine volume, correlated with decreased colour density (both sexes); (2) decreased protein and ketone levels (males); (3) increased protein, occult blood and erythrocytes in females at 12 months. Fresh urine had lower pH and osmolality than controls, with sediments in some samples collected at 1.5 months. Whilst urine contained approx. 5 µg/mL (near the solubility of hydroxyatrazine at neutral pH), some sediments contained approx. 590 ppm.

Organ wt analysis indicated that kidney weights (absolute and relative) increased at the high dose [19% (males) and 10% (females) increase in absolute wt cf. controls].

No treatment-related changes in the distribution of clinically-palpable masses were observed or in the incidence of animals with one or more palpable masses.

Treatment-related pathological lesions included:

- Kidney lesions in both sexes, only at $\geq$ 200 ppm, involving deposition of yellowish-green to slightly basophilic, transparent, amorphous to crystalline material within collecting ducts, renal pelvises and occasionally distal tubules. Renal tubules and collecting ducts which contained the material were dilated and either devoid of epithelium or lined by hyperplastic tubular epithelium. Tubular changes were often accompanied by acute inflammation and by interstitial fibrosis. Similarly, renal pelvises had multifocal transitional cell erosions.

- Ureters and/or urinary bladders of a few 400 ppm animals with aggregates of the same material found in the kidneys, a finding often accompanied by either transitional cell hyperplasia, submucosal fibrosis of the bladder, subacute inflammation, or chronic inflammation.

- In renal papillae, an increase in the incidence/severity of accumulation of interstitial matrix was seen in 200 ppm males and 25 ppm females (without any impairment of

renal function). In 400 ppm females there was an increase in incidence/severity of transitional cell hyperplasia in the papillae.

- Papillary lesions in both sexes were accompanied by cortical changes consistent with chronic progressive nephropathy. The incidence/severity was significantly greater than controls for 400 ppm males and $\geq$ 200 ppm females.

- Mineralisation of tissues as a consequence of calcium-phosphate imbalance (seen at 400 ppm) as a result of chronic renal failure - apart from mineralisation of tubular epithelia and basement membranes of kidneys, this included tubular epithelia and basement membranes of kidneys (both sexes), and ovaries and urinary bladders (females).

Secondary effects of chronic progressive nephropathy at 400 ppm were considered to include:

- Renal lymph nodes for 400-ppm animals frequently showed congestion, sinusoidal ectasia, and accumulation of pigmented macrophages;
- parathyroid hyperplasia;
- fibrous osteodystrophy (reabsorption of bone and replacement by fibrous connective tissue, and presence of activated osteoclasts);
- polyarteritis nodosa (transmural inflammation of muscular arteries, with fibrinoid necrosis, endothelial proliferation and perarterial fibrosis);
- progressive cardiomyopathy (patchy myocardial necrosis with chronic or chronic-active inflammation and fibrosis);
- testicular degeneration and necrosis.

Specific immunostaining with anti-hydroxyatrazine monoclonal antibody was demonstrated for the crystalline material seen in 400 ppm sections, with strong staining for the presence of polysaccharides, probably in the form of glycoproteins.

There was a reduced incidence of mammary tumours and other non-neoplastic lesions at the high dose, probably a consequence of the early deaths of these animals. No decrease of mammary tumour onset time was observed in this study.

The kidneys and lower urinary tract were the target organs for hydroxyatrazine toxicity in rats. The NOEL for hydroxyatrazine in this study was 25 ppm in males (0.96 mg/kg bw/d) and 10 ppm in females (0.47 mg/kg bw/d), based on kidney toxicity and pathology at the next highest dose (200 ppm in males, 25 ppm in females). No decrease of mammary tumour onset time was observed in this study. A maximum-tolerated dose was clearly exceeded at 400 ppm, based on decreased survival, reduced bodywt gains and the extent of renal damage.

7. REPRODUCTIVE TOXICITY

7.1 3-Generation rat dietary study (80W formulation)

Hollingsworth RL, Woodard MW & Woodard G. (1966) Atrazine - Three-Generation Reproduction Study in Rats. Geigy Agricultural Chemicals. Lab: Woodard Research Corp. Laboratory & Consulting Service, Herndon, VA. Report date 29 June 1966

Weanling Charles River albino rats (10 males and 20 females/dose group) were fed atrazine in the diet at levels of 0, 50 and 100 ppm (calculated on the active ingredient). Atrazine 80W, an 80% wettable powder (number FL-2446, ARS 1655A-64, Item 684) was used. At about 107 days of age and after 74 days of dietary administration, parent animals were mated (one male mated with two females, each in consecutive 10-day periods). Approximately 13 days after weaning of F1a litters, females were again mated (different males from the same group) to produce F1b litters. Ten males and twenty females were selected from the F1b litters for subsequent mating to produce F2a and F2b litters. Similarly, animals from F2b litters were selected to produce F3a and F3b litters.

Test and control groups of rats were comparable in terms of survival, mean body weights, general appearance and behaviour, and reproductive performance. Examination of test and control litters of the three generations indicated that 100 ppm (estimated 5-10 mg/kg bw/d) was an appropriate overall NOEL for litters/group, total still-births, live young/litter, birth weight, percentage alive at weaning, mean weaning weight and, where measured in the F3b offspring, organ weights (liver, kidney and heart) and microscopic pathology. No teratogenic effects were noted. There was an absence of statistical analyses but otherwise the study appeared to be reasonably well reported.

7.2 2-Generation rat dietary study

Mainiero J, Youreneff M, Giknis MLA & Yau ET (1987) Two-Generation Reproduction Study in Rats. Lab: Ciba-Geigy Pharmaceuticals Div., Summit, NJ. Study No. 852063. Toxicol/Pathol Report no. 87076. Study completion date 17 Nov. 1987 (GLP; US EPA)

Atrazine technical (Batch FL 841802; purity not stated) was administered to 2 generations (F0, F1) of Charles River (CRCD, VAF/PLUS) rats from Charles River Labs, Kingston, NY (30/sex/group) at dietary concentrations of 0, 10, 50 or 500 ppm. Analyses showed good admixture stability and homogeneity, with concentrations within 93-105% of target at all times.

Animals were dosed for 10 weeks prior to a mating period (1:1) of up to 3 weeks. F0 (P1) females were allowed to deliver and, following weaning, 30 weanlings/sex/gp were selected as the P2 generation. After 12 weeks of dietary exposure, animals were allowed to mate as before (littermate matings avoided).

All F2 litters were sacrificed on lactation day 21. Five F2 pups/sex/group underwent necropsy.

Clinical signs were monitored twice daily in all animals. Bodyweights of males were recorded weekly. Female food consumption and bodyweights were record weekly, and on days 0, 7, 14 and 20 of gestation, and on days 0, 4, 7, 14 and 21 of lactation. The number and sex of viable and stillborn pups was recorded on the day of delivery. All pups removed at the time of culling, and all parents were necropsied. Tissues exhibiting gross abnormalities were examined microscopically. Testis and ovary weights were determined and histopathology (on grossly abnormal tissues, and specimens of vagina, cervix, uterus, ovaries, testes, epididymes, seminal vesicles, prostate, pituitary and coagulation gland) was conducted .

There were no compound-related effects on mortality, clinical signs, reproductive parameters, and no perinatal or postnatal effects on the F1 and F2 generations. During the pre-mating period, food consumption was reduced in F0 and F1 animals of both sexes at 500 ppm, and bodyweights and bodyweight gains were also reduced. Bodyweights were also reduced for females of both generations during gestation and lactation. There were no gross or microscopic pathological findings in any of the reproductive organs and all other pathology was normal.

Atrazine at dietary concentrations up to 500 ppm did not cause any impairment in the reproductive performance of rats in this two-generation study. The NOEL was 50 ppm [between. 2.73 (male) to 3.45 (female) mg/kg bw/d], on the basis of reduced food consumption, bodyweights, and bodywt gains in parental animals, at the next higher dose of 500 ppm.

7.3 2-Generation mouse study (pesticide mixture)

National Toxicology Program (1993) NTP Technical Report on Toxicity Studies of Pesticide/Fertilizer Mixtures Administered in Drinking Water to F344/N Rats and B6C3F1 Mice. NIH Publication 93-3385. Date July 1993

and

Heindel JR, Chapin RE, Gulati DK, George JD, Price CJ, Marr MC, Myers CB, Barnes LH, Fail PA, Grizzle T, Schetz BA & Yang RS (1994) Assessment of the Reproductive and Developmental Toxicity of Pesticide/Fertilizer Mixtures Based on Confirmed Pesticide Contamination in California and Iowa Groundwater. Fund Appl Toxicol 22: 605-621

Two mixtures of pesticides and a fertilizer (ammonium nitrate), representative of established groundwater contamination in California and Iowa, were both tested (at 1x, 10x and 100x the median concentration found in the groundwater surveys) in a continuous breeding study in CD1 mice.

The 1x mixtures were made up as follows (ng/mL concentration given in brackets, unless otherwise stated):-

alachlor (0.9); atrazine (0.5); cyanazine (0.4); metolachlor (0.4); metribuzin (0.6); ammonium nitrate (10 µg/mL); propylene glycol (512 µg/mL; used as solubilizer) (IOWA)

aldicarb (9.0; 1;1:1 ratio of aldicarb, sulfoxide and sulfone); atrazine (0.5); dibromochloropropane (0.01); 1,2-dichloropropane (4.5); EDB (0.9); simazine (0.3); ammonium nitrate (10 µg/mL); propylene glycol (512 µg/mL; used as solubilizer) (CAL)

The Reproductive Toxicity study was conducted using the NTP protocol, Reproductive Assessment by Continuous Breeding, according to FDA GLP guidelines. COBS Crl:CD-1 (ICR) VAF/Plus outbred albino Swiss mice (from Charles River Breeding Labs, Raleigh, NC or Portage, MI) were 11 weeks of age at the start of the continuous breeding phase of the studies, each of which consisted of controls (40 breeding pairs) and three dose groups (20 pairs/gp). Following 7 days of premating exposure to dosed drinking water, animals were housed as breeding pairs for 98 days, all the while with continuous exposure. Randomly-selected F1 pups were weaned and exposed to dosed drinking water, with breeding for 7 days at age 74 $\pm$ 10 days, then housed singly until delivery of litters. Endpoints for F0 and F1 matings were clinical signs, parental bodywt, fertility (number of litters/number of breeding pairs), live pups/litter, proportion of pups born alive, pup birth weights, and food and water consumption. F1 mice were necropsied after breeding and selected organs, including testes and epididymes, weighed and examined histopathologically.

There were no significant effects on any parameter measured in the reproductive toxicity studies, even at doses of IOWA and CAL mixtures at 100x mean groundwater contamination levels. It was specifically noted that epididymal sperm concentration, percent motile sperm, percent abnormal sperm, and testicular spermatid head count were not affected, nor was there any testicular or epididymal pathology. Oestrous cyclicity, as measured by vaginal cytology in mice treated with IOWA water was not affected.

7.4 Mouse sperm morphology assay

Osterloh J, Letz G, Pond S & Becker C (1983) An Assessment of the Potential Testicular Toxicity of 10 Pesticides using the Mouse-sperm Morphology Assay. Mutation Res 116: 407-415

No testicular toxicity, as assessed by sperm morphology, sperm counts and testicular weights, was seen in mice treated daily for 5 days with ip atrazine at doses of 38-600 mg/kg/day. At higher doses (1200 and 2400 mg/kg bw/d) the herbicide was lethal. A positive control (methylmethanesulfonate) was used.

8. DEVELOPMENTAL TOXICITY

8.1 Atrazine

8.1.1 Rat oral teratology study

Fritz H (1971) Rat Segment II Reproduction Study - Test for Teratogenic or Embryotoxic Effects. Ciba-Geigy Ltd, Basle. Expt no. 22710600. Report date 29 Oct. 1971

Pregnant rats (strain?) were given atrazine (batch no. 4314) at oral dose levels of 0, 100, 500 and 1000 mg/kg bw/d on days 6-15 of gestation; dams were necropsied on day 21(day 0 is the day on which either sperm or a plug is found in the vagina). There were 30 dams in test groups and 40 in the control group.

During the first 5 days of dosing, clinical signs were seen at the high dose including ventrocumbency, dyspnoea and sedation. It was stated that there were 7/30 deaths at this dose; autopsy revealed stomach ulceration in these animals. However, no comments or autopsy data were provided on 7/40 dams from the control group which died. With respect to the clinical signs, they were noted at the mid dose. It was stated that "symptoms of intolerability were found to be less evident in the low-dose group", but no further details were given; thus it is not completely clear whether some clinical signs were seen at the low dose or not. There was a slightly reduced bodywt gain at 500 and 1000 mg/kg, partially due to a marked decrease in average fetal weight. Food consumption was reduced in all dosed groups, recovering to control levels by study termination only in the low-dose group. At the mid- and high dose, early ("embryonic") resorptions were increased (0.36%, 0.38%, 0.60% and 1.13% in the control to high-dose groups respectively; not statistically significant at the mid dose), as were late ("fetal") resorptions (0%, 0%, 0.95% and 0.38% in the respective dose groups). Mean live fetal weight was dose dependently reduced at the mid- and high dose (5.05, 5.07, 4.29 and 3.93 g respectively, control - high-dose) and two high-dose litters aborted.

At the high dose, 5/119 fetuses exhibited anasarca (oedema in the head and neck region). At 500 and 1000 mg/kg, there was delayed ossification of the hind limbs and, at the high dose, incomplete ossification of the sternebral centres.

A NOEL of 100 mg/kg bw/d for embryotoxic effects can be set, based on decreased mean live fetal body weight, increased early and late resorptions, and retarded ossification at the next higher dose of 500 mg/kg. The developmental deficits are attributed to maternal toxicity, with reduced body wt gain (mid- and high doses), reduced food consumption (all doses), and prominent clinical signs of toxicity (mid- and high dose and possibly the low dose group, but the study report is too brief to absolutely confirm this).

Results of this study indicated that atrazine was without teratogenic effects.

8.1.2 Rat dietary & SC teratology study

Peters JW & Cook RM (1973) Effects of Atrazine on Reproduction in Rats. Bull Environ Contam Toxicol 9: 301-304

This study was conducted by the Dept of Dairy Science, Michigan State University, East Lansing, Mich., USA. After confirmation of mating, rats (strain not stated) were fed at:-

(a) 0, 50, 100, 200, 300, 400, 500 and 1000 ppm of atrazine in the diet throughout the gestation period (4/group);

(b) injected sc with atrazine (in 1 mL of DMSO) at 0, 50, 100, 200, 800, 1000 or 2000 mg/kg on days 3, 6 and 9 of gestation (injection was a suspension at the two highest doses) (4/group);

(c) injected sc with 0, 1000 or 2000 mg/kg on day 3, 6 or 9 of gestation (5-7 rats/group).

The only parameters assessed were pups/litter, weaning weight and resorptions.

No changes were observed following oral administration of atrazine up to 1000 ppm in the diet. Following sc administration, the NOEL was 200 mg/kg for decreased litter size and increased resorptions. At 2000 mg/kg sc, no live pups were born although uterine resorption sites were readily apparent. This embryotoxic effect was greatest when atrazine was administered on day 6 of gestation. Because of the limited parameters recorded, this study is of limited regulatory use and it does not examine atrazine for potential teratogenic effects.

8.1.3 Rat oral teratology study

Infurna RN & Arthur AT (1984) A Teratology Study of Atrazine Technical in Charles River Rats. Ciba-Geigy Corp., Greensboro, New Jersey. Report No. 60/84 Expt. date 12 Sept. - 1 Oct. 1983. Report date 18 Sept. 1984 (GLP; USA)

Pregnant Charles River Crl:COBS CD(SD) BR rats (27/group) were gavaged with technical-grade atrazine (Ciba-Geigy batch FL-830288, purity unstated; prepared in 3% aqueous corn starch containing 0.5% Tween-80) at 0, 10, 70 and 700 mg/kg/day from days 6-15 of pregnancy, with necropsy on day 20. The protocol was in accordance with OECD test guideline no. 414.

Maternal toxicity was noted at both the mid and high doses. At both these doses there were decreases in food consumption (at the mid-dose, only statistically different from controls during the first two days of dosing), body weights and body weight gain, and an apparent increase incidence of alopecia. At the high dose there was a high mortality rate (21/27 dams; otherwise, there was only one other death, a control which died prior to commencement of the dosing period); gross clinical signs (salivation, ptosis, swollen abdomen, oral/nasal discharge, bloody vulva); and gross pathological changes

(discoloured lungs, enlarged stomachs [of unknown aetiology], and enlarged adrenals). In low-dose animals there was a transient increase in food consumption during the first half of the dosing period, with an accompanying increase in bodywt gain.

There were no observed adverse effects on the following reproductive parameters viz. pregnancy rate, numbers of corpora lutea, implantation sites, resorptions, viable fetuses, percentage of pre-implantation loss, and the mean number of live fetuses (noting that only 5 litters were examined at the high dose). Fetal toxicity was observed at the mid and high doses. Fetal size and weight were severely reduced in the high-dose group (mean fetal weight of 1.89 g cf. 3.44 g for controls). At the mid-dose there was a significant increase in the incidence of skeletal variants viz. incomplete ossification of a number of bones, considered to be developmental delays. (Skeletal examinations were not conducted on high-dose pups because of the severe reduction in fetal size and weight.) A NOEL of 10 mg/kg bw/d for fetotoxicity is appropriate, based on developmental delays in ossification. Maternotoxicity was apparent at the two higher doses at which fetotoxicity was observed.

Note: The results of this study were also published in:- Infurna R, Levy B, Meng C, Yau E & Traina V (1988) Teratological Evaluations of Atrazine Technical, A Triazine Herbicide, in Rats and Rabbits. J Tox Env Hlth 24: 307-319.

8.1.4Rat oral teratology study

Giknis MLA (1989a) Atrazine Technical. A Teratology (Segment II) Study in Rats. Ciba-Geigy, Greensboro, NJ, USA. Study no. 882049. Study completion Date 23 Feb. 1989 (GLP; US EPA & FDA, Jap. MAFF, OECD)

Virgin female Charles River [crl:COBS CD(SD)BR] rats (26/group) at approximately 2.5 months of age were administered atrazine technical (batch no. FL 841802; purity not stated) in 3% aqueous cornstarch and 0.5% Tween-80 by gastric intubation on gestation days 6-15 at doses of 0, 5, 25 or 100 mg/kg bw/d. Stability, homogeneity and concentration of suspensions were checked prior to study commencement. Mortality, bodyweight and clinical signs were monitored. Uteri and foetuses were examined at day 20. Fetuses were examined for gross, visceral and skeletal abnormalities.

Maternal toxicity was evident as reduced food consumption, body weights and body weight gain, and increased salivation at 100 mg/kg bw/d only. The incidence of minor foetal skeletal variations was also increased at the highest dose. All other parameters were normal. There was no evidence of embryotoxicity or teratogenicity. Based on these findings, the overall NOEL for maternotoxicity and fetotoxicity in this study was 25 mg/kg bw/d.

8.1.5 Rabbit oral teratology study

Arthur AT & Katz R (1984) Segment II Teratology Study in New Zealand White Rabbits. Ciba-Geigy Corp., Greensboro, New Jersey, USA. Report no. 68-84 Expt. Date 19 Sept. - 13 Oct. 1983. Report Date 18 Sept. 1984 (GLP; USA)

Artificially-inseminated NZ White rabbits (19 per group) were given technical-grade atrazine (Batch no. FL-821014, purity not stated; prepared in 3% aqueous corn starch containing 0.5% Tween-80) by gavage on days 7-19 of pregnancy at doses of 0, 1, 5 and 75 mg/kg bw/day; necropsy was on day 29. The protocol was in accordance with OECD test guideline no. 414.

There were three deaths, all at the low dose, two apparently the result of dosing accidents (days 17 and 19), whilst the third was found dead on day 26 (possibly aborting). Three females were killed because they were aborting, one at the mid-dose (day 21) and two at the high-dose (day 20 and 25). Signs of maternotoxicity at the mid-dose included decreased food consumption during the dosing period (only statistically different from controls on days 17 and 19), decreased body weight gain during the dosing period, and, at the high dose, bloody vulvae, changes in stool characteristics (absent or small and soft), decreases in food consumption, decreased body weight and body weight gain during the period of compound administration. Embryotoxicity and fetotoxicity was evident at the high dose and included increases in resorptions and percent post-implantation losses, decreases in fetal viability, decreases in fetal bodyweight, and delayed ossification. No significant differences were noted in the numbers of corpora lutea, implantation sites, pre-implantation loss and percentage of pre-implantation loss.

Based on these findings, the NOEL for embryotoxicity and fetotoxicity was 5 mg/kgbw/d. There was no evidence of teratogenicity.

Note: The results of this study were also published in:- Infurna R, Levy B, Meng C, Yau E & Traina V (1988) Teratological Evaluations of Atrazine Technical, a Triazine Herbicide, in Rats and Rabbits. J Tox Env Hlth 24: 307-319.

8.1.6 Rat oral teratology study (pesticide mixture)

National Toxicology Program (1993) NTP Technical Report on Toxicity Studies of Pesticide/Fertilizer Mixtures Administered in Drinking Water to F344/N Rats and B6C3F1 Mice. NIH Publication 93-3385. Date July 1993
and
Heindel JR, Chapin RE, Gulati DK, George JD, Price CJ, Marr MC, Myers CB, Barnes LH, Fail PA, Grizzle T, Schetz BA & Yang RS (1994) Assessment of the Reproductive and Developmental Toxicity of Pesticide/Fertilizer Mixtures Based on Confirmed Pesticide Contamination in California and Iowa Groundwater. Fund Appl Toxicol 22: 605-621

Two mixtures of pesticides and a fertilizer (ammonium nitrate), representative of established groundwater contamination in California and Iowa, were both tested (at 1x, 10x and 100x the median concentration found in the groundwater surveys) in teratology studies in Sprague Dawley rats.

The 1x mixtures were made up as follows (ng/mL concentration given in brackets, unless otherwise stated):-

alachlor (0.9); atrazine (0.5); cyanazine (0.4); metolachlor (0.4); metribuzin (0.6); ammonium nitrate (10 µg/mL); propylene glycol (512 µg/mL; used as solubilizer) (IOWA)

aldicarb (9.0; 1;1:1 ratio of aldicarb, sulfoxide and sulfone); atrazine (0.5); dibromochloropropane (0.01); 1,2-dichloropropane (4.5); EDB (0.9); simazine (0.3); ammonium nitrate (10 µg/mL); propylene glycol (512 µg/mL; used as solubilizer) (CAL)

The developmental toxicity studies utilized female Sprague Dawley Crl:CD BR VAF/Plus outbred albino rats (CD, Charles River, Raleigh, NC), of approximately 10 weeks of age at the start of the study (gestation day 0). Dams were exposed to normal water, water plus vehicle, or dosed water on gestation days 6 to 20. Animals (23-24 dams/group) were necropsied on day 20 and underwent uterine assessment. Live fetuses underwent visceral and skeletal examination.

There were no maternal deaths and no signs of maternal toxicity, apart from a significant increase in maternal water consumption in the 100x CAL dose group during gestation, although a trend to increasing water intake was seen pre-dosing (days 0-6). The only other statistically significant finding was an increase in late fetal deaths at the 100x concentration in the IOWA study; the percent of litters with late fetal deaths was 4.8% (or 0.3% late fetal deaths/litter) cf. 0% in all other groups, an observation not considered to reflect an effect related to dosing.

Thus, under the conditions of these well conducted and reported studies, there were no significant effects on any parameter measured in the developmental

toxicity studies, even at doses of IOWA and CAL mixtures at 100x mean groundwater contamination levels.

8.1.7 Sheep dietary teratology study

Binns W & Johnson AE (1970) Chronic and Teratogenic Effects of 2,4-D and Atrazine to Sheep. Proc. N Cent Weed Control Conf 25: 100 (Abstr)

This study (reported as abstract only) was conducted at ARS, VSR Division, Poisonous Plants Research Lab., Logan, Utah, USA. Two groups of 6 ewes were fed atrazine at 15 and 30 mg/kg bw/d mixed in alfalfa meal, by stomach tube, throughout gestation plus 30 days postpartum. All high-dose animals died during gestation between day 36 and 60; one had failed to conceive, three had had embryonic deaths, and two had normal fetuses. At 15 mg/kg bw, all ewes delivered full-term, normal, live lambs which nursed without clinical signs of poisoning.

Because of the limited reporting in this study, it is not of use in establishing any regulatory standards but is confirmatory of atrazine's lack of teratogenic activity.

8.1.8 Overview of developmental studies

Johnson EM (1993) 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.

This paper (unpublished?), provided by Ciba-Geigy, does not give any indication as to the affiliation of the author or the sponsor of the assessment.

The review of 29 published papers led the author to the conclusion that atrazine did not produce congenital malformations in rats or rabbits at doses causing overt maternal toxicity; the only developmental effects were developmental delays of skeletal maturation. The NOAEL (no observable advers effect level) was considered to be 5 mg/kg bw/d for these effects.

8.2 Desethylatrazine

8.2.1 Rat oral teratology study

Fritz H (1972) Reproduction Study - G 30033 (Desethylatrazine). Segment II (Test for Teratogenic or Embryotoxic Effects) Ciba-Geigy Ltd, Basle, Switzerland. Expt no. 22 71 08 00. Report date 4 Jan. 1972

Desethylatrazine (purity and batch no. not stated), an atrazine metabolite, was administered to mated female rats (strain not stated) (24 animals/group) by oral gavage in 2% CMC at doses of 0, 30, 100 and 200 mg/kg bw/d (10 mL/kg) on days 6-15 post-coitum. Homogeneity, stability and test-article concentration in

the dosing mixtures were determined; formulations were homogeneous and all concentrations were within 7% of target. Clinical signs, mortalities and bodyweights were monitored daily, food consumption at regular intervals. Dams were sacrificed at day 21 of gestation and uterine reproduction parameters (early and late resorptions, abortion sites, dead fetuses and litter details) recorded, and gross pathology and fetal examinations of skeletal and soft tissues conducted; approximately half the fetuses were processed for skeletal examination, the other half for visceral examination.

Food consumption was reduced, particularly at the mid- and high-dose, after 4 days of treatment. Otherwise, there were no other signs of compound intolerability. There was a small decrease in final mean bodywt cf. control, considered to be due to a decrease in fetal weights.

The mid- and high-dose groups displayed a significant increase in the number of embryonic resorptions; mean resorptions were 1.25, 0.96, 2.04 and 2.04 in the control - high-dose groups respectively. At the high dose, the mean weight of fetuses was lower than controls; mean weights were 4.99, 5.01, 5.03 and 4.36 g in the respective groups.

There was an increase in the number of not yet ossified or incompletely ossified skeletal elements at the mid and high dose; at 100 mg/kg there was an increase in not-yet-ossified phalangeal nuclei of the hind leg, as well as incompletely ossified sternebrae. In addition, at the high dose, the phalangeal nuclei of the foreleg showed a more frequent absence of ossification. Also at the high dose, about 35% of fetuses examined displayed an absence of ossification of sternebrae 2 and 5. There were no organ malformations.

The maternal NOEL may be taken as 30 mg/kg bw/d, based on the small reductions in food consumption after 4 days of treatment at 100 and 200 mg/kg bw and an increase in embryonic resorptions. The fetal NOEL was 30 mg/kg bw/d, based on an increase in the numbers of not-yet-ossified or incompletely ossified skeletal elements at the next highest dose of 100 mg/kg/d. There was no evidence of teratogenicity at any of the doses tested.

8.2.2Rat oral teratology study

Marty JH (1992a) Developmental Toxicity (Teratogenicity) Study in Rats with G 30033 Technical (Desethylatrazine). Ciba-Geigy Ltd, Basle, Switzerland. Test no. 901265. Expt termination date 5 June 1991 (GLP; OECD, US EPA, Japan)

This study was conducted according to OECD Guideline 414 and US EPA Guideline 83-3. **Desethylatrazine** (batch no. FL 901515; 95.7% purity), an atrazine metabolite, was administered to approx. 2-month-old female Tif: RAI f (SPF) rats (hybrids of RII/1 x RII/2) (24 mated animals/group) by oral gavage in 3% w/w aqueous cornstarch at doses of 0, 5, 25, and 100 mg/kg bw/d (10 mL/kg) on days 6-15 post-coitum; these doses were based on a dose range-finding study (Test no. 901266). Homogeneity, stability and test-article

concentration in the dosing mixtures were determined; formulations were homogeneous and all concentrations were within 7% of target. Clinical signs, mortalities and bodyweights were monitored daily, food consumption on days 6, 11, 16 and 21. Dams were sacrificed at day 21 of gestation and uterine reproduction parameters (early and late resorptions, abortion sites, dead fetuses and litter details) recorded, and gross pathology and foetal examinations of skeletal and soft tissues conducted; approximately half the fetuses were processed for skeletal examination, the other half for visceral examination.

One 25 mg/kg dam died post-dosing on day 10 post-coitum. One high-dose dam showed hunched posture on days 10-14. Maternal weight was slightly reduced (about 4.4% at day 21) at 100 mg/kg bw/d from days 7 to 20; bodywt gain was significantly reduced at 100 mg/kg throughout the treatment period and at 25 mg/kg during days 6-11. At 25 and 100 mg/kg there was a dose-related reduction in food consumption over days 6-11, but in the post-treatment period, high-dose intake exceeded control intake.

Maternal post-mortem examination did not reveal any treatment-related necropsy findings. Three were not pregnant (1 control, 1 low-dose, 1 mid-dose); allowing for the mid-dose dam which died, dams with viable fetuses were 23, 23, 22 and 24 in the control to high-dose groups, respectively. Reproduction data (mean numbers of corpora lutea, implantation sites, pre-implantation loss) and early and late resorptions (post-implantation loss) were comparable for all groups. There were no abortions or dead fetuses, and sex ratios and mean fetal body weight were comparable.

Two of 313 fetuses examined at 25 mg/kg (from separate litters) had external malformations; both had omphalocoele and one had hind-limb agenesis with bilateral missing os ischium, os pubis, tibia, fibula and hind paws. These were not considered treatment-related. Position anomaly of the hindlimb was seen in 1/334 low-dose and 3/313 mid-dose fetuses, probably related to uterine constriction and not compound-related. At visceral examination, 1/313 mid-dose fetuses had unilateral pelvic dilatation and 1/303 high-dose fetuses had bilateral hydronephrosis. At skeletal examination, there was a significant increase in the incidence of fused sternebrae -1 and -2 at the high dose, and of poor ossification of the proximal phalanx of digit-5 at the high dose; this is a minor defect attributable to slight developmental delay, most likely reflecting maternotoxic effects.

The maternal NOEL was 5 mg/kg bw/d, based on the small reductions in food consumption and body weight gain during the first half of the treatment period at the next highest dose of 25 mg/kg. The fetal NOEL was 25 mg/kg bw/d, based on an increase in the incidence of fused sternebrae-1 and -2, and poor ossification of the proximal phalanx of posterior digit-5 at the next highest dose of 100 mg/kg/d. There was no evidence of teratogenicity at any dose tested (up to 100 mg/kg bw/d).

8.3 Desisopropylatrazine

8.3.1

Marty JH (1992b) Developmental Toxicity (Teratogenicity) Study in Rats with G 28279 Technical [Desisopropylatrazine] (Oral Administration). Ciba-Geigy Ltd, Basle, Switzerland. Test no. 901262. Study completion date 1 June 1992 (GLP; OECD, US EPA, Japan, Switzerland)

This study was conducted according to OECD Guideline 414 and US EPA Guideline 83-3. **Desisopropyatrazine** (batch no. P.1; 97.4% purity), an atrazine metabolite, was administered to approx. 2-month-old primigravid female Tif: RAI f (SPF) rats (hybrids of RII/1 x RII/2) from Ciba-Geigy Animal Production, Stein (24 animals/group) by oral gavage in 3% w/w aqueous cornstarch at doses of 0, 5, 25, and 100 mg/kg bw/d (10 mL/kg) on days 6-15 post-coitum; these doses were based on a dose range-finding study (Test no. 901263). Homogeneity, stability and test-article concentration in the dosing mixtures were determined; formulations were homogeneous and all concentrations were within 9% of target. Clinical signs, mortalities and bodyweights were monitored daily, food consumption on days 6, 11, 16 and 21. Dams were sacrificed at day 21 of gestation and uterine reproduction parameters (early and late resorptions, abortion sites, dead fetuses and litter details) recorded, and gross pathology and fetal examinations of skeletal and soft tissues conducted; approx. half the fetuses were processed for skeletal examination, the other half for visceral examination.

One control dam accidentally died on day 13 post-coitum. There were no observations which were attributed to treatment. Mean maternal weight was slightly but significantly reduced (about 5.7% at day 18) at 100 mg/kg from days 7 to 18 and at 25 mg/kg on days 9-13 (about 3.6% at day 13); bodywt gain was dose-dependently reduced at 25 and 100 mg/kg during days 6-11 and increased at the high dose during the post-treatment period. Mean net body weight (bodywt minus gravid uterus weight) was 8.1% lighter than the control value at necropsy but gravid uterine weights were not affected. There was a reduction in food consumption over days 6-11 at 25 mg/kg and over the whole treatment period at 100 mg/kg, but in the post-treatment period, high-dose intake slightly exceeded control intake.

Maternal post-mortem examination did not reveal any treatment-related necropsy findings. Four animals were not pregnant (1 control, 3 low-dose) and two had total implantation loss (1 mid-dose, 1 high dose); the numbers of dams with viable fetuses at necropsy were 22, 21, 23 and 23 in the control to high-dose groups, respectively. Reproduction data (mean numbers of corpora lutea, implantation sites, pre-implantation loss) and early and late resorptions (post-implantation loss) were comparable for all groups. Mean numbers of live fetuses, sex ratios and mean fetal body weight were comparable in all groups.

1/320 Fetuses examined at 25 mg/kg had the external malformation omphalocoele. Two of 320 fetuses at 25 mg/kg (separate litters) and 1/336 100 mg/kg fetuses had a unilateral hindlimb position anomaly; since it was not dose-related, it was probably related to uterine constriction.

Visceral examination reported several findings regarded as incidental and not related to treatment viz. dilated nasal cavities (1/155 fetuses examined at 25 mg/kg), renal pelvic dilatation (4/148 control fetuses, including 3 from one litter and 2/164 fetuses at 100 mg/kg).

At skeletal examination, there were no skeletal malformations but there was as a significant and dose-related increase in the incidence of fused sternebrae -1 and -2 at the mid- and high dose (only slightly above historical controls at the mid dose). An increased incidence of variants at 100 mg/kg included poor ossification of sternebrae -2, absent ossification of the proximal phalanx of posterior digit 2,3, 4 and 5, and absent ossification of metatarsal 1. The increase in variants concerned with poor or absent ossification is consistent with a slight developmental delay.

The maternal NOEL was 5 mg/kg bw/d, based on the small reductions in food consumption and body weight gain during the first half of the treatment period at the next highest dose of 25 mg/kg. The fetal NOEL was 5 mg/kg bw/d, based on an increase in the incidence of the anomaly, fused sternebrae-1 and -2 at the next highest dose of 25 mg/kg/d. There was no evidence of embryotoxicity or teratogenicity.

8.4 Diaminochlorotriazine

8.4.1 Rat oral teratology study

Hummel H, Youreneff M, Giknis MLA & Yau ET (1989) A Teratology (Segment II) Study in Rats. Ciba-Geigy, Summit, NJ, USA. Study No. 872177. Report date 15 Aug. 1989 (GLP; USA)

This study was conducted according to US EPA guidelines no. 83-3. **Diaminochlorotriazine** (batch no. FL 871423), an atrazine metabolite, was administered to approx. 10-week old primigravid female Charles River Crl: COBS CD (SD) BR rats (26 sperm-positive animals/group) by oral gavage in 3% cornstarch at doses of 0, 2.5, 25, 75 or 150 mg/kg bw/d on days 6-15 of gestation. Clinical signs, bodyweights and food consumption were monitored. Dams were sacrificed at day 20 of gestation and gross pathology and fetal examinations of skeletal and soft tissues conducted; approx. half the fetuses were processed for skeletal examination, the other half for visceral examination. Analysis of the test article noted that formulations were homogeneous and all concentrations were within 10% of target.

There were no unscheduled deaths or treatment-related clinical signs. Food consumption and maternal weight/weight gain was reduced at 150 mg/kg bw/d, and transiently at 75 mg/kg and 25 mg/kg; at the low dose there was an approximate 10% reduction in food consumption and a small reduction in body weight gain during days 6-8, although these were not statistically significant. The incidences of resorptions and post-implantation losses were increased at 150 mg/kg (2.61 $\pm$ 3.65 mean total resorptions cf. 0.77 $\pm$ 0.69 in controls, and 18.86 $\pm$ 23.78 mean percentage post-implantation loss cf. 5.56 $\pm$ 4.83 in

controls. Foetal weights were decreased at 75 and 150 mg/kg bw/d (about 8% and 19% respectively), and the incidence of skeletal variations was increased at $\geq$ 25 mg/kg. These included (1) not completely ossified parietal bones and non-ossified hyoid bones at 25 mg/kg: (2) not completely ossified parietal, interparietal, presphenoid, nasal, occipital, and metatarsal bones; non-ossified hyoid, teeth, metatarsal bones, sternebrae, and hindpaw distal phalanges; wavy ribs and rudimentary 14th ribs; and an increased incidence of pooled variations excluding the forepaw and metacarpal at 75 mg/kg/d: (3) not completely ossified parietal, interparietal, presphenoid, occipital, nasal, frontal, metatarsal and basisphenoid bones, mandible, centrum vertebrae, tympanic bullae and os pubis; non-ossified hyoid, teeth, metacarpal, metatarsal and presphenoid bones, sternebrae, forepaw and hindpaw distal phalanges, teeth, sternebrae and centrum vertebrae; wavy ribs and rudimentary 14th ribs; bipartite centrum vertebrae; and an increased incidence of pooled variations excluding the forepaw and metacarpal at 150 mg/kg.

Embryotoxicity was evidenced by an increase in resorptions and post-implantation loss at 150 mg/kg and fetotoxicity by decreased mean fetal weights of both sexes at 75 and 150 mg/kg, and an increase in skeletal variations. There was no evidence of teratogenicity. The maternal and fetal NOEL was 2.5 mg/kg bw/d, based on the transient slight reductions in food consumption and body weight gain, and an increase in the incidence of skeletal variations, at the next highest dose of 25 mg/kg/d.

8.5 Hydroxyatrazine

8.5.1 Rat oral teratology study

Giknis MLA (1989b) Hydroxyatrazine Technical: A Teratology (Segment II) Study in Rats. Ciba-Geigy, Summit, NJ, USA. Study No. 872202. (Pathology Report no. 88099; Statistics Report 88053) Study date 2 - 19 Nov. 1987, Report date 14 Feb. 1989 (GLP; USA)

Hydroxyatrazine technical (Batch SL910, lot no. FL 870869; 99.1% purity) was administered by gavage to Crl: COBS CD (SD) BR rats (26/gp) in 3% aqueous cornstarch with 0.5% Tween 80 at doses of 0, 5, 25, 125 mg/kg bw/d on gestation days 6 to 15. Mortality (observed twice daily), clinical signs (once daily), bodyweights (gestational days 0, 6, 8, 12, 16 and 20) and fetal visceral and skeletal development (day 20) were recorded. Gross pathology was performed on all dams. Approximately half the fetuses underwent visceral examination, the other half, skeletal examination.

Food consumption and bodyweight gain were reduced in high dose dams only and fetal weights were slightly reduced in this group; the reduction in food consumption averaged approx. 8% over the dosing period, whilst there was a 24% reduction in body weight gain during gestation days 8-12, with an overall 11% reduction in bodywt gain at day 20. The effect on fetal weight was less than 5% and was probably a result of the larger litter size in the high-dose group. The incidences of incompletely ossified hyoid and interparietal bones

and non-ossified forepaw metacarpals and proximal phalanges were increased in the high dose group, and may be attributed as secondary to maternotoxicity.

There were no compound effects on any other foetal or reproductive parameters. Thus there was no evidence of embryotoxicity or teratogenicity in this study and the overall NOEL for maternal and fetal effects was 25 mg/kg bw/d.

Note: The purity of the hydroxyatrazine used was reported in a short supplementary report to the rat teratology study.

9. GENOTOXICITY STUDIES

9.1 Gene Mutation Assays

9.1.1 Atrazine

9.1.1.1 ***Simmon VF & Poole D (1977) In vitro and in vivo microbiological assays of six Ciba-Geigy chemicals. Ciba-Geigy (Japan) Ltd. Stanford Research Institute, Menlo Park, CA. March 1977***

Atrazine (batch and purity details not stated) was tested at concentrations of 10 - 5000 µg/plate, with and without metabolic activation using S9 prepared from Aroclor-induced rat liver, and compared with appropriate positive controls. *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were used. Atrazine was negative for mutagenic activity whilst positive controls gave the expected responses. There was no comment on the toxicity of atrazine for the tester strains although it appeared that the highest concentration used had some toxicity in most tester strains.

9.1.1.2 ***Arni P & Mueller D (1978) Salmonella/Mammalian-microsome Mutagenicity Test with G 30 027. Ciba-Geigy Ltd, Basle, Switzerland. Test No. 861172. Expt No. 78/2527 18 July 1978***

Salmonella typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 were used. Technical atrazine (G 30 027) in DMSO was tested at concentrations of 10 - 810 µg/plate, with and without metabolic activation using S9 prepared from Aroclor-induced rat liver, and compared with appropriate positive controls. Test plates were run in triplicate. Atrazine was negative for mutagenic activity whilst positive controls gave the expected responses. There was no comment on the toxicity of atrazine for the tester strains.

9.1.1.3 ***Ciba-Geigy Japan (1979) In vitro Microbial Assays for Mutagenicity Testing of Atrazine. Project No. NRI-79-2884. Nomura Research Inst. Japan. Project no. NRI-79-2884. Aug. 1979***

Atrazine (98.8% purity; lot no. G 30027 from Ciba-Geigy, Japan) was negative in the following mutagenicity assays: rec-assay with *Bacillus subtilis* H17 and

M45 (atrazine tested at up to 10000 µg/well), reverse mutation with *E. Coli* B/r WP2 $Try^{-}Hcr^{-}$ (up to 5000 µg/plate atrazine) with or without S9 prepared from rat liver induced with polychlorobiphenyl, and reverse mutation with *S. typhimurium* strains TA 1535, 100, 1537, 1538 and 98 (up to 10000 µg/plate atrazine) with or without S9 mix. At the highest concentrations tested, crystal formation was noted in wells and plates.

9.1.1.4 ***de Bertoldi M, Griselli M, Giovannetti M & Barale R (1980) Mutagenicity of Pesticides Evaluated by Means of Gene-conversion in Saccharomyces cerevisiae and in Aspergillus nidulans. Environ Mutagen 2: 359-370***

No mutagenic effects of atrazine were seen in *S. cerevisiae* with or without metabolic activation after exposure at 1000-4000 ppm for 1-16 h. No mutagenic effects were seen after 1-4 h exposures to 500-8000 ppm in *A. nidulans*.

9.1.1.5 ***Ciba-Geigy Switzerland (1986) G30027 Tech: Salmonella/Mammalian-microsome Mutagenicity Test. Ciba-Geigy Ltd, Basle, Switzerland. Test No. 861172. 5 Dec. 1986 (GLP; US EPA)***

In replicate studies performed according to OECD test guideline 471, atrazine technical (98.2% purity; Ciba-Geigy lot no. 210200) did not induce histidine-prototrophic mutations in *S. typhimurium* strains TA 98, 100, 1535 or 1537, with or without metabolic activation (S9 prepared from Aroclor-induced rat livers), over a concentration range of 20 - 5000 µg/0.1 mL (mutagenicity test); preliminary toxicity tests (range 0.08 - 5000 µg/0.1 mL) were used to establish the test doses.

9.1.1. 6 ***Butler MA & Hoagland RE (1989) Genotoxicity Assessment of Atrazine and Some Major Metabolites in the Ames Test. Weed Science Lab, US Dept of Agriculture, Stoneville, Mississippi. Bull Environ Contam Toxicol 43: 797-804***

All compounds used were >99% purity. It appears that experiments using *S. typhimurium* strains TA100, 97 and 98 were conducted in the absence of a microsomal activating system; duplicate assays were performed, each conducted in triplicate. Atrazine, hydroxyatrazine and 2-chloro-6-isopropyl amino-s-triazine (desethylatrazine) were negative when tested over the concentration range 0.01 to 10 µmol/plate. Two other metabolites, F702 (2-chloro-4,6-diamino-s-triazine) and F703 (2-chloro-4-amino-6-ethylamino-s-triazine; desisopropylatrazine) were also without mutagenic activity. It was claimed that tests using strains TA1535, 1537 and 1538 were similarly negative (data not given). It was stated that none of the compounds were toxic to the cells at the concentrations tested (data not given). Positive control compounds (9-aminoacridine, sodium azide, 2-nitrofluorene) gave the expected mutagenic responses.

9.1.1.7 ***Simmon VF & Poole D (1977) In Vitro and in Vivo Microbiological Assays of Six Ciba-Geigy Chemicals. Ciba-Geigy (Japan) Ltd. Stanford Research Institute, Menlo Park, CA. March 1977***

Atrazine (batch and purity details not stated) was tested at three dose levels in mouse host-mediated assays (using male Swiss Webster mice). Treated mice received either a single dose (acute exposure) or 5 consecutive daily doses (subacute) of atrazine by oral intubation. Simultaneously, *Salmonella typhimurium* strains TA 1535 (which detects base-pair substitution mutations) and TA 1538 (which detects frameshift mutations) were injected subcutaneously. After 4 h, total viable and mutant cells were enumerated by plating serial dilutions of peritoneal exudate. Positive control compounds were also used, DMNA with TA 1535 and 2-anthramine with TA 1538.

Doses of atrazine tested were 500, 1000 and 2000 mg/kg in the acute study and 275, 5500 and 1100 mg/kg in the repeat-dose study with TA 1535. Doses were 550, 1100 and 2200 mg/kg in the acute study, and 275, 5500 and 1100 mg/kg in the repeat-dose study with TA 1538.

Results were calculated as histidine-positive revertants/mL and per 10^8 cells. Atrazine was negative for host-mediated mutagenic activity whilst positive controls gave the expected responses.

9.1.1.8 ***Weisenburger DD, Joshi SS, Hickman TI, Walker BA & Lawson TA (1988) Mutagenesis Tests of Atrazine and N-Nitrosoatrazine. Compounds of Special Interest to the Midwest. Dept of Pathology & Microbiology and the Eppley Institute for Research in Cancer, Uni. of Nebraska Medical Center, Omaha, NE. Proc AACR 29: 106 (Abstract 421)***

Study details were limited in this abstract. In a modified Ames test with *S. typhimurium* (strains TA98 and 100), atrazine was not mutagenic but N-nitrosoatrazine (NNAT) increased the mean number of revertants by several-fold at concentrations tested up to 1 mg/plate. In the Chinese hamster V79 assay, NNAT (concentration unstated) apparently produced 3.4-times more mutants/10^6 survivors than did dimethylnitrosamine (100 µg/mL), leading to the conclusion that NNAT was only weakly mutagenic in the Ames assay but a strong mutagen in the mammalian V-79 assay.

9.1.2 Desethylatrazine

9.1.2.1 ***Deparade E (1989a) Salmonella and Escherichia/Liver-microsome Mutagenicity Test: G 3033 tech. Ciba-Geigy, Basle. Study No. 891236. Completion date 18 Dec. 1989 (GLP; Switzerland, USA, Japan, OECD)***

This study was conducted according to OECD, US, EEC and Japanese test guidelines.

Desethylatrazine [4-chloro-2-isopropyl-s-triazine] technical (G 30033; batch no. FL 891543; 99.3% purity), an atrazine metabolite, was tested in *S. typhimurium* strains TA 98, 100, 1535, 1537 and *E. coli* WP2uvrA at concentrations of 313 to 5000 µg/0.1 mL, with or without a microsomal fraction prepared from Aroclor-induced rat liver. In a preliminary toxicity test (20 to 5000 µg/0.1 mL), the highest concentration tested was found to be suitable for the main assay. In the main test (duplicate assays on separate days, with 3 plates/assay), there was no evidence of mutagenic activity at any concentration tested. Negative (DMSO solvent) and positive controls (4-nitroquinoline-N-oxide, daunorubicin, sodium azide, 9(5)-aminoacridine, nitrofluorene, 9-aminoacridine, 2-aminoanthracene and cyclophosphamide) gave the expected results.

9.1.2.2 ***Butler MA & Hoagland RE (1989) Genotoxicity Assessment of Atrazine and Some Major Metabolites in the Ames Test. Weed Science Lab, US Dept of Agriculture, Stoneville, Mississippi. Bull Environ Contam Toxicol 43: 797-804***

See Section 9.1.1.6

9.1.3 Desisopropylatrazine

9.1.3.1 ***Deparade E (1990) Salmonella and Escherichia/liver-microsome Test - G28279 Tech. Ciba-Geigy Ltd, Basle, Switzerland. Test no. 891243. Study completion date 18 Jan. 1990 (GLP; OECD, USA, Japan)***

This study was conducted according to OECD, US EPA, EEC, and Japanese guidelines.

G28279 technical, **desisopropylatrazine**, an atrazine metabolite (Batch P1; 97.4% purity) in DMSO vehicle was not mutagenic in Ames tests on *S. typhimurium* strains TA98, TA100, TA1535 and TA1537, and *E. coli* WP2uvrA at concentrations of 313, 625, 1250, 2500 and 5000 µg/0.1 mL, with or without microsomal activation. A preliminary toxicity test was conducted in the concentration range 20 to 5000 µg/0.1 mL. Positive controls (4-nitroquinoline-N-oxide, 2-nitrofluorene, 9-aminoacridine, cyclophosphamide and 2-aminoanthracene) gave the expected results.

9.1.3.2 ***Butler MA & Hoagland RE (1989) Genotoxicity Assessment of Atrazine and Some Major Metabolites in the Ames Test. Weed Science Lab, US Dept of Agriculture, Stoneville, Mississippi. Bull Environ Contam Toxicol 43: 797-804***

See Section 9.1.1.6

9.1.4 Diaminochlorotriazine

9.1.4.1 ***Deparade E (1987) Salmonella/Mammalian-microsome Mutagenicity Test. Technical diaminochlorotriazine. Ciba Geigy, Basle. Study No. 871372. 10 Nov. 1987 (US GLP)***

Diaminochlorotriazine technical (G 28273; batch no. FL 871776; 97% purity), an atrazine metabolite, was tested in *S. typhimurium* strains TA 98, 100, 1535, 1537 at concentrations up to 5000 mg/0.1 mL, with or without a microsomal fraction prepared from Aroclor-induced rat liver. In a preliminary toxicity test (0.08 to 5000 µg/0.1 mL), the highest concentration tested was found to be suitable for the main assay. Tests were conducted according to EPA Guideline no. 84-2 and OECD Guideline 471. In the main test (duplicate assays on separate days, with 3 plates/assay), the highest concentration (5000 µg/0.1 mL) precipitated in the soft agar. There was no evidence of mutagenic activity at the next highest concentration tested of 3000 µg/0.1 mL. Negative (DMSO solvent) and positive controls (4-nitroquinoline-N-oxide, daunorubicin, sodium azide, 9(5)-aminoacridine, 2-aminoanthracene and cyclophosphamide) gave the expected results.

9.1.5 Hydroxyatrazine

9.1.5.1 ***Arni P & Mueller D (1981) Salmonella/Mammalian-Microsome Mutagenicity Test with G 34048 (Technical Hydroxyatrazine). Ciba-Geigy Ltd, Basle. Project report no. 791690. Report Date 28 Jan. 1981 (no GLP statement)***

Hydroxyatrazine technical (batch no. and purity not stated) was tested in *S. typhimurium* strains TA 98, 100, 1535, and 1537 at concentrations of 5.7, 17 and 51 µg/plate, with or without a microsomal fraction prepared from Aroclor-induced rat liver. In a repeat experiment with S9, concentrations of 51 and 102 µg/plate were assayed. The compound was microsolized in DMSO and 1% Tween 20. There was no evidence of any mutagenic activity in any of the strains at any of the concentrations tested. Negative (DMSO solvent) and positive controls (4-nitroquinoline-N-oxide, daunorubicin, sodium azide, 9(5)-aminoacridine, N-methyl-N'-nitro-N-nitrosoguanidine) gave the expected results.

9.1.5.2 ***Deparade E (1988) Salmonella/Mammalian-microsome Mutagenicity Test. Technical hydroxyatrazine. Ciba-Geigy, Basle. Study No. 871376. report Date 15 Feb. 1988. (GLP; US EPA)***

Tests were conducted according to EPA Guidelines no. 84-2 and OECD Guideline 471.

Hydroxyatrazine technical (G 34048; batch no. FL 870869; 99% purity), an atrazine metabolite, was tested in *S. typhimurium* strains TA 98, 100, 1535, and 1537 at concentrations of 20 to 5000 mg/0.1 mL, with or without a microsomal fraction prepared from Aroclor-induced rat liver. In a preliminary toxicity test (0.08 to 5000 µg/0.1 mL), the highest concentration tested was found to be suitable for the main assay. In the main test (duplicate assays on separate days, with 3 plates/assay), the concentrations of 313 µg/0.1 mL and above showed some precipitation in the soft agar. There was no evidence of any mutagenic activity in any of the strains at any of the concentrations tested. Negative (DMSO solvent) and positive controls (4-nitroquinoline-N-oxide, daunorubicin, sodium azide, 9(5)-aminoacridine, 2-aminoanthracene and cyclophosphamide) gave the expected results.

9.1.5.3 ***Butler MA & Hoagland RE (1989) Genotoxicity Assessment of Atrazine and Some Major Metabolites in the Ames Test. Weed Science Lab, US Dept of Agriculture, Stoneville, Mississippi. Bull Environ Contam Toxicol 43: 797-804***

See Section 9.1.1.6

9.2 Chromosomal Effects Assays

9.2.1 Atrazine

9.2.1.1 ***Murnick MR & Nash CL (1977) Mutagenicity of the Triazine Herbicides Atrazine, Cyanazine and Simazine in Drosophila melanogaster. J Tox Environ Health 3: 691-697***

No conclusions were reached since the authors suggested that the increase in dominant lethals after injection was due to toxic effect to sperm, and in the larval feeding experiments in which mutagenic signs were produced, repetition using larger samples was required before confident conclusions could be reached.

9.2.1.2 ***Hool G, Langauer M & Mueller D (1981) Nucleus Anomaly Test in Somatic Interphase Nuclei - G 30 027: Chinese Hamster. Ciba-Geigy Ltd, Basle, Switzerland. Expt no. 78027. Report date 20 Jan. 1981***

Chinese hamsters (6/sex/group) were given two daily oral doses of 0 (vehicle), 282, 564 and 1128 mg/kg atrazine (lot no. 6663) dissolved in 0.7% aqueous CMC (20 mL/kg). Animals were sacrificed 24 h after the second dose and bone marrow harvested from femurs; 1000 cells were scored from 3

animals/sex/group. Cyclophosphamide (128 mg/kg) was used as a positive control.

There was no increase in the cells displaying nuclear abnormalities following atrazine. Cyclophosphamide caused a marked increase in anomalies viz. single Jolly bodies, fragments of nuclei in erythrocytes, micronuclei in erythroblasts and leucopoietic cells, and polyploid cells.

9.2.1.3 ***Hool G & Mueller D (1981a) Chromosome Studies in Male Germinal Epithelium - G 30 027: Mouse: (Test for Mutagenic effects on Spermatocytes). Ciba-Geigy Ltd, Basle, Switzerland. Expt no. 800209. 25 Jan. 1981***

In a mammalian cytogenetics test in male mice, germinal epithelium was examined for chromosomal aberrations following *in vivo* dosing with atrazine. NMRI-derived male mice (15/dose group; 12 controls) were given atrazine (lot no. 6663; 98.9% purity) at oral doses (20 mL/kg in 0.7% aqueous CMC) of 0, 444 and 1332 mg/kg/day orally for 5 consecutive days. The high dose was chosen on the basis of the LD50 in this mouse strain being determined as 3992 mg/kg (both sexes). Mice were killed 5 days after the first dose and 3 h after an ip injection of colcemide (10 mg/kg). The testes of 10/group were processed and drop preparations made of the testicular parenchyma. One hundred metaphase plates from 8 animals/group were examined for chromatid aberrations, chromosomal aberrations, chromatid gaps and chromosomal pulverisation.

No specific chromosome abnormalities were seen in the spermatocytes.

9.1.2.4 ***Hool G & Mueller D (1981b) Chromosome studies in male germinal epithelium - G 30 027: Mouse (Test for Mutagenic effects on Spermatogonia). Ciba-Geigy Ltd, Basle, Switzerland. Expt no. 800210. 28 Jan. 1981***

In a second mammalian cytogenetics test in male mice, germinal epithelium was examined for chromosomal aberrations following *in vivo* dosing with atrazine. The experiment was exactly as detailed above, except that *in vivo* dosing occurred over 10 days, with the same doses as above given orally on days 0, 2, 3, 5 and 9 of the study.

No specific chromosome abnormalities were seen in the spermatogonia.

9.1.2.5 ***Hool G & Mueller D (1981c) Dominant Lethal Test - Mouse. Ciba-Geigy Ltd, Basle, Switzerland. Expt no. 801380. 8 Sept. 1981 (QA Statement)***

Mature NMRI-derived male Tif.MAGf (SPF) mice (20/group; 3-4 months old and 34-47 g bodyweight) were given single oral doses of 0, 444 and 1332 mg/kg of technical atrazine (lot 6663: 98.9% purity) followed by mating with females (2-3 months old) at weekly intervals for 6 weeks; each male was mated with two females for one week, the period commencing approx. 6 h after

treatment. The compound was administered in 0.5% aqueous sodium CMC (0.2 mL/10 g bodywt). Females were autopsied on day 14 of pregnancy.

There were no reported clinical signs in males and no effects on pregnancy rate, or the numbers of implantations, live embryos or embryonic deaths.

9.1.2.6 ***Ceresa C, Langauer M & Arni P (1988a) G30027 Tech. (Atrazine): Micronucleus Test, Mouse. Ciba-Geigy Ltd, Basle, Switzerland. Test No. 871546. Report date 31 May 1988***

This study was conducted according to OECD Guideline 474. Atrazine technical (Batch no. 210200; 98.2% purity) was administered to groups of male and female mice (Tif: MAGF, SPF) from Ciba-Geigy Tierfarm, Sisseln, by oral gavage (0.5% CMC vehicle) at a dose of 2250 mg/kg bw and sacrificed at 16, 24 and 48 h, or was administered at doses of 562.5, 1125 and 2250 mg/kg bw, with sacrifice after 24 h. Two animals/sex were used in the tolerability study, 24/sex in the first test (control and dosed group; 8/sex at each time point), 8/sex in the second study, and 8/sex for positive controls (cyclophosphamide). Polychromatic erythrocytes from bone marrow were then analysed for micronuclei. The percentages of micronuclei were not increased significantly in any of the treated groups.

9.1.2.7 ***Hertner Th (1993) Structural Chromosome Aberration Test. Dominant Lethal Test, Mouse, 8 Weeks. Ciba-Geigy Ltd, Basle, Switzerland. Lab. Study no. 911247. 7 Jan. 1993 (GLP; OECD, Switzerland, USA, Japan)***

This study was conducted according to US EPA Guidelines no. 84-2, OECD Guideline 478 and EEC guidelines. Young adult NMRI-derived male Tif:MAGf (SPF) mice from Ciba-Geigy, Sisseln (30/group; 31-45 g bodyweight) were given single oral gavage doses of 0, 500, 1000, 2000 and 2400 mg/kg of technical atrazine (batch SG8029BA10; FL 881692: 97.4% purity) in corn oil, followed by mating with two virgin females (2-3 months old) at each the following intervals; days 1-4, days 4-8, days 8-12, and weeks 3, 4, 5, 6, 7 and 8. Females were autopsied on day 13-15 of pregnancy.

In a preliminary tolerability test at doses of 1400 to 2000 mg/kg (2 males/dose), 1 of 2 animals each at 1400 and 1800 mg/kg, and 2/2 from the 2200 mg/kg group died.

In the 500 mg/kg group, there were no signs of toxicity. 6/30 at 1000 mg/kg piloerection and/or reduced locomotor activity. 2/30 at 2000 mg/kg showed piloerection. At 2400 mg/kg, piloerection and/or reduced locomotion was seen at 15/30 males. No significant effects on mating frequency, pregnancy rate, or the numbers of implantations, live embryos or embryonic deaths were seen. The positive control, cyclophosphamide, gave the expected results.

Atrazine technical did not induce dominant lethal mutations in male mice at single doses as high as 2400 mg/kg bw.

9.1.2.8 *National Toxicology Program (1993) NTP Technical Report on Toxicity Studies of Pesticide/Fertilizer Mixtures Administered in Drinking Water to F344/N Rats and B6C3F1 Mice. NIH Publication 93-3385. Date July 1993*

This report focuses primarily on 26-week toxicity studies in F344/N rats and B6C3F1 mice given pesticide/fertilizer mixtures in their drinking water. Additionally, *in vivo* genetic toxicology assays were conducted on peripheral blood erythrocytes from female mice at the 13-week interim evaluation of the toxicity study (induction of micronuclei) and on splenocytes from male rats and female mice at the 13-week interim evaluation (induction of micronuclei and SCEs).

Two mixtures of pesticides and a fertilizer (ammonium nitrate), representative of established groundwater contamination in California and Iowa, were both tested at 0.1x, 1x, 10x and 100x the median concentration found in the groundwater surveys).

The 1x mixtures were made up as follows (ng/mL concentration given in brackets, unless otherwise stated):-

alachlor (0.9); atrazine (0.5); cyanazine (0.4); metolachlor (0.4); metribuzin (0.6); ammonium nitrate (10 µg/mL); propylene glycol (512 µg/mL; used as solubilizer) (IOWA)

aldicarb (9.0; 1;1:1 ratio of aldicarb, sulfoxide and sulfone); atrazine (0.5); dibromochloropropane (0.01); 1,2-dichloropropane (4.5); EDB (0.9); simazine (0.3); ammonium nitrate (10 µg/mL); propylene glycol (512 µg/mL; used as solubilizer) (CAL)

Results of tests for induction of micronuclei in peripheral blood erythrocytes of female mice treated with CAL water were negative. With the IOWA mix, significant increases were seen at 10x and 100x concentrations but they were within the normal range of micronuclei in historical control animals. SCE frequencies in splenocytes of male rats and female mice were marginally increased in mice and rats receiving the CAL mixture but neither species exhibited increased frequencies of micronucleated splenocytes. None of the above changes were considered of biological significance.

9.1.2.9 ***Meisner-LF; Roloff-BD; Belluck-DA (1993) In Vitro effects of N-nitrosoatrazine on chromosome breakage. State Laboratory of Hygiene, University of Wisconsin, Madison, USA. Arch Environ Contam Toxicol 24: 108-12***

Exposing human lymphocyte cultures to concentrations of N-nitrosoatrazine (NNAT) as low as 0.0001µg/mL resulted in significant elevations in chromosome breakage as well as an increased mitotic index. In contrast, 1,000-10,000-fold greater concentrations of nitrates, nitrites, and/or atrazine was required to produce comparable chromosome damage. Simultaneous exposure to atrazine and nitrate or nitrite was non-clastogenic. None of atrazine, nitrate, nitrite or a combination of these increased mitotic indices whereas NNAT did cause elevations of the mitotic index. This study illustrates that metabolic conversion of contaminants with no or minimal genotoxicity or mitogenicity can give rise to compounds such as NNAT which are genotoxic. It was stated that NNAT can be formed from atrazine and nitrite under acidic ocnditions but no data was cited on whether NNAT is formed in the environment in water contaminated with atrazine and nitrates, or whether it is formed in human stomach after ingestion of such water.

9.2.2 Desethylatrazine

9.2.2.1 ***Ogorek B (1991a) G 30033 (Desethylatrazine) Tech: Micronucleus Test, Mouse. Ciba-Geigy Ltd, Basle, Switzerland. Test No. 901309. Report date 25 March 1991 (GLP; Switzerland, OECD, US EPA, Japan)***

This study was conducted according to OECD Guideline 474, EEC and US EPA guidelines. **Desethylatrazine** technical (Batch no. FL 891543; 99.3% purity) was administered to groups of male and female mice (Tif: MAGF, SPF) from Ciba-Geigy Tierfarm, Sisseln by oral gavage (0.5% CMC vehicle) at doses of (1) 480 mg/kg, with sacrifice at 16, 24 and 48 h and (2) 120, 240 and 480 mg/kg with sacrifice at 24 h. Two animals/sex were used in the tolerability study, 24/sex in the first test (control and dosed group; 8/sex at each time point), 8/sex in the second study, and 8/sex for positive controls (cyclophosphamide). Polychromatic erythrocytes from bone marrow were then analysed for micronuclei (5/sex/group evaluated in the two parts). It was concluded that desethylatrazine showed no evidence of clastogenic or aneugenic effects under the test conditions.

9.2.3 Desisopropylatrazine

9.2.3.1 ***Ogorek B (1991b) G 28279 (Desisopropylatrazine) Tech: Micronucleus Test, Mouse. Ciba-Geigy Ltd, Basle, Switzerland. Test No. 901307. Report date 23 Feb. 1991 (GLP; Switzerland, OECD, US, Japan)***

This study was conducted according to OECD Guideline 474, EEC and US EPA guidelines. **Desisoproipylatrazine** technical (Batch no. P1; 97.4% purity) was administered to groups of male and female mice (Tif: MAGF, SPF) from Ciba-Geigy Tierfarm, Sisseln, by oral gavage (0.5% CMC vehicle) at

doses of (1) 480 mg/kg, with sacrifice at 16, 24 and 48 h and (2) 120, 240 and 480 mg/kg with sacrifice at 24 h. Two animals/sex were used in the tolerability study, 8/sex at each concentration and time point in the main and replicate study), and 8/sex for positive controls (cyclophosphamide). Polychromatic erythrocytes from bone marrow were then analysed for micronuclei (5/sex/group evaluated in the two parts). It was concluded that desisopropylatrazine showed no evidence of clastogenic or aneuploidy-inducing effects under the test conditions.

9.2.4 Diaminochlorotriazine

9.2.4.1 ***Strasser F (1988) G 28273 (Diaminochlorotriazine): Micronucleus Test, Mouse. Ciba-Geigy Ltd, Basle, Switzerland. Test No. 871369. Report date 30 March 1988***

This study was conducted according to OECD Guideline 474. **Diaminochlorotriazine** technical (Lot no. FL 871776; 97% purity) was administered to groups of male and female mice (NMRI-derived Tif: MAGF, SPF) from Ciba-Geigy Tierfarm, Sisseln by oral gavage (0.5% CMC vehicle) at doses of (1) 5000 mg/kg, with sacrifice at 16, 24 and 48 h and (2) 1250, 2500 and 5000 mg/kg with sacrifice at 48 h. Two animals/sex were used in the tolerability study, 24/sex in the first test (control and dosed group; 8/sex at each time point), 8/sex in the second study, and 8/sex for positive controls (cyclophosphamide). Polychromatic erythrocytes from bone marrow were then analysed for micronuclei. It was concluded that DACT showed no evidence of clastogenic effects under the test conditions.

9.2.5 Hydroxyatrazine

9.2.5.1 ***Ceresa C, Langauer M & Puri E (1988) Hydroxyatrazine. Structural Chromosomal Aberration Test (Micronucleus Test), Mouse. Ciba-Geigy Ltd, Basle, Switzerland. Study no. 871373. Study completion date 31 August 1988 (GLP; USA)***

This test was conducted according to OECD Guideline no. 474. The atrazine metabolite, **hydroxyatrazine** (G-34048 tech; Batch no. FL 870869; 99% purity) was administered by gavage to Tif: MAGF SPF NMRI-derived mice (24/sex/gp) at 5000 mg/kg bw in carboxymethyl cellulose (CMC). Controls (24/sex) received CMC alone (negative controls) while 8/sex received 64 mg/kg cyclophosphamide (positive controls). In a second phase of the study, mice (8/sex/group) received 1250, 2500 and 5000 mg/kg bw of hydroxyatrazine and positive controls were given 65 mg/kg cyclophosphamide. All doses were administered in 20 mL/kg 0.5% CMC. Animals from the first phase of the study were sacrificed 16, 24 and 48 h after dosing. In the second phase, all animals were sacrificed 24 h after treatment and bone marrow smears prepared.

In neither phase of the study did bone marrow smears show any significant increase in micronucleated polychromatic erythrocytes (MNEs) compared to negative controls. The positive control experiments in both phases of the study

gave an average of 2.4% (phase 1) and 1.6% (phase 2) MNEs, a significant increase from negative controls (0.08% and 0.04%, respectively).

It can be concluded that the atrazine metabolite, hydroxyatrazine, is not genotoxic in this assay.

9.3 Other Genotoxic Effects

9.3.1 Atrazine

9.3.1.1 ***Puri E & Mueller D (1984a) Autoradiographic DNA Repair Test on Rat Hepatocytes. Ciba-Geigy, Basle. Report no. 831171. Report Date 16 May 1984***

Cultures of freshly-isolated rat hepatocytes [male Tif:RAIf(SPF)] were exposed to technical atrazine (G 30 027; Batch no. P 210200; 98.2% purity; dissolved in ethanol) for 5 h at concentrations of 1.2, 6, 30 and 150 µg/mL (4 cultures/group). The highest dose used produced a slight precipitation in the culture but no toxicity was seen in preliminary toxicity tests. No unscheduled DNA synthesis (assessed by incorporation of ^{3}H-thymidine) consequent upon DNA damage by atrazine was observed at any of the concentrations used. Positive controls utilised dimethylnitrosamine.

9.3.1.2 ***Hertner Th (1992) Autoradiographic DNA-Repair Test on rat Hepatocytes. G 30027 Tech. (Atrazine). Ciba-Geigy, Basle, Switzerland. Study No. 911246. 14 April 1992 (GLP; US, Switzerland, OECD)***

Tests were conducted according to guidelines of the EEC, US EPA, and OECD Guideline 482.
Atrazine technical (batch no. SG 8029 BA 10; 97.1% purity), was negative in an *in vitro* unscheduled DNA repair test in primary hepatocytes freshly isolated from male rats [Tif: RAIf(SPF) strain] over a concentration range of 15.5 to 1670 µg/mL (preliminary test) and 15.5 to 1670 µg/mL (duplicate test; 4 cultures per group in each independent test). Concentrations tested were chosen on the basis of two preliminary cytotoxicity tests (1.6 to 1670 and 15.5 to 1670 µg/mL) in which 1670 µg/mL was shown to be the solubility limit in the DMSO solvent used, a concentration which allowed 27% cell viability and which did not inhibit replicative DNA synthesis. Negative (DMSO solvent) and positive controls (2-AAF at 45 µM) gave the expected results.

Atrazine technical was negative for unscheduled DNA synthesis in adult male rat hepatocytes *in vitro*, at concentrations up to 1670 µg/mL.

9.3.1.3 ***Puri E & Mueller D (1984b) Autoradiographic DNA Repair Test on Human Fibroblasts. Ciba-Geigy, Basle. Report no. 831172. Report Date 16 May 1984***

Cultured human fibroblasts (CRL 1121 from "The American Type Culture Collection, Rockville, MD, USA) were exposed to technical atrazine (G 30

027; Batch no. P 210200; 98.2% purity; dissolved in ethanol) for 5 h at concentrations of 1.2, 6, 30 and 150 µg/mL (5 cultures/group). No unscheduled DNA synthesis (assessed by incorporation of ^{3}H-thymidine) consequent upon DNA damage by atrazine was observed at any of the concentrations used. Positive controls used 5 µM 4-nitroquinoline-N-oxide.

9.3.1.4 ***Pino A et al. (1988) DNA Damage in Stomach, Kidney, Liver and Lung of Rats treated with Atrazine. Mutation Res 209: 145-147***

Sprague-Dawley rats received a single 875 mg/kg bw oral dose of atrazine (from Ciba-Geigy) suspended in 1% CMC in saline, and were killed at 12, 24, 36, 48 and 72 h for assessment of DNA damage. DNA damage was assessed in liver, kidney, stomach and lung by the technique of alkaline elution, which indirectly measured the number of single strand breaks. DNA fragmentation was also evaluated after administration of 5 or 15 successive daily doses of 350 mg/kg, with sacrifice 12 h after the last dose.

DNA lesions were detected in stomach and kidney, and at a lower level in liver, but not in lung. DNA breaks/lesions were present 24 h after a single dose, reaching a maximum 12 h later in liver and kidney, and 72 h later in stomach mucosa. With repeat-dosing, the DNA elution rate was significantly increased after 5 days treatment in kidney and stomach, and after 15 days in liver.

The authors suggested that atrazine may be activated *in vivo* by extra-hepatic enzymes.

9.3.2 Desethylatrazine

9.3.2.1 ***Geleick D (1991a) G 30033 (Desethylatrazine) Tech: Autoradiographic DNA Repair Test on Rat Hepatocytes. Ciba-Geigy Ltd, Basle, Switzerland. Study No. 901310. Study completion date 26 April 1991 (GLP; Switzerland, OECD, US, Japan)***

Tests were conducted according to US EPA and EC guidelines, and OECD Guideline 482. Cultured of freshly-isolated rat hepatocytes [male Tif:RAIf(SPF) from Ciba-Geigy Tierfarm, Sisseln] were exposed to technical **desethylatrazine** (G 30 033; Batch no. FL891543; 99.3% purity; dissolved in DMSO) for 16-18 h at concentrations of 9.25, 27.7, 83.3, 250, 500 and 1000 µg/mL (duplicate experiment; 4 cultures/group). These doses were based on preliminary cytotoxicity tests which used concentrations from 1.7-1800 µg/mL and 9.25 -1000 µg/mL. No significant increase in unscheduled DNA synthesis (assessed by incorporation of ^{3}H-thymidine) caused by desethylatrazine was observed at any of the concentrations used. Positive controls utilised 2-AAF.

9.3.3 Deisopropylatrazine

9.3.3.1 ***Geleick D (1991b) G 28279 (Desisopropylatrazine) Tech: Autoradiographic DNA Repair Test on Rat Hepatocytes. Ciba-Geigy Ltd, Basle, Switzerland.***

Study No. 901308. Study completion date 12 April 1991 (Amended report date 21 Dec. 1993) (GLP; Switzerland, OECD, US, Japan)

Tests were conducted according to US EPA and EC guidelines, and OECD Guideline 482. Cultured of freshly-isolated rat hepatocytes [male Tif:RAIf(SPF) from Ciba-Geigy Tierfarm, Sisseln] were exposed to technical **desisopropylthylatrazine** (G 28279; Batch no. P.1; 97.4% purity; dissolved in DMSO) for 16-18 h at concentrations of 7.4, 22.2, 66.6, 200, 400 and 800 µg/mL (duplicate experiment; 4 cultures/group). These doses were based on preliminary cytotoxicity tests which used concentrations from 1.4 to 1500 µg/mL (cell toxicity test) and 7.4 to 800 µg/mL (test for inhibition of replicative DNA synthesis). No significant increase in unscheduled DNA synthesis (assessed by incorporation of ^{3}H-thymidine) caused by desethylatrazine was observed at any of the concentrations used. Positive controls utilised 2-AAF (45 µM).

9.3.4 Diaminochlorotriazine

9.3.4.1 ***Meyer A (1987) Autoradiographic DNA Repair Test on Human Fibroblasts. Technical Diaminochlorotriazine Ciba-Geigy, Basle, Switzerland. Study No. 871371. 20 Nov. 1987***

Tests were conducted according to EPA Guidelines no. 84-2 and OECD Guideline 471.

Diaminochlorotriazine technical (G 28273; batch no. FL 871776; 97% purity), an atrazine metabolite, was negative in an unscheduled DNA repair test in human fibroblasts (CRL 1521, from 'the American Type Culture Collection', Rockville, MD) *in vitro*, over a concentration range of 5.56 to 600 µg/mL (duplicate tests, with 4 cultures per test group); visible compound precipitation was seen at 300 and 600 µg/mL. Concentrations tested were chosen on the basis of a preliminary cytotoxicity test (7.5 to 1000 µg/mL). Negative (DMSO solvent) and positive controls (4-nitroquinoline-N-oxide) gave the expected results.

9.3.4.2 ***Hertner Th & Puri E (1988) Diaminochlorotriazine: Autoradiographic DNA Repair Test on Rat Hepatocytes Ciba-Geigy Ltd, Basle, Switzerland. Study no. 871370. Report date 10 March 1988 (GLP)***

The atrazine metabolite, **diaminochlorotriazine** (DACT), was tested for DNA-damaging effects on primary rat hepatocytes *in vitro;* hepatocytes were isolated from male Tif: RAIf(SPF) rats. Concentrations from 0.10 up to 400 µg/mL of the test substance (G-28278 tech.) in DMSO vehicle were used in triplicate experiments (each with 4 cultures/group). Unscheduled DNA synthesis (UDS) was assessed by ^{3}H-thymidine incorporation and autoradiography. Negative controls (DMSO or cell culture vehicle only) and positive controls (4-aminophenyl, at 25 or 50 µM) were used. Viability tests on the cells showed that very high levels of cell death occurred at concentrations of >350 µg/mL. There was no evidence of a biologically significant increase in UDS in the

cultures treated with diaminochlorotriazine but UDS was 2.3-10.3 times negative control levels in the positive controls.

9.3.5 Hydroxyatrazine

9.3.5.1 ***Meyer A (1988) Autoradiographic DNA Repair Test on Human Fibroblasts. Technical Hydroxyatrazine. Ciba-Geigy, Basle, Switzerland, Study No. 871375. 11 Jan. 1988. (GLP; US EPA)***

Tests were conducted according to EPA Guidelines no. 84-2 and OECD Guideline 482.

Hydroxyatrazine technical (G 34048; batch no. FL 870869; 96-99% purity), an atrazine metabolite, was negative in an unscheduled DNA repair test in human fibroblasts (CRL 1521, from 'the American Type Culture Collection', Rockville, MD) *in vitro*, over a concentration range of 13.89 to 1500 µg/mL (duplicate tests, with 4 cultures per test group); visible compound precipitation was seen from 125 to 1500 µg/mL. Concentrations tested were chosen on the basis of a preliminary cytotoxicity test (31.25 to 2000 µg/mL). Negative (DMSO solvent) and positive controls (4-nitroquinoline-N-oxide) gave the expected results.

9.3.5.2 ***Hertner Th (1988) Autoradiographic DNA-repair Test on Rat Hepatocytes. Technical Hydroxyatrazine. Ciba-Geigy, Basle, Switzerland. Study No. 871374. 22 Jan. 1988 (GLP; US EPA)***

Tests were conducted according to EPA Guidelines no. 84-2 and OECD Guideline 482.

Hydroxyatrazine technical (G 34048; batch no. FL 870869; 96-99% purity), an atrazine metabolite, was negative in an *in vitro* unscheduled DNA repair test in primary hepatocytes freshly isolated from male rats [Tif: RAIf(SPF) strain] over a concentration range of 13.89 to 1500 µg/mL (preliminary test) and 3.125 to 1500 µg/mL (duplicate test; 4 cultures per group in each independent test). Visible compound precipitation was seen at all concentrations in the first test. In the second test there was no precipitation at the two lowest concentrations (3.125 and 6.25 µg/mL) but it was evident from 12.5 µg/mL. Concentrations tested were chosen on the basis of a preliminary cytotoxicity test (69.37 to 4000 µg/mL) in which 1500 µg/mL was shown to be the highest usable concentration. Negative (DMSO solvent) and positive controls (4-aminobiphenyl at 25 and 50 µM) gave the expected results.

9.4 Cell Transformation Assays

No studies cited.

9.5 Reviews of atrazine genotoxicity

9.5.1 *Brusick DJ (1987) An Assessment of the Genetic Toxicity of Atrazine: Relevance to Human Health and Environmental Effects (unpublished manuscript)*

and

Brusick DJ (1994) An Assessment of the Genetic Toxicity of Atrazine: Relevance To Human Health and Environmental Effects. Mutation Res 317: 133-1440

Atrazine genotoxicity studies were reviewed by a quantitative 'weight of evidence' approach, and by a detailed review of conflicting studies. The weight of evidence approach (as developed by the International Commission for Protection Against Environmental Mutagens and Carcinogens) scored individual genotoxicity tests of atrazine, from which an overall negative consensus score for atrazine genotoxicity was derived (methods not explicit).

The review of conflicting studies considered *in vivo* mouse bone marrow metaphase analyses, *Drosophila* sex-linked recessive lethal assays, microbial host-mediated assays, and mouse dominant lethal assays. It was not possible to reconcile all the test-response conflicts.

Bone marrow studies with atrazine have shown a positive result at 2000 mg/kg bw when administered by oral gavage in olive oil in mice, and negative results with corn oil at the same dose and with Chinese hamsters at 500 mg/kg bw. It was suggested that the different vehicles might have influenced the test outcomes. In the case of *Drosophila* sex-linked recessive lethal assays, it was indicated that the positive studies had used non-concurrent controls. One of the two positive microbial host-mediated assays was unconventional, with injection of cells into the testes of rats. Other conventional studies were negative. The positive mouse dominant lethal assays reportedly used higher doses (1500 or 2000 mg/kg bw) than the negative studies, and the vehicle was olive oil (see comment on bone marrow study, above).

The reviewer concluded that not all of the conflicting genotoxicity studies for atrazine could be reconciled on the basis of differences in study methods, atrazine purity, or route of administration, however the overall conclusion was that in most test systems atrazine was not genotoxic and that use of the weight-of-evidence approach results in a conclusion that atrazine does not pose a mutagenic hazard.

Data suggesting that atrazine and its plant metabolites have the ability to induce germ-cell damage in plants exposed *in situ* was considered, but this genotoxicity cannot be viewed as evidence for human health hazards.

9.5.2 ***Adler ID (1980) A Review of the Coordinated Research Effort on the Comparison of Test Systems for the Detection of Mutagenic Effects, Sponsored by the EEC. Mutation Res 74: 77-93***

The review, conducted under the Environmental Research Programme of the Commission of the European communities, indicated that atrazine was negative in a variety of *in vitro* tests for genotoxicity viz. reversion assays in *S. typhimurium*, forward mutation assays in *E. coli* (tested at 0.5 to 2.0 mM), *S. typhimurium* (0.23 to 1.15 mM), *S. pombe* (6 mM), and V79 cells (1.25 to 10 mM), mitotic gene conversion in *S. cerevisiae* (2.5 to 10 mM), and SCEs in CHO cells (1.25 to 10 mM), but was positive in forward mutation tests with *A. nidulans* ($8AG^R$ locus; activation with potato microsomes) and *S. coelicolor* ($Strp^R$ locus; potato microsomes), in a mitotic cross-over test in *A. nidulans* (tpa A1; potato microsomes) and in a UDS test in EVE cells (3 mM; potato microsomes). In *in vivo* studies, results were negative in recessive lethal tests in *Drosophila* (5 and 10 mM) and in host-mediated assays for histidine-reversion in *S. typhimurium* (1000 mg/kg). However, it gave positive results in a host-mediated assay for forward mutations in *E. coli* (100 mg/kg bw po, in 10% ethanol) and in *S. pombe* (1000 mg/kg po) , for chromosome breakage in mouse bone marrow cells (2000 mg/kg bw in olive oil), and for dominant lethals in mouse spermatids (1500 mg/kg po in olive oil).

10. Special Studies

10.1 Studies Related to Tumours in Sprague Dawley cf. Fischer F344 Rats

10.1.1 ***Hasegawa R & Ito N (1992) Liver Medium-term Bioassay in Rats for Screening of Carcinogens and Modifying factors in Hepatocarcinogenesis. First Dept of Pathology, Nagoya City University Medical School, Nagoya 467, Japan. Fd Chem Toxic 30(11): 979-992***

Male F344 rats (6 weeks old) were dosed with diethylnitrosamine (DEN) ip at 200 mg/kg bw and two weeks later treated with chemicals incorporated in the diet for 6 weeks, before undergoing necropsy; all rats underwent partial (2/3rds) hepatectomy at week 3. Hepatocarcinogenic potential was assessed by comparing the number and area of glutathione-S-transferase (placental form)-positive foci in the livers cf. controls given DEN alone.

A large number of chemicals were tested; of the known liver carcinogens, 14/14 compounds which tested positive in the Ames test gave positive results in this DEN-PH test while 10/12 non-mutagenic compounds gave positive results. Two peroxisome proliferators were negative, carcinogens other than hepatocarcinogens gave 5/17 positive results, and only 1/13 non-carcinogens (malathion) gave positive results.

Atrazine at 500 ppm in the diet of 15 rats was negative in this hepatocarcinogenicity assay.

10.1.2 ***Cabral R, Hoshiya T, Hakoi K, Hasegawa R & Ito N (date?) The Use of a Medium-term Bioassay for the Detection of Carcinogenicity in Pesticide Mixtures. [Abstract - source not identified] Ist Dept of Pathology, Nagoya City University Medical School, Nagoya 467, Japan***

The potential carcinogenicity of mixtures of pesticides was investigated in a short-term test for hepatocarcinogenic potential (detailed above). Pesticide mixtures were alachlor plus atrazine, and glyphosate, alachlor, atrazine and "inerts". All mixtures proved positive. When tested individually, alachlor was positive, glyphosate was borderline, and atrazine was negative. There was no information on the concentration of chemicals in the diet or on the number of animals/test.

10.1.3 ***Wetzel LT, Luempert LG, Breckenridge CB, Tisdel MO, Stevens JT, Thakur AK, Extrom PC & Eldridge JC (1994) Chronic Effects of Atrazine on Estrus and Mammary Tumour Formation in Female Sprague-Dawley and Fischer 344 Rats. Dept Toxicology, Ciba-Geigy, Greensboro, NC and Dept Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC. J Toxicol Env Hlth 43: 169-182***

This paper supports the hypothesis that atrazine acts to accelerate reproductive ageing of the neuroendocrine system in female Sprague Dawley rats, resulting in an earlier onset of mammary tumours of mammary tumours stemming from prolonged oestrogen exposure, and argues that a threshold can be established for this effect.

10.1.4 ***Eldridge JC, Fleenor-Heyser DG, Extrom PC, Wetzel LT, Breckenridge CB, Gillis JH, Luempert LG & Stevens JT (1994) Short-Term Effects of Chlorotriazines on Estrus in Female Sprague-Dawley and Fischer 344 Rats. Dept Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC and Dept Toxicology, Ciba-Geigy, Greensboro, NC. J Tox Env Hlth 43: 155-167***

Atrazine and simazine were administered by gavage to female Sprague-Dawley 2and Fischer 344 rats for 2 weeks at 0, 100 and 300 mg/kg bw in order to evaluate effects on body weight, endocrine and/or sex accessory tissue, oestrous cycle, vaginal cytology and serum hormone levels.

Significant dose-related reductions in bodywt occurred in both strains with both compounds at both dose levels; the magnitude of the effects was less in Fischer than in Sprague-Dawley rats, while the effects of simazine were less pronounced than those of atrazine at the same dose. Significant reductions in ovarian and uterine weights were observed in SD and Fischer rats given atrazine (both doses) but not in rats given simazine. Similarly, significant increases in adrenal weights were observed in SD and Fischer rats given atrazine (both doses) but not in rats given simazine (apart from a small increase in Fischer rats at 300 mg/kg). The increased adrenal weights may, together with reduced bodywts, reflect the stressful nature of the treatment.

There was a dose-related decrease in circulating oestradiol levels after atrazine dosing in SD rats (see also Section 4.1.4), with only a marginal decrease, if any, in Fischer rats. After simazine dosing, decreases in plasma oestradiol levels were minimal, and not statistically significant. There were no noteworthy changes in progesterone, prolactin or corticosterone levels.

For SD rats treated with atrazine, examination of vaginal cytology revealed a lengthening of the oestrous cycle, an enhancement of the percent of days spent in oestrus and a reduction of those in dioestrus. Fischer rats also exhibited a trend towards cycle lengthening, but showed a reduction in the percent of cycle days spent in oestrus and a higher percent of dioestrual days. These oestrous cycle observations are consistent with those made in long-term toxicity studies [see Sections 6.1.10 and 6.1.11(SD rats) and 6.1.13, 6.1.14 and 6.1.15 (F344 rats)]. Effects of simazine in both strains were similar to, but less pronounced than with atrazine.

The lack of a prolactin (PRL) secretory response to triazine administration is noteworthy because rodent mammary tumours are promoted by factors that enhance endogenous PRL secretion; these results would appear to remove prolactin as a significant contributor to mammary tumour development (although it would be useful to follow PRL levels in longer-term feeding studies).

While the reduction in oestradiol in SD rats contrasts with the increases noted in long-term SD rat studies (see Sections 6.1.10 and 6.1.11, and Appendix VI), the increase in oestrous cycle length, and days spent in oestrus suggests that the triazines (and atrazine much more than simazine) may hasten ageing of the endocrine system in SD rats.

10.1.5 ***Tennant MK, Hill DS, Eldridge JC, Wetzel LT, Breckenridge CB and Stevens JT (1994a) Chloro-S-Triazine Antagonism of Estrogen Action: Limited Interaction with Estrogen Receptor Binding. Dept Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC and Dept Toxicology, Ciba-Geigy, Greensboro, NC. J Toxicol Env Hlth 43: 197-211***

Note: This paper was also submitted as a manuscript prepared for J Fund Appl Tox.

Studies were conducted on the interaction of atrazine, simazine and diaminochlorotriazine (DACT), a common metabolite, with rat uterine oestrogen receptors (ER).

None of the compounds at concentrations up to 100 mM competed with radioactive oestradiol binding (5 nM) to ER in extracted uterine tissue under equilibrium conditions (at 4°C). The triazines were about 10^5 less potent than oestradiol itself in causing 50% reduction in labelled oestradiol binding to ER (at 25°C). If ER was preincubated with 100 mM triazines for 30 min before addition of labelled oestradiol, its association with ER was slowed. Similarly,

very high concentrations of triazines promoted a dissociation of oestrogen bound to ER (but at a much lower rate than that caused by excess unlabelled oestradiol).

Labelled atrazine possibly underwent some limited binding to the 4S isoform of ER. In 'in vivo' studies in ovariectomised rats, PO doses of 300 mg/kg/d of atrazine, simazine and DACT for 2 days reduced uterine ER binding capacity about 33%, 39% and 24%, respectively ie. rat uterine tissue had a diminished ability to take up or retain oestrogen; it was not possible from the results to know whether this reduction was due to a triazine occupation of receptor sites or whether the ER population was diminished in the tissue of treated animals.

The authors concluded that responses to triazine treatment would be best explained by effects on events other than, or in addition to, effects on ER binding of oestrogen.

10.1.6 Tennant MK, Hill DS, Eldridge JC, Wetzel LT, Breckenridge CB and Stevens JT (1994b) Chloro-S-Triazine Interaction with Estrogen Receptor Binding. Dept Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC and Dept Toxicology, Ciba-Geigy, Greensboro, NC. (Manuscript submitted to J Fund Appl Toxicol)

This manuscript appears to cover the same issues and studies as discussed in the previous paper.

10.1.7 ***Stevens JT, Breckenridge CB Wetzel LT, Gillis JH, Luempert LG & Eldridge JC (1994) Hypothesis for Mammary Tumorigenesis in Sprague-Dawley Rats Exposed to Certain Triazine Herbicides. Dept Toxicology, Ciba-Geigy, Greensboro, NC and Dept Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC. J Toxicol Env Hlth 43: 139-153***

This is a review of the carcinogenicity of the chloro-triazines substituted in the 2-position, and tertiary butyl-s-triazine, terbutryn, compounds which have been shown to evoke an increase in the incidence of mammary tumours in female Sprague-Dawley rats.

The following data were summarised:-

2-Chloro-s-triazines

Atrazine	mammary gland adenocarcinomas but not fibroadenomas) were increased at 70, 500 and 1000 ppm, with decreased survival at 500 ppm and above (Mayhew, 1986)
Propazine	adenocarcinomas increased at 1000 ppm (Jessup, 1980)
Simazine	increase in adenocarcinomas and fibroadenomas at 100 and 1000 ppm, accompanied by decreased survival (McCormick & Arthur, 1988
Terbuthylazine	no statistically significant effect on mammary tumour incidence at up to 750 ppm (Gfeller, 1983)

2-Thiomethyl-s-triazines

Ametryn	did not induce any mammary neoplasms; survival increased in 1000 ppm females (Hazelette & Green, 1987b)
Prometryn	did not induce any mammary neoplasms (Chau et al, 1991)
Terbutyrn	increased combined incidence of mammary fibroadenomas and adenocarcinomas; however, incidence of fibroadenomas at 3000 ppm well within historical control range (Jessup, 1979)

2-Methoxytriazines

Prometon	negative with regard to mammary tumours (O'Connor et al, 1988)
Terbumeton	negative with regard to mammary tumours at up to 500 ppm (Rolofson, 1981)

However, the reviewer drew attention to the fact that the mammary tumour response to atrazine observed in various female Sprague-Dawley rat studies (those conducted according to current standards) has not been consistent. Thus, the first study (Spindler & Sumner, 1981) revealed a non dose-related increase in the incidence of fibroadenomas (at 10 and 1000 but not 100 ppm). In a second study (Mayhew, 1986) there was a dose-responsive increase in adenocarcinomas but no effect on fibroadenomas. In a subsequent study (female offspring culled from the F2 generation of a 2-generation reproduction study), there was no increase in mammary tumours (Rudzki McCormick & Arthur, 1991; Ackerman, 1991: Section 6.1.8 & 6.1.9). Considering the large variation noted in the spontaneous occurrence of mammary tumours in SD rats (Haseman et al., 1986), it was considered that the inconsistent response was not surprising. The results of recent studies (eg. Wetzel et al, 1994; see Section 10.1.3) showing an earlier onset of mammary tumours (without an increase in total tumour incidence) has led to the hypothesis that certain triazines can produce an endocrine-mediated imbalance which results in precocious

reproductive ageing, with the possible earlier onset or increased incidence of mammary tumours.

10.1.8 *Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel G and Anton-Culver H (1993) Medical Hypothesis: Xenoestrogens as Preventable Causes of Breast Cancer. Env. Hlth Perspect 101, 372-377: 1994*

The authors **hypothesise** that substances such as xenoestrogens (chlorinated organic compounds, polycyclic aromatic hydrocarbons, triazine herbicides, and some pharmaceuticals) increase the risk of breast cancer by mechanisms which include interaction with breast cancer susceptibility genes.

The following experimental observations are cited:-

(1) the progression of chemically-induced mammary tumours in rats is inhibited by ovariectomy, and stimulated by reintroduction of oestrogen implants;

(2) oestradiol metabolism proceeds primarily through two mutually exclusive pathways, pathway I to 2-hydroxyestrone (2-OHE1), which has minimal oestrogenic activity and is claimed non-genotoxic, or pathway II to 16-alpha-OHE1, a fully potent oestrogen which is claimed to be genotoxic (Telang et al, 1992)[3]

It is suggested that substances which inhibit pathway I (eg. DMBA, benzo[a]pyrene, oncogenes, tumour viruses) or elevate pathway II (eg. alcohol, linoleic and arachidonic acids) increase tumour risk, while the converse is true for those factors which inhibit pathway II (eg. severe caloric restriction) or elevate pathway I (eg. dietary supplements such as indol-3-carbinol).

The paper cites Ghinea et al. (1988) as providing evidence of the oestrogenicity of atrazine, stating cryptically in a Table that 'hormone release' [was] increased by atrazine; this paper is assessed below.

The paper proposes a series of major epidemiological studies to evaluate the hypothesis and suggests that screening tests for oestrogenicity could become critical tools to assess the potential health consequences of new and existing chemicals.

[3] Subsequent to the completion of the draft atrazine review, a paper by Stephen Safe [Safe S (1997) Is there an association between exposure to environmental oestrogens and breast cancer. Env Hlth Perspect 105, Suppl 3, 675-678] concluded, on the basis of *in vitro* studies in MCF-7 cells, that the significance of 16 - and 2-hydroxy-E2/E1 ratios as prognostic indicators for mammary cancer is controversial and requires further investigation. This is supported by preliminary epidemiological studies in postmenopausal women [Ursin G, London S, Stanczyk F, Gentzschein E, Paganini-Hill A, Ross RK & Pike MC (1997) A pilot study of urinary estrogen metabolites (16 -OHE1 and 2-OHE1) in postmenopausal women with and without breast cancer. Env Hlth Perspect 105, Suppl 3, 601-605].

10.1.9 ***Ghinea E, Dumitriu L, Stefanovici G, Pop A, Damian A, Handoca A and Stanciu R (1988) Protein Content and Thyroid Hormone Release 'in Vitro' by Differentiated Thyroid Cancer Cells in the Presence of Estradiol, Dehydroepiandrosterone, Polypeptidic Hormones and Pesticides. 'CI Parhon' Institute of Endocrinology, Bucharest, Romania. Review Roum. Med - Endocrinol 26, 165-171: 1988***

This study was reviewed because Davis et al (see 10.1.8 above) claimed that it provided experimental evidence on the oestrogenicity of atrazine.

This is a poorly reported study, with the data reported in several complicated graphs, without adequate analysis of the findings, or of any discussion.

The authors measured the 'reactivity' of cultured human thyroid cancer cells treated 'in vitro' with oestradiol and dehydroepiandrosterone in the presence of other (thyroid hormones and insulin) and some pesticides including atrazine. The only findings reported on atrazine (1 µg per 1.5(?)mL culture) were:-

(1) a small inhibition of release of thyroglobulin, reversed by 10 µg oestradiol (not by dehydroepiandrosterone);
(2) an inhibitory effect on protein synthesis in the cell cultures (effect largely independent of the presence of oestrogen or dehydroepiandrosterone).

Therefore this finding was opposite to the above-reported "increase" in hormone release, and certainly does not seem to indicate an oestrogenic action of atrazine; if anything, it could suggest an antagonistic or competitive effect of atrazine on oestrogen.

10.1.10 ***Tennant MK, Hill DS, Eldridge JC, Wetzel LT, Breckenridge CB and Stevens JT (1994c) Possible Anti-Estrogenic Properties of Chloro-s-Triazines in Rat Uterus. Dept Physiol. and Pharmacol., Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC and Dept of Toxicology, Ciba Plant Protection, Ciba-Geigy Corporation, Greensboro, NC. J Tox Env Hlth 43(2): 183-196***

Note: This paper was also submitted as a manuscript with the title' Anti-Estrogenic Properties of Chloro-s-Triazines in Rat Uterus'.

In direct tests of estrogenic bioactivity, atrazine (97.7% purity), simazine (96.9% purity) or the common metabolite, diaminochlorotriazine (DACT) (98.2% purity), at po doses (in 0.5% CMC) of 20, 100 and 300 mg/kg/day over 3 days did not significantly increase the uterine weight of ovariectomised female SD rats; the highest dose (approx. 10% of the LD50) did cause bodywt loss viz. about 14% for atrazine, 11% for simazine and 17% for DACT cf. about 1% loss for controls, over the three days.

When administered as above, in ovariectomised SD rats, but with SC injections of oestradiol (2 µg/kg) on days 2 and 3, 300 mg/kg of the chlorotriazines reduced uterine weight in comparison to animals given estrogen alone ie.

appeared to antagonise the uterotrophic effects of the replacement oestrogen; the average reduction was 27% below the control uteri. DACT was also significantly active at 100 mg/kg. Body weight losses were similar to those in the previous experiment.

None of the chloro-s-triazines at 300 mg/kg/day po stimulated incorporation of [^{3}H]thymidine into uterine DNA of immature SD female rats; however, doses of 50 mg/kg bw/d or more, in a dose-related manner, significantly reduced thymidine incorporation into uterine DNA extracted from immature rats given single injections of 0.15 µg oestradiol.

Expression of progesterone receptor binding is an even more specific test of oestrogen action. Oral doses of 300 mg/kg (but not 50 mg/kg) of the three triazines significantly reduced expression of progesterone receptor binding in cytosol fractions prepared from uteri of ovariectomised rats injected SC with 1 µg oestradiol (using labelled synthetic progesterone ligand, 17 ,21-dimethyl-19-norpregna-4,9-diene-3,20-dione[17 -methyl-^{3}H]). Furthermore, uterine progesterone receptor levels were not stimulated in rats given po doses up to 300 mg/kg of these triazines without oestradiol injections.

Overall, results from these three studies suggest that these three triazines possess no intrinsic oestrogenic activity but are capable of weak inhibition of oestrogen-stimulated responses in the rat uterus; this inhibition may play a role in the changes in reproductive endocrine function previously observed in female SD rats. It was claimed that these findings differentiate the triazines from eg. DDT, methoxychlor, chlordecone. The observation that high levels (>50 mg/kg) of atrazine are needed to produce any anti-oestrogenic effects, the fact that the metabolite DACT has similar effects suggests that its storage and clearance should be taken into account when assessing risks from triazine exposure.

10.1.11 ***Morseth SL (1996a) Evaluation of the Luteinizing Hormone (LH) Surge in Female Sprague-Dawley Rats - Pilot Study. Ciba Crop Protection, Greensboro, NC. Lab: Corning Hazleton Inc., Vienna, Virginia. Laboratory study no. CHV 2386-109. Report date 18 Jan. 1996 (non-GLP study)***

and

10.1.12 Morseth SL (1996b) Evaluation of the Luteinizing Hormone (LH) Surge in Female Sprague-Dawley Rats - Method Validation. Ciba Crop Protection, Greensboro, NC. Lab: Corning Hazleton Inc., Vienna, Virginia. Laboratory study no. CHV 2386-110. Report date 18 Jan. 1996 (non-GLP study)

These studies were preliminary to a 4-week study to examine the effects of atrazine on the LH surge in female Sprague Dawley rats. Atrazine (Batch no. SG8029BAI0; 97.1% purity) was used, prepared in 0.5% CMC in deionised water. The -oestradiol benzoate used had a purity of approximately 98%.

-Oestradiol was administered sc via a surgically-implanted capsule to ovariectomised female SD Crl: Cd BR rats from Charles River Laboratories,

Raleigh, NC at an age of about 8 weeks. Atrazine (300 mg/kg) was administered by oral gavage for 3 days, beginning the day after surgery. The animals were subsequently bled at designated intervals and discarded without necropsy. Plasma was analysed for LH and prolactin (PRL) by RAI. Ten females/group were assigned to the vehicle control group or the atrazine group, with bleeding at 0700, 0900, 1100, 1400 and 1800 h, corresponding to 1100, 1300, 1500, 1800 and 2200 h in terms of the animals' biological time.

In control animals the LH surge was apparent at 1500 h, with a peak at 1800 h (biological time, 9 h after the light cycle began) and did not return to baseline by the final collection period (2200 h). Atrazine attenuated the LH surge at 1500 h, the time range in which the LH peak is expected to occur in young intact animals. The data also suggest that the LH surge may have been delayed in the atrazine-treated animals cf. controls (in a similar manner to the age-related delay seen in middle-aged animals when compared to young animals).

PRL levels rose over the course of the day; the failure of PRL levels to drop as expected late in the day in either control or atrazine-treated animals is likely to be related to the stress of repeated bleeding of the animals in this experiment (5 time points each). Atrazine did not affect the level of this hormone.

In conclusion, atrazine (300 mg/kg/d for 3 days po) decreased the LH surge in female SD rats.

10.1.13 ***Morseth SL (1996c) Evaluation of the Luteinizing Hormone (LH) Surge in Atrazine-Exposed Female Sprague-Dawley Rats. Ciba Crop Protection, Greensboro, NC. Lab: Corning Hazleton Inc., Vienna, Virginia. Lab. Study no. CHV 2386-111. Report date 25 Jan. 1996 (Interim Report) (GLP)***

This study was designed to elucidate the hormonally-mediated mechanisms underlying the earlier onset of mammary tumours in female SD rats. Atrazine technical (Lot no. SG8029BA10; % purity) in 0.5% CMC was administered to female Sprague Dawley Crl:CD BR rats from Charles River Labs, Raleigh, NC (90/group) at 0, 2.5, 5, 40 and 200 mg/kg bw/day by oral gavage. Analyses for homogeneity, stability and concentration were performed; all values were within 10% of target. Animals were ovariectomised after 28-31 days of dosing and 10 days prior to sacrifice. Oestradiol was administered via a subcutaneous capsule, implanted 3 days before decapitation (non-repeat bleed animals) or bleeding via the jugular vein (repeat bleed animals) ie. 7-days after the ovariectomies.

Lower mean bodywt values were noted for 40 mg/kg animals at weeks 5 and 6 and the 200 mg/kg animals at weeks 2-6. At these two doses, mean bodywt changes were lower than control values over weeks 1-6. Bodywts/bodywt gains were not affected at 2.5 and 5 ppm.

Atrazine at 40 and 200 mg/kg decreased the LH surge and prolactin (PRL) levels and affected oestrous-cycle patterns by causing persistent dioestrus as well as episodes of prolonged oestrus. A dose of 5 mg/kg/d did not disrupt

oestrous cycle patterns or affect plasma PRL, but inconclusive results were obtained for LH levels; no LH suppression was noted in repeat-bleed animals whereas LH levels appeared to be decreased in the nonrepeat bleed animals. A dose of 2.5 mg/kg/d was a clear NOEL for atrazine effects on the LH surge, PRL, and oestrous cycles.

The study clearly demonstrated that young female SD rats dosed with high levels of atrazine began to display one of the early events of reproductive senescence, namely, reduced surges of pituitary LH. This type of neuroendocrine failure leads to oestrous cycle disruption which, in SD females, produces an endocrine environment which is favourable for mammary tumour growth later in life.

10.1.14 ***Safe S (1996) Failure of Chloro-s-triazine Derived Compounds to Induce Estrogenic Responses in vivo and in vitro. Dept of Veterinary Physiology & Pharmacology, Texas A & M University, College Station, TX, and Dept Pharmacology & Toxicology, University of Western Ontario, Canada. Fund Appl Toxicol (in press)***

Some of the funding support from this study came from Ciba-Geigy Corp.

The potential oestrogenic activity of atrazine and simazine (each greater than 97% pure) was investigated in the immature female rat uterus and in the oestrogen-responsive MCF-7 human breast cancer cell line and the PL3 *S. Cerevisiae* yeast strain which is dependent on the presence of oestrogenic substances to grow on selective media.

Oral treatment of immature 21-day-old female Sprague Dawley rats (from Harlan Sprague Dawley, Houston, Texas) with 50, 150 or 300 mg/kg/day of atrazine or simazine (dissolved in DMSO and suspended in 5% aqueous hydroxypropylcellulose) for 3 days did not significantly induce rat uterine weight or cytosolic progesterone receptor levels (animals sacrificed 20 h after the last dose). Uterine peroxidase activity was decreased by both compounds at the two higher doses. 17 -Oestradiol (E2) injected intraperitoneally in corn oil (10 µg/kg/d) caused a 6.2-, 8.9- and 9.7-fold increase in rat uterine weight, cytosolic progesterone levels and uterine peroxidase activity, respectively.

In rats co-treated with i.p. E2 plus po atrazine or simazine, some doses slightly inhibited E2-induced cytosolic progesterone receptor binding and uterine peroxidase activity but there was no dose-response relationship (and no inhibition at the high dose of atrazine). The high dose of simazine produced a small inhibition of the E2-induced increase in uterine wet weight.

In MCF-7 cells, atrazine and simazine did not affect E2-induced cell proliferation or nuclear progesterone receptor levels (measured in two different types of assays). Data suggest that for these two responses neither chloro-s-triazine exhibits oestrogen agonist or antagonist activity.

Luciferase activity in MCF-7 cells transiently infected with the Gal4-oestrogen receptor chimeric construct and a Ga14-regulated luciferase reporter gene exhibited dose-dependent increases following E2 treatment and several other endocrine receptor agonists (including the weak bisphenol A and nonylphenol). However, neither atrazine nor simazine (tested up to 10 µM) had any significant effect on luciferase activity. Furthermore, in cells co-treated with E2 plus atrazine or simazine, the triazines did not inhibit the E2-induced responses.

Engineered PL3 cells contain the URA3 gene which encodes for orotidine-5'-monophosphate decarboxylase, an enzyme involved in uracil synthesis; the regulatory region of this gene contains three tandem oestrogen responsive elements and OMP decarboxylase can be induced in the presence of functional oestrogen receptors in the presence of oestrogenic substances. These cells were transformed with a vector containing human oestrogen receptor cDNA (YEp10-HEGO). Neither atrazine or simazine (at concentrations as high as 10 µM) promoted the growth of PL3 cells on minimal media lacking uracil, whereas growth was observed on similar media supplemented with 1 nM E2. Evidence was cited to suggest that their lack of activity was not due to their inability to penetrate the cells. (The weak industrial oestrogens, bisphenol A and *p*-nonylphenol were also capable of producing transformant growth.)

Overall, these results indicate that atrazine and simazine do not exhibit 17 -oestradiol-mediated oestrogenic agonist activity in 7 different measured oestrogen-regulated responses. In co-treatment studies, the two chloro-s-triazines did not inhibit E2 responses in *in vitro* cellular assays, but weakly inhibited some 17 -oestradiol-induced responses in the rat uterus *in vivo*.

It is possible that this weak anti-oestrogenic activity may be to an indirect interaction with endocrine-receptor-mediated signal transduction pathways eg. TCDD and related compounds exhibit a broad spectrum of ant-oestrogenic activities which interact between the ER and aryl hydrocarbon (Ah) receptor-induced endocrine-response pathways.

10.2 Other Studies

10.2.1 *Santa-Maria C, Moreno J & Lopez-Campos JL (1987) Hepatotoxicity Induced by the Herbicide Atrazine in the Rat. J Appl Tox 7: 373-378*

Adult male Wistar rats (240-280 g) were given 0, 100, 200 and 400 mg/kg bw/day atrazine by gavage (in gum arabic) for 14 days and 600 mg/kg bw for 7 days. At necropsy (under light ether anaesthesia), blood was taken for determination of serum total lipids, glucose, ALT and SAP. The liver was examined grossly and microscopically.

Bodywt loss (cf. initial bodywts) in the control to high-dose groups respectively were: 1.6%, -23.3%, -25.2%, -35.6% and -30.1%. There was a small increase in the weight of liver relative to bodywt (from 4.13% of bodywt to 4.66% at the high dose) possibly indicating some hypertrophy since relative liver weight loss

is normally observed with bodywt loss. A dose-dependent decrease in serum glucose and an increase in total serum lipids was seen in all groups. In 600 mg/kg animals, serum ALT increased (approx. 60%) as did serum AP (approx. 200%). Electron microscopy did not reveal any changes in the liver at 100 mg/kg but at higher doses there was hepatocytic proliferation, degeneration of smooth endoplasmic reticulum (ER), vacuolation of hepatocytes (primarily in the centrilobular parenchymal cells), lipid accumulation, some swollen mitochondria, and alteration of bile duct cannuli, proportional to dose and duration of treatment. The morphological changes in the ER may represent an adaptive compensatory reaction to the fatty liver.

10.2.2 *Babic-Gojmerac T, Kniewald Z & Kniewald J (1989) Testosterone Metabolism in Neuroendocrine Organs in Male Rats under Atrazine and Deethylatrazine Influence. J Steroid Biochem 33: 141-146*

Three-month old male Fisher rats received 60 or 120 mg/kg bw/d pure atrazine or desethylatrazine in paraffin oil for 7 days. On the 8th day the anterior pituitary or hypothalamic tissue was removed and immersed in Krebs-Ringer solution containing 4-^{14}C testosterone, for measurement of testosterone conversion into metabolites. *In vitro* measurements of testosterone conversion were also conducted on the anterior pituitary or hypothalamus from 90-95 day old male Fisher rats, in the presence of 0.92 µM of atrazine or deethylatrazine.

Atrazine *in vivo* (120 mg/kg bw/d for 7 days) significantly increased the weight of the pituitary gland, with hyperaemia and hypertrophy of chromophobic cells with vacuolar degeneration. 3-Oxo-steroid- 4-ene-dehydrogenase (5 -R) activity in the anterior pituitary was significantly inhibited by both atrazine and desethylatrazine, whereas 3 - and 17 -hydroxysteroid dehydrogenase (3 - and 17 -HSD) levels were unchanged.

Atrazine *in vitro* significantly inhibited 5 -R, 3 -HSD and 17 -HSD activities in the anterior pituitary. Deethylatrazine produced similar, but less marked effects.

Atrazine *in vivo* significantly inhibited 5 -R and 17 -HSD activities in the male hypothalamus. Desethylatrazine produced similar effects; 3 -HSD activity in the hypothalamus was not affected by either compound.

10.2.3 *National Toxicology Program (1993) NTP Technical Report on Toxicity Studies of Pesticide/Fertilizer Mixtures Administered in Drinking Water to F344/N Rats and B6C3F1 Mice. NIH Publication 93-3385. Date July 1993*

This report focuses primarily on 26-week studies in F344/N rats and B6C3F1 mice given pesticide/fertilizer mixtures in their drinking water. Additionally, in vivo genetic toxicology, reproductive, and developmental toxicity studies with these mixtures were reviewed (see assessments of these latter studies in other Sections of this review).

Two mixtures of pesticides and a fertilizer (ammonium nitrate), representative of established groundwater contamination in California and Iowa, were both tested at 0.1x, 1x, 10x and 100x the median concentration found in the groundwater surveys).

The 1x mixtures were made up as follows (ng/mL concentration given in brackets, unless otherwise stated):-

alachlor (0.9); atrazine (0.5); cyanazine (0.4); metolachlor (0.4); metribuzin (0.6); ammonium nitrate (10 µg/mL); propylene glycol (512 µg/mL; used as solubilizer) (IOWA)

aldicarb (9.0; 1;1:1 ratio of aldicarb, sulfoxide and sulfone); atrazine (0.5); dibromochloropropane (0.01); 1,2-dichloropropane (4.5); EDB (0.9); simazine (0.3); ammonium nitrate (10 µg/mL); propylene glycol (512 µg/mL; used as solubilizer) (CAL)

The 26-week studies in mice and rats, conducted at the Southern Research Institute, Birmingham, AL, used 20/sex/dose group, with clinical pathology performed on 10/sex/group. Neurobehavioural and neuropathological studies were performed on rats and mice (10/sex/gp). In addition to organ weight analysis and histopathological assessments, examination of the reproductive system included sperm morphology and vaginal cytology. Sperm motility, numbers of motile and non-motile sperm, sperm density, testicular spermatid head count (to quantify spermatogenesis) were assessed.

In the 26-week drinking water studies in mice, 1/20 control females (IOWA mix), 1/20 females (IOWA mix at 100x) and 1/20 male mice (CAL mix at 100x) died early but it could not be determined if the deaths in the 100x groups was related to pesticide/fertiliser intake. Water consumption and bodywt gains were not affected and no signs of toxicity were seen in clinical observations or neurobehavioural assessments. No clear adverse effects were seen in clinical pathology, reproductive system, organ weight, or histopathological evaluations.

All rats survived the 26-week drinking water studies with both mixtures and there were no effects on bodywt gains or on water consumption. There were no clinical signs and no neurobehavioural effects (as measured by a functional observation battery, motor activity evaluations, thermal sensitivity evaluations, and startle response). Clinical pathology (including cholinesterase) did not reveal any adverse effects, nor did organ weight analysis or histopathological assessment (including a detailed examination of the reproductive system). With the IOWA mixture, there possibly was a marginal increase in absolute and relative liver weights with increasing dose; in males the relative organ weight (mg organ wt /g bodywt) was 34.27 for controls, increasing to 39.77 at the 100x dose. For females, the respective values were 30.57 and 34.28. This increase, albeit statistically significant (at 1x, 10x and 100x doses) may not be biologically significant, particularly when compared with the relative liver wts in the control animals in the CAL mixture study viz. 38.27 (males) and 32.62 (females).

10.2.4 ***Fournier M, Friborg J, Girard D, Mansour S & Krzystyniak K (1992) Limited Immunotoxic Potential of Technical Formulation of the Herbicide Atrazine (AAtrex) in Mice. Departement des Sciences Biologiques, Universite du Quebec a Montreal, Canada. Toxicol Lett 60: 263-74***

Immunotoxicity of the atrazine formulation, AAtrex, was examined in C57Bl/6 female mice following a sublethal exposure to equivalent 1/2-1/64 LD50 doses of the herbicide. Animal weight was not affected by the herbicide exposure. No dose-related changes could be concluded for fluctuations in organ weight, changes in the spleen cell number and cell viability. Furthermore, cytofluorometric studies showed no significant changes in the frequency of L3T4-positive and Lyt-2-positive T-cells. Functional *in vitro* assays of mitogen activation showed no marked effects of AAtrex exposure on lymphocyte stimulation by lipopolysaccharide (LPS), phytohemagglutinin (PHA) and concanavalin A (Con-A). In addition, sublethal exposure to AAtrex did not affect interleukin-2 (IL-2) production by splenic cells. Furthermore, no dose-related effect could be concluded from a transient suppression of a primary humoral IgM response to sheep erythrocytes (SRBC) as well as from a transient inhibition of a specific T-cell response to alloantigens in mixed lymphocyte reaction (MLR). Exposure to equivalent 1/2-1/16 LD50 doses augmented phagocytic activity of peritoneal macrophages, without any visible AAtrex dose-related effect. Normal humoral and cellular responses were restored at 14-40 days after the herbicide exposure. Overall, transient and reversible immunosuppression of humoral-mediated and cell-mediated responses and activated macrophage phagocytic activity could not be attributed to the direct chemical-related effect of sublethal exposure to Aatrex.

10.2.5 ***Mencoboni M, Lerza R, Bogliolo G, Flego G & Pannacciulli I (1992) Effect of Atrazine on Hemopoietic system. Dipartimento di Medicina Interna, Universita di Genova, Italy. In-Vivo 6(1): 41-4***

This paper investigated the toxicity of the s-triazine herbicides to haemopoiesis. The effect of atrazine on mouse haemopoietic progenitors (CFU-S and GM-CFC) and on peripheral blood (leukocytes and reticulocytes) after a single ip injection of 58.65 mg/kg bw was studied. The peripheral blood leukocyte level was not modified but reticulocytes dropped severely with prompt recovery. Haemopoietic progenitors were severely affected but they recovered and reached normal levels in a few days. These results demonstrate a haemotoxic effect of atrazine after a large acute dose.

10.2.6 ***Wolff NL, Zepp RG, Gordon JA & Fincher RC (1976) N-Nitosamine Formation from Atrazine. Southeast Environmental Research Lab., US EPA, Athens, USA. Bull Environ Contam Toxicol 15: 342-347***

This paper noted that, under synthetic reaction conditions, atrazine could undergo nitrosation. N-Nitrosoatrazine in water underwent photodecomposition, with the half-life near the surface of water in sunlight calculated as being less than 10 min. It was also rapidly decomposed under

fluorescent light. The study did not encompass any environmental monitoring to indicate whether it was formed in the environment and, if so, whether it was stable in soil or in water.

10.2.7 ***Yoder J, Watson M & Benson WW (1973) Lymphocyte Chromosome Analysis of Agricultural Workers during Extensive Occupational Exposure to Pesticides. EPA, Idaho State Dept of Environmental & Community Services, Boise, USA. Mutation Res 21: 335-340***

Lymphocyte cultures from 42 pesticide applicators and 16 controls were examined for chromosomal aberrations during mid-winter and again during the peak summer period of intense spraying activity. Cultures from exposed individuals were reported to show a marked increase in the frequency of chromatid lesions, especially noticeable among workers exposed primarily to herbicides. The number of chromatid gaps (per person per 25 cells scored) in herbicide-exposed individuals at mid-season ($\pm$ SEM) was 1.38 $\pm$ 0.22 cf. mid-winter values of 0.38 $\pm$ 0.10 (n =26), whilst the number of chromatid breaks was 1.81 $\pm$ 0.35 (n=26) cf. mid-winter values of 0.07 $\pm$ 0.05 (n=26). Whilst there would appear to be a 26-fold increase in chromatid breaks in exposed individuals, the number of chromatid breaks in controls (unexposed individuals) ranged between 0.31 to 0.44 (winter to summer) ie. much higher than the mid-winter value in exposed workers. On this data, it could be argued that herbicide exposure might offer compensatory protection against chromosome damage. Also noted was the relatively limited number of cells scored per individual. Furthermore, it is not possible to make any firm conclusion about the chromosome damaging properties of atrazine because the 26 workers examined were exposed to over 11 herbicides and 3 fungicides, in addition to some exposure to a range of insecticides.

11. HUMAN STUDIES

11.1 Epidemiological Studies

11.1.1 Farm Herbicide Use

11.1.1.1 ***Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R et al. (1986) Agricultural Herbicide Use and Risk of Lymphoma and Soft-tissue Sarcoma. J Amer Med Assoc 256(9): 1141-1147***

The incidences of soft-tissue sarcoma (STS), Hodgkins disease (HB) and non-Hodgkins lymphoma (NHL) in white male Kansas residents aged 21 or older, from 1986 to 1982, were studied in relation to farm herbicide use. Farm herbicide use was determined by phone interviews with patients and controls, or their next of kin, and herbicide suppliers to the subjects.

NHL was found to be associated with farm herbicide use, and the relative risk of NHL increased significantly with duration (years) or frequency (days/year) of herbicide use.

Comments: The study overestimated risks of NHL by comparing exposed farmers with non-farmers, rather than unexposed farmers. Study data shows an increased risk of NHL in non-exposed farmers - when exposed farmers are compared with unexposed farmers the risks of NHL are statistically the same. Furthermore, the relative risk associated with exclusive use of triazines is reduced, and is no longer statistically significant. The data may suggest confounding factors associated with farming.

11.1.1.2 ***Donna A, Crosignani P, Robutti F, Betta PG, Bocca R, Mariani N, Ferrario F, Fissi R & Berrino F (1989) Triazine Herbicides and Ovarian Epithelial Neoplasms. Scand J Work Env Hlth 15: 47-53***

All women histologically confirmed to have primary malignant epithelial tumours of the ovary (serous, mucinous, endometrioid, clear cell, Brenner tumours, mixed epithelial and unclassified epithelial) between 1980-1985 in Alessandria Province, Italy, were compared with randomly selected (plus or minus five years, no bilateral oophorectomy) referents. There were 65 cases (27 decedents) and 126 referents in the study. Subjects, or descendants relatives were interviewed to determine occupational history and herbicide exposure. 'Exposed' individuals were defined as those who were involved in the preparation or use of triazine herbicides or who worked in corn cultivation with reported use of herbicides (triazines were reportedly used in all herbicide treated corn cultivation). Reproductive risk factors for ovarian cancer such as age, number of live births, use of oral contraceptives, miscarriages, abortions etc. were recorded.

Risk factors for ovarian tumours were increased by use of oral contraceptives and short menstrual cycles, whereas parity had a protective role. Relative risks were 2.7 for 'definitely exposed' subjects, and 1.8 for 'possibly exposed' subjects. Risk factors increased with increased years of exposure. The risk factor for unexposed agricultural workers was one ie. no different than from unexposed non-agricultural people.

Comments: It should be noted that triazine exposure was not quantified. Although a number of reproductive factors for ovarian tumours were controlled, other known non-reproductive factors such as obesity, smoking and alcohol were uncontrolled, and could affect the results. The use of 90%, rather than 95% confidence intervals throughout the study did not affect the study conclusions based on relative risk factors.

A critique of the above paper, from a member the Department of Social & Preventive Medicine (Minder, 1990) concluded, on the basis of statistical aspects, classification of the exposed women, and possible confounders and biases, that the case for an association between ovarian cancer and triazines was weak, and for a causal link, even weaker.

A second, unpublished critique (apparently solicited by Ciba-Geigy) was prepared by JV Watson, a Consultant Oncologist with the MRC, Cambridge, UK ('Report on Triazine Herbicides and Ovarian Epithelial Neoplasms').

Whilst not containing many specific points which would raise major concerns about the study, it highlighted the problems and pitfalls in the conduct of such epidemiological studies, and concluded that the paper raised more questions than it answered.

11.1.1.3 *Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM, Burmeister LF, Van Lier S & Dick F (1990) Pesticide Exposures and Other agricultural Risk Factors for Leukemia Among Men in Iowa and Minnesota. Cancer Res. 50: 6585-6591*

The authors conducted a case-control interview study of 578 white men with leukaemia and 1245 controls living in Iowa and Minnesota. There were slight but significantly elevated risks for farmers cf. non-farmers for all leukaemia (odds ratio 1.2) and for chronic lymphocytic leukaemia (OR 1.4).

The following results were given for atrazine or triazines:-

	Cases/controls	Odds Ratio	95% CI
All triazines			
risk of leukaemia according to ever use	67/172	1.1	0.8-1.5
Atrazine			
risk of leukaemia for mixing, handling or applying	38/108	1.0	

Thus there was no evidence of a linkage between atrazine use and leukaemia in white males.

11.1.1.4 *Hoar-Zahm S, Weisenburger DD, Cantor KP, Holmes FF & Blair A (1993) Role of the Herbicide Atrazine in the Development of Non-Hodgkin's Lymphoma. Scand J Environ Hlth 19, 108-114*

The role of atrazine in the development of Non-Hodgkin's Lymphoma (NHL) was investigated in three case-referent studies conducted in 4 mid-western states (Nebraska, Iowa, Minnesota and Kansas); a total of 993 white men with NHL and 2918 population-based referents were interviewed. While the odds ratio for NHL with atrazine use was 1.4 (95% CI 1.1-1.8) in the combined studies, when adjusting for the use of 2,4-D and OP insecticides, the association was much reduced (to less than unity in all but one state). The authors concluded that the data provide little evidence that atrazine use is associated with NHL in white males.

11.1.2 Agricultural Chemical Production Workers

11.1.2.1 *Cronan AB (1988) Ciba-Geigy Interoffice Correspondence. 6 June 1988* and

Charters JE (1989) Ciba-Geigy Interoffice Correspondence. 30 May 1989

Correspondence included in the submission from medical officers at the Ciba-Geigy Corporation's plants at St Gabriel and McIntosh, USA, certify that no cases of skin irritation or other illness due to atrazine have been seen in these Ciba-Geigy plants.

11.1.2.2 ***Gass R & Stalder GA (1990/1993) Atrazine - A Epidemiological Study at the Schweizerhalle Plant. Ciba-Geigy AG, Basle. Report date Dec. 1990 & 15 Jan. 1993***

The epidemiological study (Dec. 1990) was conducted by Ciba-Geigy at their plant, with comment from Prof. Dr GA Stalder, Head of the Gastroenterology Division of the Internal Medicine Dept of the University Kantonsspital, Basle (15 Jan. 1993).

Studies on 154 pairs of employees to determine if there was any increase in health disorders amongst those working with atrazine did not find any changes in clinical parameters measured. With regard to all diseases that occurred since 1975, gastritis ("occasional curable gastritis or gastroenteritis") was diagnosed in a higher number of cases, but independent of the duration of exposure. Dr GA Stalder concluded that "there is no indication that there could be any causal relationship'.

11.1.2.3 ***Delzell E, Druschell C, Iyer V & Cole P (1989) A Follow-up Study of Triazine Herbicide Manufacturing Workers. Ciba-Geigy Corp., Greensboro, NC. Lab: University of Alabama at Birmingham, Dept of Epidemiology. Study completion date 15 Sept. 1989***

Mortality rates were studied in 1472 workers (6 months or more of herbicide production-related work) at a triazine manufacturing plant in Louisiana, and compared with general population mortality rates. Overall mortality of workers was much lower than the general population (13 observed vs 28 expected total). Cancer rates were normal (3 observed vs 3.7 expected cancer deaths). Deaths due to non-Hodgkins lymphoma initially appeared higher than normal in workers (observed/expected = 2/0.2) however one case of nasopharyngeal cancer was originally misreported. Low employee mortality was attributed to selection of healthy workers.

Comment: The study did not establish whether any of the workers had physical contact with herbicides during manufacturing. The low mortality (13 deaths in total) would severely limit the capacity to discern cause-specific mortalities.

11.1.2.4 ***Sathiakumar N, Delzell E, Austin H & Cole P (1992) A Follow-up Study of Agricultural Chemical Production Workers. Dept of Epidemiology, School of Public Health, University of Alabama at Birmingham, USA. Amer J Indust Med 21: 321-330 (1992)***

and

11.1.2.5 Delzell E & Sathiakumar (1992) Combined Analysis of Mortality among Workers at Ciba-Geigy Corporation's McIntosh and St Gabriel Plants. Department of Epidemiology, School of Public Health, University of Alabama at Birmingham, USA

An initial epidemiological study by the above investigators of a group of workers at the Ciba-Geigy herbicide production plant at St Gabriel, Louisiana, was inconclusive and could be criticised on the grounds that the cohort of workers followed-up was relatively small (1472) and that no attempt was made to distinguish workers who actually had physical contact with the herbicide during their employment. Two more recent studies include a study on a larger cohort of workers (4323) at another Ciba-Geigy plant at McIntosh in Alabama, and a combined re-analysis of both the St Gabriel and the McIntosh data. In addition to evaluating the overall larger cohort (4917 workers, after some exclusions), the investigators assigned all the workers to two groups viz. those with definite/probable exposure (2683) and those with possible exposure (2234), based on job codes.

McIntosh study

The McIntosh-only study evaluated the mortality of 4323 men employed at the McIntosh production plant in Alabama, USA. The plant primarily manufactured agricultural chemicals including insecticides (organochlorines and organophosphates), herbicides (triazines and others), fungicides, herbicides, miticides, and micronutrients; DDT was produced for 10 years from 1952-1962. Phenoxyacetic acid herbicides were never manufactured.

The subjects were all employed at the plant, in production-related jobs, for at least one month between 1951 and 1987. The mortality rates of the study subjects were compared with either those of United States or Alabama men, using standardised mortality ratios (SMRs) as a measure of association ie. the ratio of observed to expected numbers of deaths. The follow-up started either from Jan. 1951 or from one-month after the day of hire, and ended in the date of death, loss to the study, or the closing date of the study (Jan. 1987). Workers were further classified as white (3600), black (663), hourly payroll (2835) or salaried (823) and according to duration of employment and duration of follow-up (1692 of the subjects were followed up for at least 20 years). Overall, the cohort of subjects was rather young, with 83% of the total person-years of observation occurring before 45 years of age. Cohort members were primarily short-term employees, with 61% having fewer than 5 years of cumulative employment.

At the end of the follow-up period 3928 (91%) of the workers were presumed living, 280 (6%) had died, while status was unknown for 115 (3%). The observed causes of death for all white employees versus expected values for the whole United States or for Alabama are shown in the Table below.

The subcohort of 2835 hourly employees showed very similar results to the total cohort except that the deaths from accidents was slightly higher. By contrast, salaried workers had a marked decrease in accidental deaths, from all causes combined, and from cancer.

Observed and expected causes of death for McIntosh production plant workers

Cause of death	Observed US[1]	Observed AL[2]	Expected US[1]	Expected AL[2]	SMR
All causes	233	240	277	97	84
All cancer	54	45	49	120	109
• Buccal cavity & pharynx	5	1		388	
• Esophagus	4	1		417	
• Other digestive system	7	9		78	
• Lung	22	15		150	
• Central nervous system	4	2		169	
• All lymphatic & haemopoietic tissue (LHT)	9	6		144	
• non-Hodgkin's lymphoma	4	2		203	
• other LHT	5	4		116	
• Other cancer	0	9.4		29	
Diseases of the circulatory system	65	79		82	
Diseases of the respiratory system	6	90		66	
Diseases of the digestive system	8	13		62	
External causes	75	72		104	
Accidents	53	46	64	115	83

1 Based on United States data
2 Based on Alabama data only

Separate analysis of short-term (< 5 years) and long-term (≥ 5 years) employees showed an approximately 20% higher mortality rate in short-term employees that for white United States males overall. A slight excess was also observed for all cancer and for accidents, and the excesses of deaths from esophageal, lung and CNS cancer were concentrated in this group. In contrast, men employed for over 5 years had deficits of deaths from all causes and from all cancer. Further analysis showed that increases in all cancer, lung cancer and CNS cancer were particularly concentrated in the subgroup who had worked at the plant for less than 1 year.

Black employees had fewer deaths compared to the United States black male population overall for all causes and for all cancer. Circulatory diseases and external causes were about equal and, as with the white males, accidents were higher. For more specific causes, only soft tissue sarcomas showed a statistically significant difference (increase) between observed (2) and expected

(0.8) numbers. For black and white subjects there were 3 observed vs 0.6 expected soft tissue sarcoma deaths; job histories did not suggest any shared occupational exposure.

Combined study

The results of this combined analysis are shown in the Table below.

Definite/probable exposure. The results do not show a strong correlation between definite/probable exposure and most disease categories, with a deficit in all causes and in all cancers, as well as in respiratory, circulatory and digestive diseases. The only specific cause showing an increase of observed over expected values (SMR > 100) was non-Hodgkin's lymphoma (NHL).

When a further division of the data was made into those employed for at least 10 years and those for less than 10 years, there were not any significant findings. Two of the 3 NHL subjects were white, 2 were McIntosh employees with a total of 42 days of definite triazine-related work. The third was a St Gabriel employee with 5.5 years of definite triazine work. When the definite triazine exposure employees were further analysed into less than 1 year or at least 1 year of employment, the subjects with short-term (< 1 year) employment showed a slight increase in cancer deaths (13/10 obs/exp) and the increases in LHT cancer and NHL were concentrated in this group. Using Alabama or Louisiana data as a base for expected-value calculations did not substantially alter the results.

Possible exposure. Results showed a slight increase in all cancer deaths in this group, related to slight increases in buccal cavity and pharynx (BCP), oesophageal, pancreatic, lung, CNS, and lymphatic & haemopoietic tissue (LHT) cancers. The most significant increase was for lung cancer (obs/exp - 12/8). The 'other cancer' category includes 2 observed vs 0.3 expected soft tissue sarcoma deaths (both black/McIntosh employees). Of the two men reported to have died from NHL, one actually had nasopharyngeal cancer (determined from medical records). In this group, most of the increase in cancer deaths were concentrated in the group with > 1 years employment. Using Alabama or Louisiana base data (instead of US overall) did not cause any substantial changes to results.

Observed and expected causes of death for combined cohort of McIntosh and St Gabriel production plant workers

Cause of death	Definite/probable exposure			Possible exposure (No. of deaths)		
	Obs	Exp[1]	SMR[2,3]	Obs	Exp[1]	SMR[2,3]
All causes	86	119	72	134	135	99
• All cancer	14	17	85	29	24	120
• BCP	1	0.6		2	0.8	

• Esophagus	0	0.5		1	0.7	
• Pancreas	1	0.7		2	1.1	
• Other digestive	0	2.5		4	4	100
• Lung	4	5	82	12	8	152
• CNS	0	0.9		2	1.2	
• All lymphatic and haemopoietic tissue	5	3.6	138			
• non-Hodgkin's	3	0.8	385	2[4]	1.0	
• other	2	1.8		2	2	
• Other cancer[5]	2	5		2	5.5	
Circulatory diseases	19	28	58	36	41	87
Respiratory diseases	4	4	100	2	5	40
Digestive diseases	3	6	47	4	2	52
External causes	42	50	84	49	43	114
Accidents	29	28	104			
Other	13	22	58	16	17	92
Other causes	3	13	23	10	13	77

1 Expected values calculated from person-years of observation allocated to race-specific/five-year age and calendar time categories and multiplied by corresponding general population mortality rates for the whole United States.
2 United States
3 SMRs are only quoted for observed values >3
4 Of the 2 reported NHL deaths, one was subsequently found (from medical records) to have had nasopharyngeal cancer.
5 Includes soft tissue sarcoma.

Discussion The increases in the buccal cavity and pharynx, lung and esophageal cancer in the McIntosh study are difficult to interpret. Non-occupational determinants of these conditions are smoking, alcohol consumption, and possibly dietary deficiencies. The excesses of lung and esophageal cancer were concentrated among short-term employees which, to some extent, argues against a causal link with employment at the plant.

The results for NHL and for soft tissue sarcoma (STS) are of particular interest because other epidemiological investigations have suggested a relationship between these diseases and exposure to agricultural chemicals. A previous study has evaluated NHL and exposure to triazine herbicides (Hoar et al, 1986) and found an increased risk among farmers who had used triazines compared to non-farmers. However, the data was not significant when triazine-using and non-using farmers were compared. A further study is referenced by the investigators in which atrazine was associated with a small increased risk of

NHL (relative risk (RR) = 1.4) and the RR rose with increasing duration of use (Zahm et al, 1990). The study did not, however, take into account the use of other herbicides and insecticides which could have been responsible for the results. In the present study, the increased incidence of NHL is not statistically significant. Two of the four workers who died from NHL had worked in triazine production; none had worked in DDT production (the McIntosh plant also produces DDT). Two had spent less than 6 months working at the plant and two died less than 10 years after beginning employment. Therefore there is not a strong case for a causal relationship.

STS has also been linked to exposure to herbicides and chlorophenols. There have been no reports linking triazine exposure with STS although it has been suggested that exposure to DDT is associated with an increased risk of STS. In this study none of the men with STS had worked in DDT production at the McIntosh plant. The result in this study is on the borderline of statistical significance. However, the result is based on small numbers and, of the 3 victims in the McIntosh study, two had short employment periods (2 and 4 months) and the third was a long-term employee but his specific exposures are unknown. A causal relationship between soft tissue sarcoma and employment at the plant can therefore not be proved or totally discounted.

11.2 Exposure Studies

11.2.1 *Ikonen R, Kangas J & Savilainen H (1988) Urinary Atrazine Metabolites as Indicators for Rat and Human Exposure to Atrazine. Toxicol Lett 44: 109-112*

Three groups of 5 male Wistar rats received 0.1, 0.2, 0.5 g/L commercial atrazine in drinking water for 1 or 3 weeks. Urine was collected daily and analysed for atrazine metabolites.

The principal rat urinary metabolite at the two time points was 2-chloro-4-ethylamino-6-amino- s-triazine (desisopropylatrazine), with a concentration linearly related to atrazine concentration, and not the estimated cumulative dose.

Atrazine concentrations were measured in the breathing zone of 6 railway workers engaged in weeding operations on railway lines. Urine samples after the 8 h shift were analysed for atrazine metabolites.

In railway workers, the main urinary metabolites were the didealkylated atrazine metabolite, diaminochlorotriazine and desisopropylatrazine. The sum of their concentrations in urine correlated with atrazine dust concentrations in the breathing zone. Inhaled atrazine may not be the major source and percutaneous absorption could be a more important route.

11.2.2 *Atrazine Dermal and Respiratory Exposure in Sprayers* (Unreferenced evaluation assessed in Departmental assessment on file)

Atrazine exposure was measured in respirator filters and clothing samples of 4 subjects applying atrazine at 4.5 kg ai/ha using a towed sprayer, and a wand spray gun. Each subject performed mixing/loading, boom application, and spray-gun operation. Workers wore coveralls, mid-calf boots and mid-forearm rubber gloves.

Dermal and respiratory exposures (mg/kg ai) over 20 minutes were:-

operation	dermal	respiratory
mixing-loading	272	12
spray gun	54	4
boom	3	11

Dermal exposure was highest on the forearm (*vs* chest and thigh) (686) and using a spray-gun (161). Respiratory exposures were approximately 220-times less than the inhalation LC50 value for rats.

11.2.3 ***Catenacci G, Maroni M, Cottica D & Pozzoli L (1990) Assessment of Human Exposure to Atrazine Through the Determination of Free Atrazine in Urine. Bull Environ Contam Toxicol 44: 1-7***

Atrazine exposure and excretion was measured in 4 workers during its manufacture and packaging. Subjects were monitored during 6 consecutive shifts (1 bagger), 4 consecutive shifts (1 bagger and 1 control board operator) or 6 shifts for airborne exposure only (1 control board operator). Airborne exposure was measured by pads on the neck, and under the clothes on the chest and back, and by handwashings. Urine was collected (i) for 24 h before the first workshift (ii) throughout the monitoring period (8 aliquots) (iii) one or more 12-h periods after exposure.

Air atrazine concentrations during production varied from 0.07-0.53 mg/m^3 (8 h) and skin deposition (whole body) ranged from 4.11 - 10.66 mg/h. Urinary excretion of unchanged atrazine reached a maximum (0.1-0.3 µg/h) during the workshift, and decreased to 0.01-0.04 µg/h 12 h after the shift. Assuming 20% inhalation and 10% skin absorption, total atrazine absorption was estimated to be 3-8 mg per shift. Small amounts of free atrazine in urine indicated that unchanged atrazine accounted for only a very minor part of the total absorbed dose.

11.2.4 ***Catenacci G, Barbieri F, Bersani M, Feriolo A, Cottica D & Maroni M (1993) Biological Monitoring of Human Exposure to Atrazine. Toxicol Lett 69: 217-222***

In six manufacturing workers, total atrazine exposure varied from 10 to 700 µmol per workshift (cutaneous and respiratory exposure). Urinary metabolites included bi-dealkylated metabolites (80%), desisopropylated metabolites (10%), desethylated (8%) and atrazine (2%). Fifty percent (50%) of the absorbed dose was eliminated in the first 8 h, completely in just over 24 h.

11.2.5 ***Rosenheck L, Phillips JC & Selman FB (1993) Worker Mixer/Loader and Applicator Exposure to Atrazine. Ciba-Geigy Corp., Greensboro, NC. Trial & Labs: Pan-Agriculture Labs Inc., Medera, CA. Report no. AE-91-511. Study completion date 14 Oct. 1993. (QA statement)***

The results of this study are summarised only.

The study aimed to determine the amount of atrazine residues that mixer/loaders and applicators are exposed to during commercial and homeowner turf treatment.

	Dermal exposure (mg) per kg ai handled	Inhalation exposure (mg) per kg ai handled
Aatranex Nine-O handgun		
mixer/loader	0.738	0.0545
applicator	0.952	0.0135
Scotts Bonus S Push cyclone spreader		
Loader/applicator	7.30	0.0275
Hand cyclone spreader		
Loader/applicator	46.5	0.136

Data are average adjusted figures.
For the homeowner simulation using cyclone spreaders, one person did the loading and application.
Application rate was 2 lbs ai/acre (Aatranex Nine-O) and 1.9 lb ai/acre (Scotts Bonus S).
Handgun mixer/loaders and operators used long-sleeved shirts, pants, rubber boots and gloves.
Cyclone operators wore short-sleeved T-shirts, pants, shoes and socks.

11.2.6 *Loosli R (1994) Triazines. Toxicol 91: 59-62*

This paper provides a brief overview of the toxicology of atrazine, advice on biological monitoring and first aid advice. Although ruminants apparently have a low tolerance to triazine herbicides, they have low acute toxicity in laboratory mammals, and are not teratogenic or mutagenic.

No signs or symptoms of poisoning have been seen in humans due to atrazine. Thus, there is no intervention on the basis of the atrazine component of formulations, and an antidote is neither known nor needed.

Quantitative exposure determination relies on metabolites in urine. The diaminochlorotriazine metabolite can be measured by gas chromatography. All s-triazines can be detected by chemically transforming metabolites into cyanuric acid ie. trihydroxytriazine; however, this can be generated from other sources and low urinary levels have to be interpreted with caution. Ciba-Geigy Safety Data Sheets recommend an exposure limit of 4 mg cyanuric acid per litre of urine for industrial workers who handle triazines every day, corresponding to a triazine uptake of 10 mg/person/day. Field workers do not need to be monitored.

11.2.7 ***Richards RP, Baker DB, Christensen BR & Tierney DP (1995) Atrazine Exposures Through Drinking Water: Exposure Assessments for Ohio, Illinois and Iowa. Water Quality Lab., Tiffin, Ohio; Montgomery-Watson Inc., Minnesota; Ciba-Geigy, Greensboro, NC. Environ Sci Technol 29: 406-412***

Atrazine breaks down more slowly than most other current generation herbicides and is detectable in surface waters for a longer period of time after application. It has been found in rivers draining agricultural watersheds, in groundwater and even in rain and fog.

Assessments of human exposures to atrazine through drinking water have been carried out for the populations of the US states of Iowa, Illinois, and Iowa. The assessments indicated that atrazine through exposure in drinking water does not represent a significant human health threat, based on current knowledge of atrazine toxicity. Exposures to atrazine above the lifetime health advisory level of 3.0 ppb did not exceed 0.25% of the population in any of the three states and between 94-99% of the assessed population had exposure concentrations less than 1 ppb.

In the USA, the EPA has established the following Health Advisory Levels (HALS) for atrazine:-

Exposure Duration	Popul'n Segment	HAL (ppb)	Safety Factor
1 day	child	100	100
10 day	child	100	100
7 year	child	50	100
7 year	adult	200	100
70 year	adult	3	1000

The additional 10-fold safety factor was used in calculating the lifetime HAL because the US EPA ranks atrazine as a class C (possible) carcinogen. For calculation of the lifetime (70 year) HAL, it is assumed that only 20% of atrazine exposure comes from drinking water and includes an additional 5-fold factor to account for this. However, in the USA over 30 years of use, atrazine has not been detected in edible portions of plants or livestock nor has it been detected in market-basket surveys; results suggest that at least 95% of non-occupational exposure to atrazine occurs through drinking water. Thus, the 20% assumption provides an additional safety factor which approaches 5-fold.

The maximum contaminant level (MCL) is a legally-enforceable drinking water standard; for atrazine it is equal to the lifetime HAL of 3 ppb ie. 0.003 mg/kg or 3 µg/kg.

Some of the highest groundwater concentrations observed in the study were known to be of point-source origin, arising from accidents or improper pesticide handling practices.

11.2.8 ***Hofherr W (1995) Field Operator Exposure Study. Gesaprim 500FW Herbicide. Ciba-Geigy AG, Basel. Special study 136/94. 22 March 1995 (GLP; OECD, Switzerland; analytical part of study)***

The exposure of experienced operators was monitored during the routine application of Gesaprim 500FW to maize and fallow fields using commercial spraying equipment (ground application), using all safety precautions specified (coverall, cotton head cover, cotton inner gloves, nitrile outer gloves), except carrying a face shield when handling the concentrate. Each trial consisted of 2 tank mix preparations and 2 spraying operations. The test substance was applied at 1.42-1.53 kg ai/ha (approx.3 L of formulation/ha), with an application rate in the range 186-205 L/ha. Areas treated were in the range 4.80-5.27 ha, in Switzerland and France.

When using protective clothing, operator exposure values were as follows:-

Average Total Dermal Unprotected Exposure (TUDX; coverall, outer gloves, glove wash, cap) was 51.69 mg (highest total exposure was 97.7 mg). Highest dermal exposure (DX; whole undergarment, inner gloves, handwash) for mixing/loading and spraying was 178.5 µg/kg bw, average 47.4 µg/kg bw, highest respiratory exposure (REX; first, second air filter) was 0.395 µg/kg bw, average 0.07 µg/kg.

11.2.9 ***Honeycutt RC, Bennet RM & DeGeare MA (1996) Evaluation of the Potential Exposure of Workers to Atrazine during Commercial Mixing, Loading and Spray Application to Corn - Biological Field Phase. Ciba-Geigy Corp., Greensboro, NC. Research: H.E.R.A.C. Inc, Greensboro, NC. Interim report date 22 Jan. 1996. HERAC Study no. 95-501HE; Ciba Study no. 178-95 (GLP; US EPA)***

The exposure of commercial applicators was studied at 19 US sites during both the cold and warm months of spring 1995; dermal dosimetry and urine monitoring techniques were used.

The corn spray season ranged from 8-13 days (average 20.8 d). Applicators sprayed an average of 4,443 lbs atrazine/person/season. Mixer-loaders handled an average 14,400 lbs/person/season.

The exposure monitoring data is yet to be reported.

ATTACHMENT 15

MAJOR DEGRADATION STEPS OF ATRAZINE IN PLANTS AND ANIMALS

ATTACHMENT 16

METABOLIC PATHWAY OF ATRAZINE IN THE GOAT