ATRAZINE

First draft prepared by
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Explanation

Atrazine, 6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine (International Union of Pure and Applied Chemistry, IUPAC) (Chemical Abstracts Service, CAS No. 1912-24-9), is a selective systemic herbicide of the chlorotriazine class, which is used for the control of annual broadleaf and grassy weeds. It acts as a photosynthetic electron transport inhibitor at the photosystem II receptor site. Atrazine and its chloro-*s*-triazine metabolites deethyl-atrazine (DEA), deisopropyl-atrazine (DIA) and diaminochlorotriazine (DACT) have been found in surface and groundwater as a result of the use of atrazine as a pre-emergent or early post-emergent herbicide. Hydroxyatrazine is more commonly detected in groundwater than in surface water. The relative order of concentrations of these substances measured in rural wells in the USA was generally as follows: atrazine ~ DEA ~DACT > DIA > hydroxyatrazine. However, concentrations of DEA that are severalfold those of the parent compound have been reported.

Atrazine was evaluated previously by WHO, a tolerable daily intake (TDI) of 0.0005 mg/kg bw being established in the 1993 *Guidelines for Drinking-water Quality* on the basis of a no-observed-adverse-effect level (NOAEL) of 0.5 mg/kg bw per day in a study of carcinogenicity in rats and using a safety factor of 1000 (100 for inter- and intraspecies variation and 10 to reflect potential carcinogenic risk to humans).

Atrazine has not been previously evaluated by JMPR, and no acceptable daily intake (ADI) had been established. For that reason, the WHO Drinking-water Guidelines Programme recommended that atrazine should be evaluated toxicologically by JMPR.

The database on atrazine was extensive, consisting of a comprehensive set of studies with atrazine and its four key metabolites, which complied with good laboratory practice (GLP), as well as a large number of published studies. The present Meeting did not aim to perform a review of the database de novo, but to summarize the key studies, focusing on the issues of carcinogenicity, endocrine disruption (especially neuroendocrine mode of action) and immunotoxicity. Reference was made to a number of reviews made by national and international agencies and organizations in recent years.

Summary data were provided on the occurrence of atrazine residues in water, abstracted from monitoring data collected in areas where atrazide is widely used. Globally, atrazine is most commonly used as a pre-emergent or early post-emergent herbicide in corn in the USA and Brazil. Much smaller amounts are used in sorghum (Australia and the USA), sugar-cane (Brazil and the USA), oilseed crops (Australia) and tree plantations (Australia). The non-agricultural uses of atrazine (e.g. railways, road embankments, turf, home garden) have largely been removed from atrazine labels in recent years.

Because atrazine is somewhat persistent in the environment and is reasonably mobile in soils, a number of studies have monitored the concentrations of atrazine in groundwater and surface water over the last two decades. Recent monitoring data show declining levels and incidences of detections of atrazine and its chloro-*s*-metabolites (DIA; DEA and DACT) compared with data collected

in the early 1990s; this reflects restrictions on the use of atrazine that were introduced in the late 1990s and early 2000s and the introduction of the "Good Farming Practice Programme" (GFPP) in the European Union (EU) and other parts of the world and "Best Management Practices" (BMPs) in the USA. Therefore, older monitoring data generally represent an overestimate of environmental concentrations likely to arise from current use practices. In surface water, the concentrations of the chlorotriazine metabolites of atrazine are generally less than those of atrazine itself, while the concentrations of these metabolites in rural wells are more similar to those of atrazine.

Consideration of data from monitoring carried out in a number of countries indicate that concentrations of atrazine and its chloro-*s*-metabolites in groundwater and surface water rarely exceed the current WHO guideline value of 2 ppb; levels are commonly well below the EU "parametric value" for pesticide residues in drinking-water of 0.1 ppb. Specific monitoring of drinking-water indicates that:

- recent data from the USA show that in no public-water supplies does the concentration of atrazine exceed the United States Environmental Protection Agency (EPA) Drinking Water Levels of Comparison (DWLOCs) for any age group;
- concentrations of atrazine in the United Kingdom (UK) are less than 0.1 ppb; and
- in Canada, concentrations are lower than in "raw" groundwater or surface water.

Hydroxyatrazine is a plant metabolite of atrazine. It can also be formed in acidic and humitic soils. It is reported to bind to soil to a greater extent than parent atrazine or the chlorinated metabolites. Hydroxyatrazine has been less frequently measured in monitoring programmes than parent atrazine or the chlorinated metabolites; however, in studies in which hydroxyatrazine has been measured, it was generally detected less frequently than were the chlorinated metabolites, and commonly at lower concentrations. The available data suggest that hydroxy compounds are unlikely to significantly contaminate surface water.

Evaluation for acceptable daily intake

Unless otherwise stated, the studies evaluated in this monograph were performed by laboratories that were certified for GLP and that complied with the relevant Organisation for Economic Co-operation and Development (OECD) test guidelines or similar guidelines of the EU or United States Environmental Protection Agency. As these guidelines specify the clinical pathology tests normally performed and the tissues normally examined, only significant exceptions to these guidelines are reported here, to avoid repetitive listing of study parameters.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

In a study on absorption, distribution and elimination, male Sprague-Dawley rats received daily oral doses of [^{14}C]atrazine ([U^{14}C]-triazine labelled; radiochemical purity, $\geq$ 97.5%) for up to 7 days at 0.1 mg per rat (equal to approximately 0.4 mg/kg bw) or 1.0 mg per rat (equal to approximately 4.0 mg/kg bw). Groups of three rats were killed 5, 7, 9, 10, 14 and 18 days after the start of dosing, and blood and tissues were sampled for radioactivity. From the rats killed after 18 days (11 days after last dose), faeces and urine were collected for periods of 24 h; tissues were analysed for radioactivity, and a final material balance was calculated. A material balance of 103.9% and 93.4% was achieved for the doses at 0.4 and 4.0 mg/kg bw, respectively. Recovery of radiolabel was approximately 67–73% in

urine, 24–28% in faeces, and 2–3% in the tissues, with the proportions remaining constant between the doses. Approximately 95% of the administered dose was eliminated within 48 h of the final dose; however, 11 days after the final dose appreciable residues were still present in muscle and erythrocytes. Elimination half-lives of 4 days for most tissues, 10 days for brain, and 25–30 days for erythrocytes and muscle were derived. Binding to erythrocytes was postulated, with the decline in this tissue correlating to the half-life for erythrocyte turnover (Ballantine et al., 1985).

In a study on absorption, distribution and elimination, groups of five male and five female Sprague-Dawley rats received [^{14}C]atrazine ([U^{14}C]-triazine labelled; radiochemical purity, ≥ 98%) as a single dose at 1.0 or 100 mg/kg bw, or as a single dose at 1.0 mg/kg bw after receiving unlabelled atrazine as daily doses at 1.0 mg/kg bw for 14 days. Faeces and urine were collected for up to 168 h (7 days) after administration of the radiolabel. After 7 days, rats were killed and selected tissues and carcass were harvested. The material balance achieved 103% for rats receiving single doses at 1.0 or 100 mg/kg bw, but only 88% for the rats receiving repeated doses of non-radiolabelled atrazine. Recovery of radiolabel was approximately 74% in the urine and approximately 19% in the faeces, most elimination occurring within 48 h; the residues in the tissues accounted for 4.7–7.0% of the administered dose and were predominantly associated with erythrocytes. In most tissues, a lower proportion of the administered dose remained after a dose of 100 mg/kg bw than 1 mg/kg bw (4.7% versus 7.0%); this was proposed to be evidence of tissue saturation at higher doses. The kinetics of atrazine elimination were determined using urinary radioactivity excretion data, which best fitted a linear two-compartment open model for each of the three groups. The half-lives of the alpha (distribution and elimination) phase, and of the beta (whole-body renal elimination phase) were 6.9 h and 31.1 h, respectively. The half-life of renal elimination from the central compartment was 12.4 h (Orr et al., 1987).

In a study on absorption, distribution and elimination, pairs of female Sprague-Dawley CD rats were given [^{14}C]atrazine ([U^{14}C]-triazine labelled; radiochemical purity, 97.9%; purity of unlabeled atrazine, 98.8%) by gavage daily at a dose of 1, 3, 7, 10, 50 or 100 mg/kg bw for 10 days. Blood samples were collected at 24-h intervals, and tissue samples were collected at necropsy. Urine and faeces were collected over successive 24-h periods. One rat of each pair was killed at 3 h and the second at 72 h after the tenth dose. Proportions excreted in the urine (70–76%) and faeces (13–15%) did not vary with dose. Concentrations of atrazine in plasma rose until day 8 or 9 then achieved plateau values. Concentrations of atrazine in erythrocytes continued to increase throughout the 10-day dosing period; it was estimated that steady-state would be achieved only after 30 days. Half-lives for elimination from plasma or erythrocytes were 38.6 h and 8.1 days, respectively. Tissue concentrations 3 h after the tenth dose were linearly related to plasma concentrations obtained at the same time-point for the dose range of 1−100 mg/kg bw (Khars & Thede, 1987).

In a study on absorption, distribution and elimination, groups of six Sprague-Dawley rats (three males and three females) received [^{14}C]atrazine ([U^{14}C]-triazine labelled; radiochemical purity, > 99.7%; purity of unlabeled atrazine, 99.0%) as a single dose at 1 or 100 mg/kg bw. Blood samples were taken at 17 intervals within 168 h to determine blood-level kinetics. A further two groups of 12 male rats received a single dose at 1 or 100 mg/kg bw after which three rats per group were killed at 2, 48, 168 and 336 h to determine tissue residues. From three of these males that were killed at 168 h, urine and faeces were collected at successive time-points. A further group of four males were bile-duct cannulated, given a single dose of 1 mg/kg bw and then sampled for bile, urine and faeces during 48 h. Another group of four male rats was given a single dose at 100 mg/kg bw, and one rat was killed at each time-point, 24, 72, 168 and 336 h, for whole-body autoradiography. Excreta from this study were also used for the characterization of metabolites (see section 1.2).

Maximum concentrations in the blood were achieved at 2 h at the lower dose and 24 h at the higher dose. Areas under the curve of blood concentration–time (AUC) were not different between the sexes. The half-life in blood, assuming first-order kinetics, was approximately 150 h, independent of sex and dose. Results from bile-cannulated animals showed approximately 88% absorption of the lowest dose, on the basis of the excretion via urine (65%) and bile (7%) over 48 h and the amount remaining in the carcass (16%); elimination via the faeces was about 3%. At the highest dose, elimination via the urine and faeces during 168 h accounted for approximately 66% and 20%, respectively, with approximately 85% excreted within the first 48 h.

Tissue concentrations from rats killed at four different time-points showed that the highest concentrations of atrazine occurred in the liver, kidney and erythrocytes. Elimination from the tissues was judged to be biphasic, and half-lives ranged from 59 to 300 h (assuming first-order kinetics). Elimination from the erythrocytes was monophasic, with a half-life of 320 h. There were no significant differences between the sexes or doses.

Whole-body autoradiography supported the previous toxicokinetic and tissue-residue data, with slow elimination of radioactivity from well-perfused tissues (liver, kidney, lungs, heart and spleen) owing to the presence of erythrocytes (Paul et al., 1993).

In a study on absorption, distribution, metabolism and elimination, two groups of four male Fischer 344 rats received [^{14}C]atrazine ([U^{14}C]-triazine labelled; radiochemical purity, > 98%; purity of unlabeled atrazine, 99.6%) as a single oral dose at 30 mg/kg bw with or without pre-treatment with non-radiolabeled tridiphane (purity, > 99%) as a single oral dose at 60 mg/kg bw. The plasma time-course for the radiolabel exhibited a mono-exponential decrease for both treatment groups, with an absorption and elimination half-life of approximately 3 h and 11 h, respectively. About 93% of the administered radioactivity was recovered 72 h after dosing; with approximately 67% found in the urine and approximately 18% in the faeces, and less than 10% in the carcass, skin and erythrocytes. There were no appreciable differences in the metabolite distribution between treatment groups, and the major urinary metabolite of atrazine was found to be 2-chloro-4,6-diamino-1,3,5-triazine (64–67% of total urinary radioactivity). *S*-(2-Amino-4-methylethylamino-1,3,5-triazin-6-yl)-mercapturic acid (13–14%), and *S*-(2,4-diamino-1,3,5-triazin-6-yl)-mercapturic acid (9%) were tentatively identified based on similar high-performance liquid chromatography (HPLC) retention times. The data indicated that there were no meaningful differences in the absorption, distribution, metabolism, and excretion between rats given only [^{14}C]atrazine and those given both tridiphane and [^{14}C]atrazine (Timchalk et al., 1990).

The slow elimination of [^{14}C]atrazine from rat tissue may in part be related to the extent of blood perfusion of the tissue because a metabolite of *s*-triazines binds covalently to Cys-125 in the β-chain of rodent haemoglobin rather than to the usually more reactive Cys-93, which is present in most mammalian haemoglobins. The metabolite reacted also (but to a lesser extent) with haemoglobin from chicken (Cys-126), but not with haemoglobin from humans, dogs, sheep, cows or pigs, which lack Cys-125 in their haemoglobin (Hamboeck et al., 1981). In Sprague-Dawley rats exposed to atrazine, it was shown using matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) that the adduct to Cys-125 of the β-chain of haemoglobin was DACT (G 28273) and not parent atrazine or the mono-dealkylated metabolites G 28279 or G 30033 (Dooley et al., 2006).

Monkeys

In a study on the clearance of atrazine from the blood, four female Rhesus monkeys (aged approximately 20–30 years) received [^{14}C]atrazine ([U^{14}C]-triazine labeled; radiochemical purity, 98.1%; purity of unlabeled atrazine, 96.5%) intravenously as a single dose at 0.26 mg per monkey. Samles of urine, faeces and blood were collected for 168 h after treatment. The principal route of excretion was via the urine with approximately 63% of the administered dose being excreted within

24 h. Clearance of radioactivity from the blood was best described by a two-compartment model with half-lives of 1.5 and 17.7 h, respectively; the half-life for renal clearance was 20.8 h. By the end of the 7-day collection period, about 85% of the administered dose was found in the urine and 12% in the faeces (Hui et al., 1996a).

In a study on absorption, distribution, metabolism and elimination, three groups of four female Rhesus monkeys (aged 6–25 years) received capsules containing [^{14}C]atrazine ([U^{14}C]-triazine labelled; radiochemical purity, 96.8%; purity of unlabeled atrazine, 98.7%) as a single dose at 1, 10 or 100 mg per monkey. Samples of urine, faeces and blood were collected for 168 h after treatment. Maximum blood concentrations were achieved after 2, 8 and 24 h at the lowest, intermediate and highest dose, respectively. On the basis of the comparison of AUCs after oral and intravenous administration (Hui et al., 1996a), the bioavailabilities were estimated at 92%, 75% and 78% for the lowest, intermediate and highest dose, respectively. The terminal half-lives for elimination from plasma were 31.9 h, 21.6 h and 19.7 h for the lowest, intermediate and highest dose, respectively. By the end of the 7-day collection period, excretion of radioactivity in the urine was about 57%, 58% or 53% for the lowest, intermediate and highest dose, respectively, and in faeces about 21%, 25% or 35% for the lowest, intermediate and highest dose, respectively (Hui et al., 1996b).

1.2 Biotransformation

The metabolic pathway of atrazine in the rat is illustrated in Figure 1. The experimental evidence supports a metabolic pathway dominated by oxidative removal of the alkyl side-chains with 2-chloro-4, 6-diamino-*s*-triazine (G 28273) being the major metabolite. The 2 carbon-chlorine bond is stable to enzymatic hydrolysis, but is subject to conjugation via the action of glutathione-*S*-transferase. Action on sulfur-containing metabolites gives 2-sulfhydryl-*s*-triazines which in turn are subject to methylation followed by oxidation to the corresponding *S*-oxides. Oxidation of primary positions of the alkyl side-chains to carboxyl functions is a minor alternative metabolic route.

The different species including humans appear to share this common pathway (Figure 2); however, differences in the kinetics of each step may be inferred. No difference in metabolism was found in those cases where the sexes were compared. Although in-vitro data suggest that metabolism to the bi-dealkylated metabolite by cultured hepatocytes from women is minimal, this data were not confirmed by analysis of urine in vivo in two studies in men in which the bi-dealkylated form was a major component, as in the rat. The possibility of significant post-hepatic modification in humans cannot therefore be excluded.

Rats

From a study in Sprague-Dawley rats described previously (section 1.1; Paul et al., 1993), the excreta from male rats treated with atrazine as a single dose at 100 mg/kg bw, and from bile-duct cannulated male rats treated with a single dose at 1 mg/kg bw were used for the separation and identification of the metabolites. Urine and faeces were collected for up to 168 h, and bile for up to 48 h. Metabolites were analysed by two-dimensional thin-layer chromatography (TLC). The pattern of metabolites was complex, with 26, 12 and 9 different metabolites identified in the urine, faeces and bile, respectively. The major metabolite DACT (G 28273) accounted for approximately 26% of the administered dose in the urine, 1.6% in bile and 1.3% in faeces. Approximately 1% of the administered dose was the mercapturic derivative of the DACT (CGA 10582) in the urine. Lesser amounts of the mono-dealkylated metabolites G 28279 and G 30033 were found in the urine, bile or faeces. A trace amount of parent atrazine was found in the faeces (Paul et al., 1993).

In a study on comparative metabolism of two *S*-triazines, [^{14}C]atrazine ([U^{14}C]-triazine labeled; radiochemical purity, 96.4–97.0%; purity of unlabeled atrazine, 97.5–98.9%) was given to female Sprague-Dawley rats as a single oral dose at 1 or 76 mg/kg bw. Urine and faeces were collected at 24-h intervals for 3 days. At 72 h after treatment, the rats were killed and brains were analysed for residual radioactivity. Within 72 h, approximately 69% of the radioactivity was eliminated in the urine and approximately 16% in the faeces. Residues of atrazine equivalents in brain were 0.167 and 3.85 ppm for the lower and higher dose, respectively. No parent compound or its dealkylated metabolites were found in brain. Sample of urine analysed by cation exchange chromatography, HPLC and two-dimensional TLC showed that DACT (G 28273) was the major metabolite of atrazine, accounting for approximately 24–29% of the administered dose in urine for 0–24 h, while another chloro-*s*-triazine metabolite (metabolite UD, consisting of a mixture of chloro sidechain acids) accounted for approximately 6% of the administered dose. The total of the mercapturate of atrazine (CGA-359008) and the mercapturate metabolites (CGA-246059, CGA-63079 and CGA-10582) accounted for approximately 2–3% of the administered dose (Simoneaux, 2001).

Monkeys

In a study described previously (section 1.1, Hui et al., 1996a), female Rhesus monkeys received [^{14}C]atrazine intravenously as a single dose at 0.26 mg per monkey, and samples of urine, faeces and blood were collected during 168 h after treatment. Atrazine metabolites identified in the urine by TLC included 10.7% DEA (G 30033), 13.7% DIA (G 28279), 4.4% atrazine mercapturate and 5.6% di-dealkylated atrazine mercapturate. Plasma contained trace amounts of atrazine in addition to G 30033, G 28279 and G 28273. The mercapturic acid of atrazine, as determined by enzyme immunoassay (EIA), was detected primarily in urine samples collected during the first 24 h after dosing. All four potential mercapturic acid conjugates of chlorotriazine moieties were detected in the urine using the LC/MS/MS method (liquid chromatography/mass spectroscopy). The total mercapturic acid residues in 0–24 h composite urine samples ranged from 15 to 25 ppb and the individual mercapturic acids ranged from 1 to 14 ppb. Total accountability of the mercapturates ranged from 1.6% to 3.0% of the total radioactive residues (TRR). The amount of atrazine mercapturate (CGA 359008) present in the composite sample as determined by EIA was an average of 16 times greater than the amount determined by LC/MS/MS. Based upon preliminary characterization data, it was postulated that one or more unknown chlorotriazine compounds altered in one of the side-chain alkyl groups could react with the EIA reagent to explain the higher results obtained by this method compared with the more specific LC/MS/MS method (Hui et al., 1996a).

In a study described previously (section 1.1, Hui et al., 1996b), female Rhesus monkeys received [^{14}C]atrazine orally as a single dose at 1, 10 and 100 mg per monkey, and samples of urine, faeces and blood were collected during 168 h after treatment. Samples of urine were analysed by TLC and gas chromatography/mass selective detection (GC/MSD) for atrazine and its dealkylated metabolites DEA (G 30033), DIA (G 28279), and DACT (G 28273). Both analytical methods gave similar amounts of chlorotriazine residues. An average of approximately 11% of the administered dose in all dose groups was found as chlorotriazine residues when measured in urine samples for 0–24 h, the major metabolites in urine were G 30033 and G 28273. Atrazine mercapturate (CGA 359008) accounted for 1% or less of the total radioactive residues in urine (Hui et al., 1996b).

Humans

In a study on kinetics and metabolism, six adult male volunteers received atrazine (purity not reported) as a single oral dose of 0.1 mg/kg bw. Urine was collected over 168 h. Blood samples were obtained from one man at 0, 2, 3, 4, 5, 6, 8, 24, 32, 72 and 168 h after dosing. Samples of urine and blood were analysed for atrazine and its dealkylated metabolites DEA (G 30033), DIA (G 28279)

and DACT (G 28273) using a gas chromatography method with stated detection limit of 0.005 ppm. Atrazine and G 28279 were detected in whole blood but were below the limit of quantification. The metabolite G 30033 appeared rapidly in the plasma reaching a peak after 2 h to decline rapidly thereafter with a half-life of 2.8 h. The disappearance of G 30033 corresponded with an increased plasma concentration of the metabolite G 28273 which reached a peak at 5 h after dosing and was eliminated with a half-life of 17.8 h. This suggests a step-wise dealkylation of atrazine to DEA and then to DACT. The elimination of both metabolites from the blood was consistent with one-compartment first-order kinetics at least up to 32 h after dosing. Analysis of urine identified only approximately 14.5% of the administered dose, with consistent results for all six men. The chloro-metabolites G 30033, G 28279 and G 28273 accounted for 5.4%, 1.4% and 7.7% of the administered dose, respectively. For metabolite G 28273, urinary kinetics suggested a single-compartment first-order model with a half-life of 11.5 h. For the metabolites G 30033 and G 28279, a two-compartment first-order model suggested a fast and slow phase of 2.3 h or 8.4 h and 2.4 h or 36.2 h, respectively (Davidson, 1988).

In a biological monitoring study on a group of six manufacturing workers exposed to atrazine (10–700 μmol per workshift), total urinary excretion of atrazine plus three metabolites accounted for 1–2% of the external dose, with 50% of the amount excreted in the first 8 h following the workshift. About 80% of the excreted metabolites accounted for DACT (G 28273), 10% for DIA (G 28279), 8% for DEA (G 30033), and only 1–2% was atrazine (Catenacci et al., 1993).

In a study on comparative metabolism of atrazine in vitro, cultures of hepatocytes from CD1 mice, Sprague-Dawley rats, Fischer 344 rats, guinea-pigs, goats and Rhesus monkeys, and two women were used. The isolated cells were assayed for viability and cultured with [^{14}C]atrazine (purity, 97%) at a concentration of 1, 5, 10, 25 or 100 ppm for up to 24 h. Analysis of the metabolites was by thin-layer and cation-exchange chromatography. The different species appeared to share common pathways for the metabolism of atrazine, dealkylation of the ethyl- and isopropyl moities but displayed significant differences in the kinetics of phase I and phase II metabolism of atrazine. Human and CD1 mice hepatocytes showed a marked preference for rapid *N*-deethylation, while in rats and Rhesus monkeys *N*-deisopropylation predominanted over *N*-deethylation. Sprague-Dawley rat, Fischer rat, CD1 mouse and Rhesus monkey hepatocytes rapidly metabolized atrazine to the di-dealkylated metabolite DACT (G 28273) and then further metabolized this intermediate to different conjugates. For guinea-pig, goat and human hepatocytes, the primary products were the mono-dealkylated metabolites DEA (G 30033) and DIA (G 28279) with only traces of the bis-dealkylated metabolite DACT. The bis-dealkylated metabolite DACT accounted for 50–70% of total radiolabel in 24-h incubations with hepatocytes from CD1 mice, rats (both strains) and Rhesus monkeys, but only 3–10% in incubations with guinea-pig, goat and human hepatocyte cultures. The percentages of the different metabolic products of atrazine produced by hepatocytes from different species are presented in Table 1 (Thede, 1988).

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

In a study of acute oral toxicity, groups of five male and five female Tif:RAI rats received atrazine as a single dose at 600–6000 mg/kg bw suspended in 2% carboxymethyl cellulose Clinical symptoms occurred within 2 h after treatment in all groups and included sedation, dyspnoea, exophthalmus, curved body position and ruffled fur. The surviving animals recovered between days 7 and 8 after treatment. The median lethal dose (LD_{50}) was 1869 mg/kg bw (Sachsse & Bathe, 1975b).

Table 1. Metabolism of atrazine by hepatocytes from different species in vitro

Metabolite	Species						
	Sprague-Dawley rat	F-344 rat	Mouse	Rhesus monkey	Goat	Guinea-pig	Human
Atrazine (%)	ND	ND	ND	2	60	4	19
DIA (%)	14	12	3	25	18	30	18
DEA (%)	5	6	6	10	20	32	50
DACT (%)	50	54	60	50	9%	10	< 5
Ratio of DEA to DIA	1 : 3	1 : 2	2 : 1	1 : 2.5	1 : 1	1 : 1	3 : 1
Conjugated metabolites (%)	34	29	30	9	< 1	25	11

From Thede (1988)
DACT, diaminochlorotriazine; DEA, deethyl-atrazine; DIA, deisopropyl-atrazine; ND, not detected.

In a study of acute oral toxicity conducted in compliance with GLP and US EPA test guidelines, groups of five male and five female HSD:(SD) rats received atrazine as a single dose at 4000–5500 (males) or 2000–5500 (females) mg/kg bw suspended in 2% carboxymethyl cellulose. Clinical symptoms included hypoactivity, ataxia, emaciation, lacrimation, nasal discharge, piloerection, polyuria and salivation. The surviving rats recovered within 3–6 days. No organ-specific lesions were discernible on macroscopic examination of the animals. Discoloration of the gastrointestinal tract in the rat may be related to local irritation. The male, female and combined oral LD_{50} and the 95% confidence limits (CL) were 3520 mg/kg bw (2290–5400), 3000 mg/kg bw (2090–4300) and 3090 mg/kg bw (2170–4420), respectively (Kuhn, 1991a).

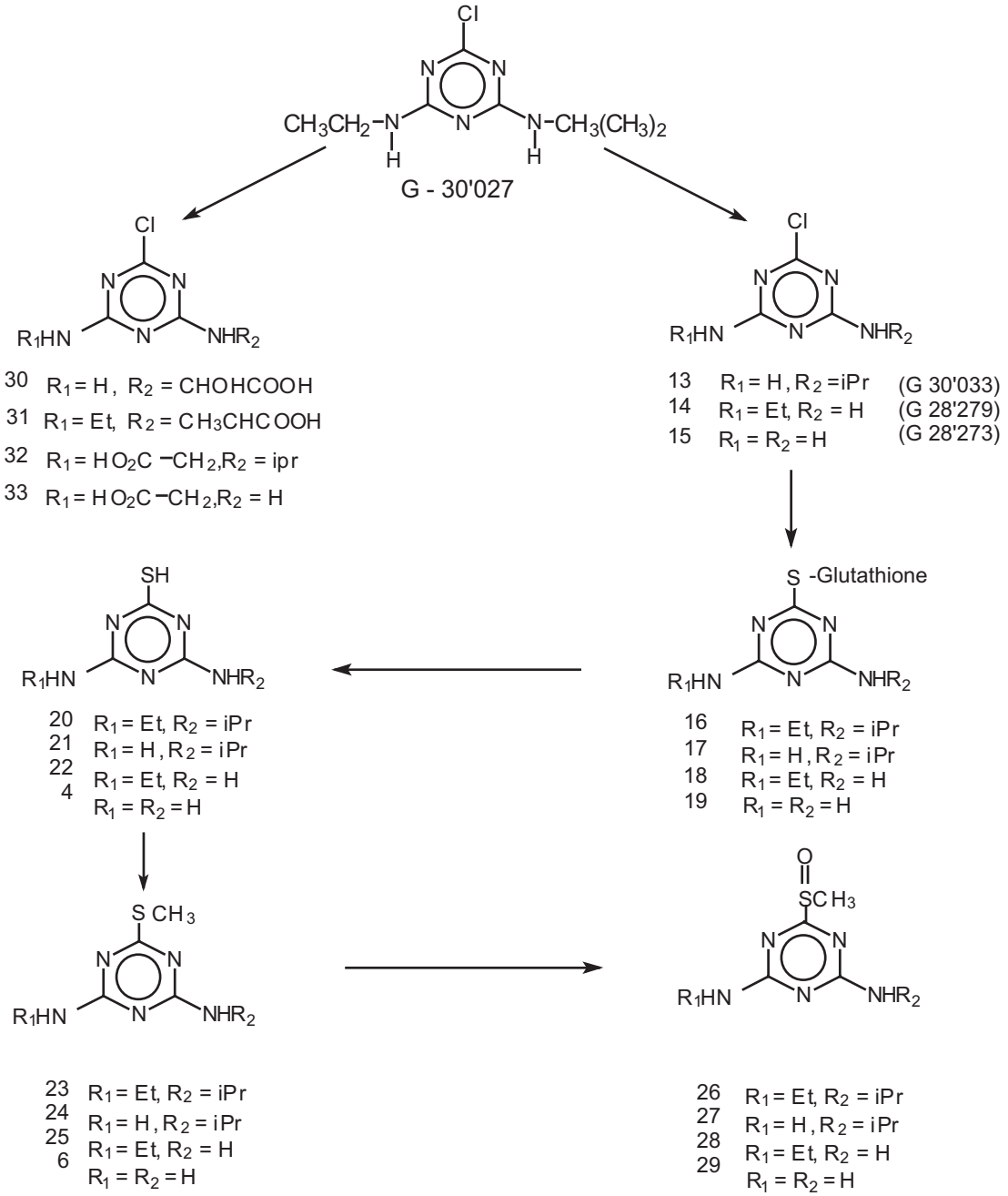
In a study of acute dermal toxicity, groups of five male and five female Tif:RAIf rats received atrazine as a single application at a dose of 2150 or 3170 mg/kg bw suspended in 2% carboxymethyl cellulose on the shaved back skin. No mortality occurred during the 14-day observation period. There were no treatment-related clinical signs or gross changes in the organs. The dermal LD_{50} was > 3100 mg/kg bw in males and females (Sachsse & Bathe, 1976).

In a study of acute dermal toxicity conducted in compliance with GLP and OECD test guidelines, one group of five male and five female Tif:RAIf rats received atrazine as a single application at a dose of 2000 mg/kg bw moistened with 0.5% carboxymethyl cellulose on the shaved skin for 24 h. No mortality occurred. Clinical symptoms including piloerection and hunched body position were seen on the first 5 days of application. No organ-specific lesions were discernible on macroscopic examination of the animals. The dermal LD_{50} was > 2000 mg/kg bw in males and females (Hartmann, 1993).

In a study of acute toxicity after exposure by inhalation that was conducted in compliance with GLP and OECD test guidelines, one group of five male and five female Tif:RAIf rats was exposed to atrazine at an average air concentration of 5148 mg/m^3 for 4 h. No mortality occurred. Rats showed piloerection, hunched posture, dyspnea, and reduced locomotor activity. All animals recovered within 5 days. No organ abnormalities were recorded. The median lethal concentration (LC_{50}) was > 5148 mg/m^3 (5.15 mg/l) (Hartmann, 1989).

In a study of acute toxicity after exposure by inhalation that was conducted in compliance with GLP and US EPA test guidelines, one group of five male and five female Sprague-Dawley rats was

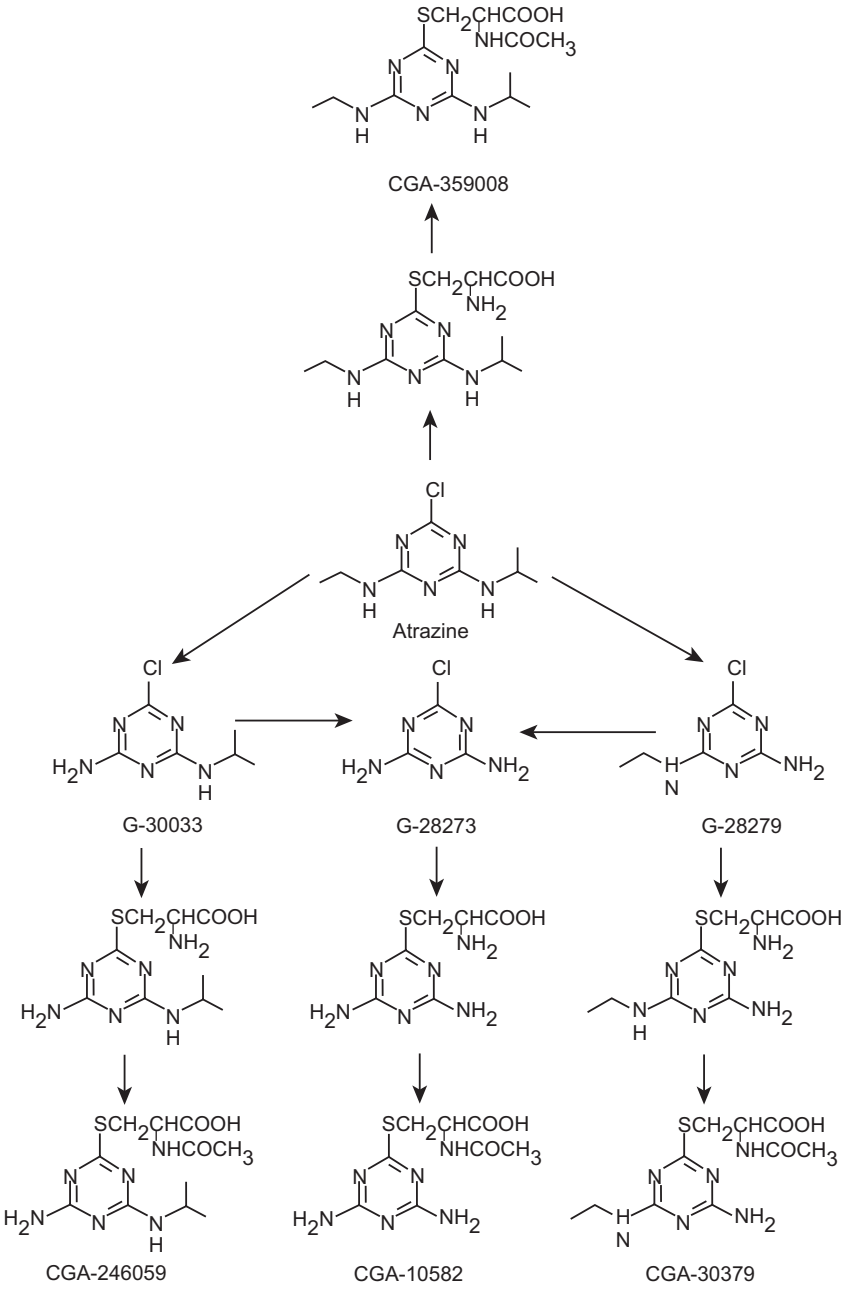
Figure 1. Proposed metabolic pathway for atrazine in the rat



exposed to atrazine at an average air concentration of 5820 mg/m³ for 4 h. No mortality occurred. Rats showed reduced activity, lacrimation, nasal discharge, piloerection, polyuria, ptosis and salivation. No organ abnormalities were recorded. The LC_{50} was > 5820 mg/m³ (5.82 mg/l) (Holbert, 1991).

In a study of acute toxicity after intraperitoneal administration, groups of five male and five female Tif:RAI rats received atrazine at doses of 147–1000 mg/kg bw as a suspension in a 2% solution of carboxymethyl cellulose by intraperitoneal injection. Within 2 h after treatment all rats showed sedation, dyspnea, exophthalmus, curved body position and ruffled fur, and at doses of 600 mg/kg bw

Figure 2. Proposed metabolic pathway for atrazine in primates and humans

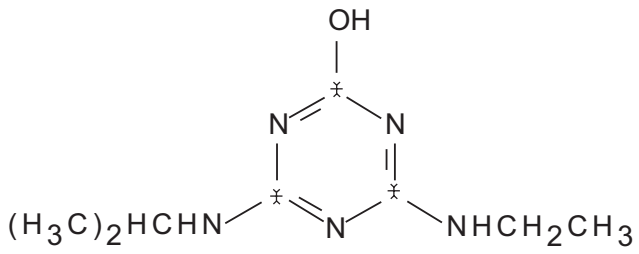


and greater, also showed chromodacryorrhoea and salivation. No treatment-related gross changes in organs were seen at necropsy. The LD_{50} was 235 mg/kg bw (Sachsse and Bathe, 1975a).

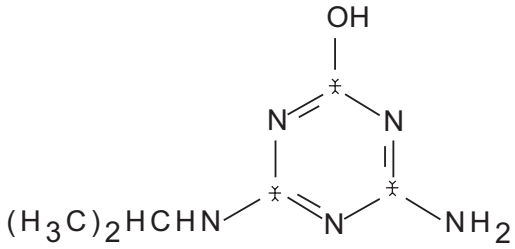
(b) Dermal irritation

In a study on skin irritation potential, atrazine powder (0.5 g) was applied to intact and abraded skin of three male and three female Himalayan rabbits. The treated area was occluded for 24 h. A very slight erythema was observed in three rabbits (abraded) at 24 h after application and in one rabbit at 72 h. The study differed from present OECD test guidelines in that the test material was not moistened, the exposure period was for 244 h not 4 h; that abraded skin sites were used in addition

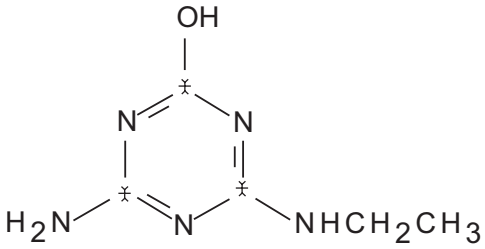
Figure 3. Primary metabolic pathway for hydroxyatrazine in the rat



(^{14}C)-hydroxyatrazine (G-34048)



[^{14}C]-GS-17794



[^{14}C]-GS-17792

Table 2. Results of studies of acute toxicity with atrazine

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg)	LC_{50} (mg/l air)	Reference
Rat	Tif:RAI	Males & females	Oral (in 2% CMC)	NR	1869	—	Sachsse & Bathe (1975b)
Rat	HSD:(SD)	Males & females	Oral (40% w/v in water)	97.7	3090	—	Kuhn (1991a)
Mouse	Tif:MAG	Males & females	Oral (in 2% CMC)	NR	3992	—	Sachsse & Bathe (1975c)
Mouse	HSD:(ICR)	Males & females	Oral (in 0.5% CMC)	97.7	> 1332	—	Kuhn (1988)
Rat	Tif:RAIf	Males & females	Dermal (in 2% CMC)	NR	> 3100	—	Sachsse & Bathe (1976)
Rat	Tif:RAIf	Males & females	Dermal (in 0.5% CMC)	97.1	> 2000	—	Hartmann (1993)
Rat	Tif:RAIf	Males & females	Inhalation	96.7	—	> 5.15	Hartmann (1989)
Rat	HSD:(SD)	Males & females	Inhalation	97.4	—	> 5.82	Holbert (1991)
Rat	Tif:RAI	Males & females	Intraperitoneal (in 2% CMC)	NR	235	—	Sachsse & Bathe (1975a)

CMC, carboxymethyl cellulose; NR, not reported.

to intact skin; and that no reading was made at 48 h after application. However, the study is adequate for the purpose intended. Very slight erythema was recorded on abraded skin of three animals and, therefore, atrazine is classified as a mild skin irritant under EPA guidelines but is not an irritant under OECD or EC criteria (Ullmann, 1976b).

(c) Ocular irritation

In a study on eye irritation potential, atrazine powder (0.1 g) was applied into the left eyes of three male and three female Himalayan rabbits, and after 30 s was rinsed out from three of these eyes. The eyes were closely examined for reaction at intervals after application. The study deviates from present OECD test guideline in that no recording was performed at 1 h. In view of the results, this does not detract from the acceptability of the study. Atrazine caused no reaction to the eye at 24, 48 or 72 h, and therefore is non-irritant to the eye (Ullmann, 1976a).

(d) Dermal sensitization

In a study on skin sensitization potential that was conducted in compliance with GLP and OECD test guidelines (optimization test), 10 male and 10 female Pirbright White (Tif:DHP) guinea-pigs received a total of 10 intradermal injections (each of 0.1 ml of 0.1% atrazine in 20% ethanol and 80% physiological saline) during the induction period, while the control group (10 males and 10 females) received the vehicle only. Two weeks later, a challenge dose of 0.1 ml of 0.1% atrazine in a mixture of 20% ethanol and 80% physiological saline was injected intradermally. Ten days later, a sub-irritant level dose of 30% atrazine in vaseline was applied on the skin under occlusive patches for 24 h. Twenty-four and 48 h after removal of the patches, 10 out of 19 animals showed skin reactions indicating sensitization potential in the test system (Maurer, 1983).

In a study on skin sensitization potential that was conducted in compliance with GLP and OECD test guidelines (Magnusson & Kligman test), 10 male and 10 female Pirbright White (Tif:DHP) guinea-pigs received three pairs of intradermal injections (adjuvant/saline 1 : 1, atrazine in oleum arachidis 1% mixture, and atrazine in adjuvant/saline mixture 1%). One week later, 0.4 g atrazine incorporated in vaseline (30%) was applied epidermally to the neck of the animals for 48 h. After a 2-week rest period (week 3 and 4) the guinea-pigs were challenged by application of 0.2 g of atrazine at a concentration of 30% incorporated in vaseline for 24 h. Twenty-four and 48 h after removal of the patches, 65% and 70% of the guinea-pigs showed skin reactions indicating sensitization potential in the test system (Schoch, 1985).

In a study on skin sensitization potential, repeated-insult patch tests were conducted on 50 (otherwise undescribed) humans given 0.5 ml of a 0.5% suspension of atrazine 80W formulation in water; the composition of the formulation was not given. Subjects received 15 consecutive 24-h, alternate-day treatments followed by a 14-day rest period before a challenge dose. None of the subjects reacted to any application or to the challenge. The tested spray dilution of atrazine did not appear to be sensitizing to humans (Shelanski & Gittes, 1965).

2.2 Short-term studies of toxicity

Rats

In a study of oral toxicity, groups of 10 male and 10 female Tif:RAIf (Sprague-Dawley derived) rats were fed diets containing atrazine (purity, 97.1%) at a concentration of 10, 50 or 500 ppm, equal to 0, 0.6, 3.3 and 34.0 mg/kg bw per day in males and 0, 0.66, 3.35 and 35.3 mg/kg bw per day in females, for 13 weeks. An additional 10 males and 10 females were allocated to the groups at the

Table 3. Dermal irritation and sensitization potential of atrazine

Species	Strain	Sex	End-point (method)	Purity (%)	Result	Reference
Rabbit	Himalayan	Males & females	Skin irritation	NR	Not irritating	Ullmann (1976b)
Rabbit	Himalayan	Males & females	Eye irritation	NR	Not irritating	Ullmann (1976a)
Guinea-pig	Pirbright White (Tif:DHP)	Males & females	Skin sensitization (optimization test)	98.2	Sensitizing	Maurer (1983)
Guinea-pig	Pirbright White (Tif:DHP)	Males & females	Skin sensitization (Magnussen & Kligman test)	98	Sensitizing	Schoch (1985)
Human	—	NR	Repeated-insult patch test	Atrazine 80 W formulation	Not sensitizing	Shelanski & Gittes (1965)

NR, not reported.

highest dose and the control group to evaluate an additional 4-week recovery period. The study was conducted in compliance with GLP and US EPA and OECD test guidelines.

There were no major treatment-related clinical signs and no deaths. A statistically significant decrease in body-weight gain, compared with controls, was found in males (−15%) and in females (−9%) of the group at 500 ppm. A slight, non-statistically significant decrease (−8%) in body-weight gain was also reported in males at 50 ppm. Food consumption was decreased in males (−10%) and females (−5%) of the group at 500 ppm and for males at 50 ppm (−5%). No treatment-related changes were found for any haematological or clinical chemistry parameters at any dose. The mean absolute liver and kidney weights were lower in the males at 500 ppm and liver weight relative to body weight was higher in females at 500 ppm than in the controls. Histologically, deposition of haemosiderin pigment in the spleen was found at an increased incidence and severity at 500 ppm. In females, this finding was still present at a higher incidence after the recovery period.

The NOAEL was 50 ppm, equal to 3.3 mg/kg bw per day, on the basis of decreased body weight and increased splenic haemosiderosis at 500 ppm (Bachmann, 1994).

Dogs

In a study of oral toxicity in beagle dogs, groups of four males and four females (lowest and intermediate dose) or six males and six females (control and highest dose) were fed diets containing atrazine (purity, 97%) at a concentration of 0, 15, 150 or 1000 ppm, equal to 0, 0.5, 5 and 33.7 mg/kg bw per day, for 52 weeks. Fixed portions of 400 g of diet were offered during a 3-h period each day. Two males and two females in the control group and in the group at highest dose were designated to be placed in a recovery group. Investigations included clinical pathology, ophthalmoscopic, auditory and electrocardiographic examinations at 3-monthly intervals throughout the study. The study was conducted in compliance with GLP and US EPA and OECD test guidelines.

Two dogs at the highest dose (one male on day 250, one female on day 113) and one male at the intermediate dose (on day 75) were killed in a moribund condition during the course of the study. The two dogs found in a moribund condition at the highest dose may have been affected by treatment-related cardiac dysfunction since compound-related myocardial lesions were observed in these animals. Treatment-related symptoms were restricted to dogs at the highest dose. Clinical symptoms at 1000 ppm included cachexia, ascites and laboured and shallow breathing. The ascites was considered related to treatment-induced myocardial degeneration, as was the laboured breathing. Marked reduction in body-weight gain (males, 61%; and females, 55% of control values) was recorded in dogs at

the highest dose, and food consumption was consistently lower in the group at the highest dose when compared with controls.

Electrocardiographic changes occurred in dogs at the highest dose and included slight to moderate increases in heart rate (primarily in males), moderate decreases in height of the P-wave, PR and QT values. Atrial premature complexes and atrial fibrillation were found in one female.

Haematology data indicated a slight but significant decrease in erythrocyte parameters in males and an increase in the number of platelets in males and females at 1000 ppm. Treatment-related changes in clinical chemistry were restricted to dogs at 1000 ppm and included a slight decrease in total plasma protein and albumin, considered to be secondary to the reduction in food consumption.

Necropsy revealed heart lesions in the group at the highest dose, these consisting of moderate to severe dilatation of right and/or left atria, and in some dogs, a fluid-filled pericardium and enlarged heart. In females at the highest dose, there was a treatment-related decrease in absolute heart weight. Histopathological findings were restricted to the heart in the group at the highest dose and included myolysis and atrophy of myocardial fibres, and oedema of the heart. These findings correlated with the clinical symptoms, the electrocardiogram results and with the gross pathology observations.

The NOAEL was 150 ppm, equal to 5 mg/kg bw per day, on the basis of decreased body-weight gain and cardiac effects at 1000 ppm (O'Connor et al., 1987).

Rabbits

In a short-term study of dermal toxicity, groups of five male and five female New Zealand White rabbits were given atrazine (purity, 97.6%) at a dose of 0, 10, 100 or 1000 mg/kg bw per day under semi-occluded conditions to the skin for 6 h per day for at least 25 consecutive days. The study was conducted in compliance with GLP and US EPA test guidelines.

At 1000 mg/kg bw per day, systemic toxicity (faecal changes, body-weight loss, decrease in food consumption) was recorded in males and females during treatment, while females at 100 mg/kg bw per day showed a transient decrease in body-weight gain during the first week of treatment. Also at 1000 mg/kg bw per day, changes in haematology and clinical chemistry parameters (slight depression of erythrocyte count and haemoglobin concentration with a minimal increase in the percentage of reticulocytes, decrease in total serum albumin and chloride values) and slight changes in absolute and relative organ weights were observed. Local effects in females at 1000 mg/kg bw per day included slight dermal irritation, an increased incidence of minimal to moderate acanthosis and focal subacute lymphocytic inflammation of the skin.

The NOAEL for systemic toxicity was 100 mg/kg bw per day on the basis of decreased body-weight gain and food intake, a slight reduction in erythrocyte parameters and increased spleen weight at 1000 mg/kg bw per day (Huber et al., 1989).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In an early study of carcinogenicity, atrazine (from commercial sources, purity not given) was administered to groups of 18 male and 18 female mice of each of the C57BL/6 × C3H/Anf and C57BL/6 × AKR strains (a total of 36 males and 36 females). At the beginning of the study, mice aged 7 days (pre-weanling) were given atrazine at a dose of 21.5 mg/kg bw per day by gavage in 0.5% gelatin, and were switched at weaning (age 4 weeks) to diet containing atrazine at 82 ppm (cited to be approximately equivalent to the dose given by gavage, but estimated in review to be approximately half this dose). Doses were not adjusted for body-weight gain, either when given by gavage or by dietary administration. The dose was selected as the highest not causing mortality in a pilot screening

Table 4. Results of studies of carcinogenicity with atrazine

Species	Strain	Sex	Dietary concentration (ppm)	Carcinogenic effect	Reference
Mouse	C57BL/6 x C3H/Anf and C57BL/6 x AKR	Males & females	21.5 mg/kg bw per day (days 7–28) thereafter 82 ppm	Negative	Innes et al. (1969)
Mouse	CD1	Males & females	0, 10, 300, 1000	Negative	Sumner (1981)
Mouse	CD1	Males & females	0, 10, 300, 1500, 3000	Negative	Hazelette & Green (1987)
Rat	Sprague-Dawley	Males & females	0, 10, 100, 1000	Males: negative Females: positive (increased incidence) at 10 and 1000 ppm	Spindler & Sumner (1981)
Rat	Sprague-Dawley	Males & females	0, 10, 70 500, 1000	Males: negative Female: positive (increased incidence) at 70–1000 ppm	Mayhew (1986)
Rat	Sprague-Dawley	Males & females	0, 10, 50, 500	Negative	Rudzki et al. (1991)
Rat	Sprague-Dawley	Females	0, 70, 400	Positive: (earlier onset) at 400 ppm No effect on incidence	Thakur (1991a)
Rat	Sprague-Dawley	Females	0, 70, 400	Positive: (earlier onset) at 400 ppm No effect on incidence	Thakur (1992a)
Rat	Sprague-Dawley	Females	0, 15, 30, 50, 70, 400	Positive: (increased incidence and earlier onset) at 400 ppm	Pettersen & Turnier (1995)
Rat	Sprague-Dawley	Females (intact)	0, 25, 50, 70, 400	Positive: (increased incidence) at 50 to 400 ppm, (earlier onset) at 400 ppm	Morseth (1998a)
Rat	Sprague-Dawley	Females (ovex)	0, 25, 50, 70, 400	Negative	Morseth (1998a)
Rat	Fischer 344	Males & females	375, 750	Inconclusive	Pintér et al. (1990)
Rat	Fischer 344	Females	0, 10, 70, 200, 400	Negative	Thakur (1991b)
Rat	Fischer 344	Males & females	0, 10, 70, 200, 400	Negative	Thakur (1992b)

procedure. A limited necropsy (cranial cavity not opened) and limited histopathology (including all macroscopic lesions) were conducted on all animals. This study was part of a screening procedure involving 120 chemicals and nearly 20 000 mice, and logistical considerations restricted the procedures conducted (including reporting). Inclusion of carcinogens acting as positive controls proved the responsiveness of the test procedure. Atrazine did not cause a significant increase in the incidence of tumours (Innes et al., 1969).

In a study of carcinogenicity, groups of 60 male and 60 female CD1 mice were given diets containing atrazine (purity, 96.4%) at a concentration of 0, 10, 300 or 1000 ppm for 21 months (males) or 22 months (females).The study data were audited by the sponsor and declared to be valid and useful for the purposes of evaluating carcinogenicity. However, various data were missing (e.g. body-weight gain for the first 2 months of the study, food consumption throughout the study) and some

mice (evaluated as roughly equivalent numbers in each group) received treatment with 1% rotenone for a mite infestation. During the early months of the study, diets were mixed sufficiently infrequently that substantial degradation of atrazine (20–30%) may have occurred before feeding. In some tissues, substantial numbers of samples were lost to autolysis. There were other practices that did not meet modern guideline requirements. Histopathology was performed by an independent laboratory. Treatment with atrazine caused a significant reduction in the body weights of mice at 1000 ppm. Survival among males was not affected by treatment although survival was significantly lower in females at 1000 ppm. The cause of death of these female decedents does not appear to have been documented. However, eventual survival and duration of this study was adequate for the evaluation of carcinogenicity. Histopathology revealed no evidence of a treatment-related change in inflammatory, degenerative, proliferative or neoplastic lesions in treated mice. Atrazine was not carcinogenic in mice, in this study suitable to be used as supportive data.

The NOAEL was 300 ppm, equivalent to approximately 30 mg/kg bw per day, on the basis of reduced survival and reduced body weight at 1000 ppm (Sumner, 1981).

In a study of carcinogenicity performed according to US EPA guidelines and in compliance with GLP, groups of 60 male and 60 female CD1 mice were given diets containing atrazine technical (purity, 97.6%) at a concentration of 0, 10, 300, 1500, or 3000 ppm for 91 weeks. Mean daily intakes were equal to 0, 1.2, 38.4, 194.0 and 385.7 mg/kg bw per day in males and 0, 1.6, 47.9, 246.9 and 482.7 mg/kg bw per day in females. Blood samples for haematology were collected from all animals at necropsy, and blood smears prepared from all survivors at weeks 52 and 78. Survival was not affected by treatment in males (59%, 55%, 60%, 65% and 60% at 0, 10, 300, 1500 and 3000 ppm) while decreased survival was reported for the females at 3000 ppm (43%, 39%, 43%, 45% and 25% at 0, 10, 300, 1500 and 3000 ppm). No treatment-related clinical signs occurred during the study. Lower mean body weights and body-weight gains were recorded for males and females at 300, 1500 and 3000 ppm, which correlated with lower food consumption at 1500 and 3000 ppm. Haematology investigations revealed a slight reduction in mean haemoglobin concentration, erythrocyte volume fraction and in erythrocyte count in males at 1500 and in males and females at 3000 ppm. Analysis of organ weight data did not reveal variations with toxicological significance. A lower brain weight in males and females at 3000 ppm was not accompanied by any histopathological correlates. Histopathology revealed an increased incidence of cardiac thrombi primarily in the atria of decedents in males at 3000 ppm and in females at 1500 and 3000 ppm. No cardiac changes were apparent to account for this change. No evidence of compound-related neoplastic lesions was found in this study.

The NOAEL was 10 ppm, equal to 1.2 and 1.6 mg/kg bw per day in males and females, respectively, on the basis of lower body weight/body-weight gain at 300 ppm and greater (Hazelette & Green, 1987).

Rats

In a study of carcinogenicity conducted before GLP, groups of 60 male and 60 female Sprague-Dawley rats were fed diets containing atrazine technical (purity, 98.0%) at a concentration of 0, 10, 100 or 1000 ppm, equal to 0, 0.35, 4 and 40 mg/kg bw per day in males and 0, 0.45, 5.5 and 60 mg/kg bw per day in females, for up to 2 years. Accuracy of diet preparation was confirmed by analysis on only three occasions during the study. Clinical pathology was not investigated in rats receiving the intermediate or lowest dose.

Body-weight gain was reduced significantly at 1000 ppm throughout the study reaching 22% and 27% lower than that of the controls for males and females, respectively. Weight gain for the rats at 100 ppm was reduced to a lesser extent (about 10% lower than that of controls). A decrease in erythroid parameters was reported at the highest dose, and no measurements were performed at the lower doses. Clinical chemistry data showed slight variations in the serum transaminases and

cholesterol of females at the highest dose at different occasions. Gross pathology investigations revealed a high incidence of enlarged pituitaries and of dermal/subdermal masses in females from all groups including controls. Histopathology of mammary tissues showed a statistically significant, but not dose-related increase in the incidence of benign fibroadenomas in females at the lowest (10 ppm) and highest (1000 ppm) dose, but not at the intermediate dose of 100 ppm (incidences were: 11 out of 54, 20 out of 52, 14 out of 54, and 22 out of 49 at 0, 10, 100 and 1000 ppm, respectively). Atrazine gave positive results for carcinogenicity in this study, which is suitable to be used for supportive data only. Because of data deficiencies (parameters not investigated in groups at the intermediate dose), a NOAEL was not identified in this study (Spindler & Sumner, 1981).

In a combined long-term study of toxicity and carcinogenicity conducted in compliance with GLP and EPA guidelines, groups of 70 male and 70 female Sprague-Dawley rats were fed diets containing atrazine (purity, 95.8%) at a concentration of 0, 10, 70, 500 or 1000 ppm, equal to 0, 0.4, 2.6, 19.9 and 41.7 mg/kg bw per day in males and 0, 0.5, 3.5, 29.5 and 64.7 mg/kg bw per day in females, for 2 years. Dietary exposure was verified by analysis. The control group and the group at the highest dose contained an additional 20 males and 20 females for interim kill, 10 males and 10 females after 12 months and 10 males and 10 females after a further 1-month recovery period. For the long-term evaluation of toxicity, 20 males and 20 females per group were used, and the study design contained appropriate end-points to satisfy guideline requirements.

The males at the highest dose (1000 ppm) showed an increased survival (67% vs 44% in controls), while a significantly decreased survival was recorded in the females at 1000 ppm when compared with the controls (26% in the treated females vs 50% in the controls) at the study termination.

Increased irritability was seen in the males, and pallor in females, at 500 or 1000 ppm. An increase in numbers of palpable masses was apparent in the females at 70, 500, and 1000 ppm. Bodyweight gains of rats at 500 ppm (weight gain after 12 months, −10% in males; −23% in females) and 1000 ppm (weight gain after 12 months, −22% in males; −34% in females) were significantly decreased throughout the study. On the basis of these weight-gain retardations, doses of 500 and 1000 ppm were considered to be excessive for carcinogenicity testing. Food consumption was slightly decreased. Haematology investigations revealed lower values for erythrocyte parameters in females at 1000 ppm. Sporadic changes in some clinical chemistry parameters, namely lower glucose and transient decrease in triglyceride concentration, were recorded in rats at 1000 ppm. The number of females with mammary gland tumours was increased in groups at 70, 500 and 1000 ppm, confirmed by histology peer review (Table 5). Time-to-tumour analyses are not reported.

Also seen was an increase in mammary acinar hyperplasia in males at the highest dose. Other non-neoplastic lesions increased with treatment were confined to the highest dose and included renal pelvic calculi, and prostate epithelial hyperplasia, in males; and urothelial hyperplasia in kidney and urinary bladder, degeneration of the rectus femoris muscle, and hepatic centrilobular necrosis in females. An increase in testicular interstitial cell tumours in males at 1000 ppm was not considered to be treatment-related, since the increased incidence was within the range of spontaneous occurrence and could also be attributed in part to the increased survival of the rats at 1000 ppm.

The 12- and 13-month interim kills were not considered for identification of the NOAEL, since these comprised 20 rats in the control group at low risk of tumours and would dilute the number of tumours in the controls relative to the group at the lowest dose, which had no scheduled interim kill. It is noted that at 10 ppm the incidence of mammary tumours was also higher than that in controls (63% vs 53%, excluding the interim kill), although not achieving statistical significance. This value was outside the cited range for historical controls of 40–51%. However, the range of values for historical controls (on the basis of four studies only) may be considered to be unusually narrow, a 40–60% range being considered to be more representative.

Table 5. Incidence of mammary tumours in female rats at terminal kill

Mammary tumours	Dietary concentration (ppm)				
	0	10	70	500	1000
12- & 13-month kill, including early decedents					
No. of animals examined	22	5	1	5	25
Adenocarcinoma	0	1	1	0	8 (32%)
All mammary tumours	0	1	1	1	9 (36%)
Terminal kill (including deaths occuring between 12 and 24 months)					
No. of animals examined	66	64	68	65	64
Adenocarcinoma	15 (23%)	15 (23%)	26* (38%)	27* (42%)	35** (55%)
Carcinosarcoma	0	0	0	0	2 (3.1%)
Fibroadenoma	29 (44%)	29 (45%)	35 (51%)	38 (58%)	42** (66%)
Adenoma	1 (1.5%)	0	1 (1.5%)	1 (1.5%)	2 (3.1%)
Malignant + benign tumours	35 (53%)	39 (63%)	47 (69%)	47* (72%)	56** (89%)
All rats combined (original report)					
No. of animals examined	88	69	69	70	89
Benign tumours	29 (33%)	29 (42%)	36 (52%)	39 (56%)	46** (52%)
Malignant tumours	15 (17%)	16 (23%)	27* (39%)	27** (39%)	45** (51%)
Malignant + benign tumours	35 (40%)	40 (58%)	48 (70%)	48** (69%)	65** (73%)
All rats combined (peer review)[a]					
No. of animals examined	88	69	69	70	89
Benign tumours	31 (35%)	35 (51%)	38 (55%)	62 (89%)	58 (65%)
Malignant tumours	15 (17%)	16 (23%)	32 (46%)	35 (50%)	56 (63%)

From Mayhew (1986)
* $p < 0.05$; ** $p < 0.01$.
[a] No statistical analysis reported.

The NOAEL was 70 ppm, equal to 2.6 mg/kg bw per day, in males, on the basis of decreased body-weight gain at 500 ppm and greater, and 10 ppm, equal to 0.5 mg/kg bw per day, in females, on the basis of an increased incidence of mammary tumours in females at doses of 70 ppm and greater (Mayhew, 1986).

In a long-term study of toxicity conducted in compliance with GLP and EPA guidelines, groups of 50 male and 50 female Sprague-Dawley rats were fed diets containing atrazine (purity, 97.6%) at a concentration of 0, 10, 50 and 500 ppm, equal to 0, 0.5, 2.3, and 23.6 mg/kg bw per day in males and 0.7, 3.5 and 37.6 mg/kg bw per day in females, for 52 weeks (males) or 104 weeks (females) commencing in utero. The rats were male and female offspring culled from the $F1_a$ generation of a previously conducted two-generation study of reproductive toxicity. Of the 50 males and 50 females per group, 10 males and 10 females were killed after 8 weeks and a further 10 females at 35 weeks. Of the remaining 30 females in the group at the highest dose, 10 were placed on withdrawal after 65 weeks, and 10 females in the control group were placed on diet containing atrazine at the highest dose, 500 ppm. Dietary exposure was verified by regular analysis of diets. Examinations conducted included ophthalmoscopy, blood and urine analyses, and estrous smears. Sections of pituitary were stained immunochemically for prolactin, follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

No treatment-related effect on survival occurred. Body-weight gain and food consumption were reduced at 500 ppm. Reductions in values for erythrocyte parameters late in the study and a persistent increase in serum cholesterol concentration occurred in females at 500 ppm. Histopathology investigations revealed an increased incidence of pituitary gland adenomas in females at 500 ppm (85% vs 45% in the controls) at termination (the control value appeared to be low in view of data for historical controls, presented in the report, of 80–89%). The incidence of pituitary adenoma was 89% in the controls placed on diet containing atrazine at 500 ppm at week 65, and 75% in the females at 500 ppm placed on control diet at week 65. All these incidences are reported among small numbers of animals and the significance is therefore obscure. Controls and females at the highest dose killed after 104 weeks had similar incidences of mammary tumours. A higher incidence of mammary tumours occurred in females from the control group that were placed on diet containing atrazine at 500 ppm at week 65 than occurred in rats exposed in utero or in the females at the highest dose placed on control diet at week 65. However, only 10 females per group were used in this part of the study. There was no obvious effect of treatment on the proportions of prolactin-, LH- or FSH-reactive cells in the pituitary.

Atrazine did not appear to be carcinogenic in female Sprague-Dawley rats in this study of exposure in utero that was of supportive value (because of low numbers of rats at termination). The NOAEL was 50 ppm, equal to 2.3 mg/kg bw per day in males and 3.5 mg/kg bw per day in females, on the basis of body weight and haematological effects at 500 ppm (Rudzki et al., 1991).

In a supplementary study of carcinogenicity, designed to investigate the effect of atrazine on the estrous cycle, selected hormone levels and the development of mammary, ovary, uterine and pituitary tumours, groups of 70 female Sprague-Dawley rats [Crl:CD BR] were fed diets containing atrazine (purity, 97.0%) at a concentration of 0, 70 and 400 ppm, equivalent to 0, 3.5 and 20 mg/kg bw per day, for 104 weeks. Interim terminations of 10 females per group were conducted at 1, 3, 9, 12, 15 and 18 months of the study, and all remaining survivors killed after 2 years. The terminations were conducted at proestrus as determined by vaginal smear, unless this phase was not reached within 7 days of the scheduled termination time-point. In order to avoid stress-related hormone changes, rats were killed by decapitation without anaesthesia. Samples of blood were collected post mortem for hormone analysis. The pituitary, mammary glands, uterus and ovaries from all rats were examined microscopically. The study was conducted in compliance with GLP and OECD test guidelines.

Statistical analysis indicated a significant trend toward early death at 400 ppm, since five deaths in the group at this dose occurred before the earliest death in the control group. At termination, however, there were no treatment-related differences in mortality. A decrease in body-weight gain associated with decreased food consumption was observed at 400 ppm. At necropsy, the incidences of fluid-filled uteri at 70 and 400 ppm and of mammary galactocoeles at 400 ppm were increased, particularly during the first 12 months of the study (Table 6).

At 400 ppm, the onset-time for initial palpation of masses in the mammary region (confirmed histologically as mammary fibroadenomas and/or carcinomas) was decreased when compared with the control group. Because of early onset-time, there was a significant positive trend in the incidence of mammary fibroadenomas or carcinomas analysed either separately or combined, and a significant increase using pairwise comparison for onset rate of fibroadenomas and carcinomas combined in the group at 400 ppm. The incidence of pituitary adenomas was increased at 400 ppm after 12 months of treatment, but not at the end of the study, indicating that the onset-time was shortened. No correlation between rats with mammary tumours and those with pituitary tumours was apparent.

Table 6. Selected findings from a study in rats fed diets containing atrazine for 104 weeks

Finding	Dietary concentration (ppm)		
	0	70	400
Unscheduled deaths, weeks 1–55 (No. of rats)	0	0	5
Unscheduled deaths, weeks 1–104 (No. of rats)	5	6	8
Palpable mammary masses,[a] weeks 1–54 (No. of rats)	6	3	14
Palpable mammary masses,[a] weeks 55–104 (No. of rats)	10	13	8
Fluid-filled uterus, weeks 1–54 (No. of rats/No. examined histopathologically)	4/40	10/40	14/40
Fluid-filled uterus, weeks 1–104 (No. of rats/No. examined histopathologically)	5/70	15/70	15/70
Mammary galactocoele, weeks 1–54 (No. of rats/No. examined histopathologically)	6/39	9/38	20/41
Mammary galactocoele, weeks 1–104 (No. of rats/No. examined histopathologically)	29/69	30/67	41/69
Mammary fibroadenoma, weeks 1–54 (No. of rats/No. examined histopathologically)	1/39	0/38	4/41
Mammary carcinoma, weeks 1–54 (No. of rats/No. examined histopathologically)	0/39	1/38	6/41
Mammary fibroadenoma, weeks 1–104 (No. of animals)	8/69	12/67	13/69
Mammary carcinoma, weeks 1–104 (No. of rats/No. examined histopathologically)	9/69	4/67	11/69
Pituitary adenoma, weeks 1–54 (No. of rats/No. examined histopathologically)	2/39	2/40	8/43
Pituitary adenoma, weeks 1–104 (No. of rats/No. examined histopathologically)	22/68	16/69	20/69

From Thakur (1991a)
[a] Histologically confirmed as tumours.

The NOAEL was 70 ppm, equivalent to 3.5 mg/kg bw per day, on the basis of increased mortality, decreased body-weight gain and an earlier onset of mammary and pituitary tumours at 400 ppm (Thakur, 1991a).

From the rats in the supplementary study of carcinogenicity described above (Thakur, 1991a), data on estrous cycles, vaginal cytology and selected serum hormone levels were also collected from rats killed at 1, 3, 9, 12, 15, 18 and 24 months. Results were evaluated as a function of treatment and as a function of treatment over time.

Normal age-related changes noted in rats in the control group included an increase in the number of days when high density cornified cells were evident in vaginal smears during 12 months, with a decrease in this index between 12 and 18 months. Beginning at approximately age 1 year, episodes of persistent vaginal estrus occurred, resulting in an increase in percentage of total days in estrus at the expense of days in diestrus. Serum estradiol (E2) concentrations increased between 1 and 9 months of the study, followed by decreased levels between months 9 and 18.

Atrazine-treated rats had a dose-related increase in percentage days in estrus at each interval between 9 and 18 months. Compared with rats in the control group, the cornified cell index in treated rats showed a similar increase to that in controls until 12 months, but a slower decline between 12 and 18 months. Episodes of persistent vaginal estrus were observed earlier in treated groups. Although serum concentrations of E2 increased in the control group and in the groups treated with atrazine, the magnitude of the increase was greater than that in the control group between 1 and 9 months in the group at 400 ppm. Overall, treatment with atrazine accelerated the development of typical age-related changes in female Sprague-Dawley rats. As a result, rats treated with atrazine were exposed to persistent endogenous estrogens for longer periods of time (Eldridge et al., 1993a).

From the rats in the supplementary study of carcinogenicity described above (Thakur, 1991a), the ovaries, uterus, vagina, mammary gland and pituitary were re-evaluated microscopically for specific indications of reproductive senescence, which might relate to the onset-time of hormonally-mediated mammary tumours.

The histomorphological evaluation of the reproductive organs and mammary and pituitary glands revealed no evidence of any exogenous estrogenic effect associated with atrazine. This observation was on the basis of the absence of histomorphological changes which occur with administration of exogenous estrogen. Vaginal epithelial morphology in treated and control groups was similar. There was no increased mitotic activity in the basal layer, no significant increase in cornification (an estrogenic response), no suppression of mucification, and no increase in exaggerated rete pegs in treated animals when compared with that in controls. The lack of exogenous estrogenicity was also evident in senescent rats that had progressed into the phase of extended diestrus (pseudopregnancy) when increased endogenous progesterone activity is expressed. E2 directly antagonizes progesterone and if exogenous estrogenicity associated with atrazine had been present, extended diestrus would have been suppressed or inhibited.

Ovarian histomorphology showed that anovulatory cycles (ovaries without corpora lutea) were slightly increased at 400 ppm at 3 months and were present in all rats at 9 months. A similar trend in anovulatory cycles was present in rats in the control group and at 70 ppm but was less frequent. Thus, the period between 3 and 9 months was critical in the development of irregular estrous cycles and development of the first stages of reproductive senescence. Morphological alterations in ovarian follicular and corpora lutea development and interstitial clear cells of rats at 400 ppm indicated a treatment-related interference of the LH surge and prolonged exposure of LH-responsive cells in the interstitial gland to endogenous LH. These changes, exacerbated in rats at 400 ppm between 9 and 12 months, suggested that atrazine had modulated LH secretion.

Changes in the mammary glands, characterized by increased acinar/lobular development, secretory activity with duct ectasia, and galactocoele formation, occurred as early as 3 months and were more frequent and severe at 400 ppm than at 70 ppm or in the control group for 1 year. After 1 year, the mammary changes were balanced in all groups. The mammary-gland changes in rats treated with atrazine were similar in type and degree to those observed during early and later phases of reproductive senescence in rats in the control group. They have been shown to occur by imbalances of endogenous estrogen, progesterone and prolactin.

The Meeting concluded that atrazine induced the earlier appearance of reproductive senescence in Sprage-Dawley rats by gradually interfering with ovulation and causing estrous cycles characterized by extended periods of proestrus/estrus. Although changes characteristic of reproductive senescence occurred earlier in rats at 400 ppm, the stages of senescence tended to equalize with those of the rats at 70 ppm and in the control group after 1 year of treatment (McConnell, 1995).

In a supplementary study of carcinogenicity designed to evaluate the oncogenic potential of atrazine in the ovaries, pituitary, uterus and mammary gland, groups of 60 female Sprague-Dawley rats were fed diets containing atrazine (purity, 97%) at a concentration of 0, 70 and 400 ppm, equivalent to 0, 3.5 and 20 mg/kg bw per day, for 104 weeks. At termination, all surviving rats were killed and uterus, ovary, pituitary, and mammary glands from all rats were examined microscopically for oncogenic effects. The study was conducted in compliance with GLP guidelines.

No treatment-related increases in clinical signs were noted in the study. A slight reduction in survival was found in the group at the highest dose. Body-weight gains were statistically significantly reduced relative to controls (12–13%) at 400 ppm during weeks 0–76. Non-neoplastic lesion findings were comparable in controls and treatment groups. At 400 ppm, the onset-time for palpable masses in the mammary region (confirmed histologically as mammary fibroadenomas and/or carcinomas)

was decreased when compared with the control group, although no statistically significant increase in mammary tumours was observed at the end of the study. The incidences of pituitary tumours did not indicate any larger numbers or earlier onset in the treated groups compared with the controls (Table 7).

The NOAEL was 70 ppm, equivalent to 3.5 mg/kg bw per day, on the basis of increased mortality, decreased body-weight gain and an earlier onset of mammary tumours at 400 ppm (Thakur, 1992a).

In a supplementary long-term study of toxicity conducted to determine the effects of atrazine on the mammary and pituitary glands, the estrous cycle, and plasma levels of E2, LH, progesterone and prolactin, groups of 55 female CD (Sprague-Dawley derived) rats were fed diets containing atrazine (purity, 97.1%) at a concentration of 0, 15, 30, 50, 70 and 400 ppm, equal to 0, 0.8, 1.7, 2.8, 4.1 and 23.9 mg/kg bw per day, for up to 12 months. The study was conducted in compliance with GLP and EPA guidelines. For estrous cycle determinations and plasma hormone concentration analysis, interim kills of 10 rats per group were conducted after 3, 6 and 9 months of treatment. Fifteen rats per group were sampled continuously throughout the study, i.e. at 3, 6, 9 and 12 months and subsequently killed for pathology evaluation at 12 months. Survivors of the remaining 10 females per group were killed after 12 months, following estrous cycle determinations and blood sample collection.

Table 7. Summary of selected findings of a study of carcinogenicity in rats fed diets containing atrazine for 104 weeks

Finding	Dietary concentration (ppm)		
	0	70	400
Mortality (No. of rats)	29	35	38*
Palpable mammary masses,[a] weeks 1–52 (No. of rats)	2	3	9*
Palpable mammary masses,[a] weeks 53–104 (No. of rats)	44	31	40
Mammary tumours (No. of rats/No. examined histopathologically)	46/60	34/59	49/60
Pituitary tumours (No. of rats/No. examined histopathologically)	44/58	46/58	47/60
Pituitary and mammary tumours (No. of animals)	34	26	39

From Thakur (1992a)
[a] Histologically confirmed as tumours.
* $p < 0.05$.

Table 8. Incidence of mammary gland tumours in groups of 55 rats fed diets containing atrazine for 12 months

Mammary gland tumour	Dietary concentration (ppm)					
	0	15	30	50	70	400
Adenocarcinoma	1	2	0	1	1	6
Adenoma	0	0	1	0	1	1
Fibroadenoma	2	2	2	1	4	4
Mammary tumours, combined	3	4	3	2	6	10*

From Pettersen & Turnier (1995)
* $p < 0.05$.

There were no effects on clinical signs, survival or organ weights. A slight retardation of body-weight gain (82–89% of controls) and feed consumption (88–95% of controls) were seen at the highest dose. The results of plasma hormone analyses were not reported. There were no statistically significant differences in the incidence of mammary gland adenomas, fibroadenomas or adenocarcinomas at up to 400 ppm. When the incidences of mammary adenomas, fibroadenomas and adenocarcinomas were combined for analysis, a statistically significant increase in the incidence of rats with mammary tumors was observed at 400 ppm (Table 8). There were no significant trends or group differences for onset-time of mammary gland adenomas, fibroadenomas or adenocarcinomas. However, a significant positive trend for onset-time existed when mammary gland adenomas and fibroadenomas were combined or when adenomas, fibroadenomas and adenocarcinomas were combined. No trend was evident when the group at the highest dose (400 ppm) was excluded from the analysis.

The NOAEL for incidence of mammary tumours and onset-time was 70 ppm, equal to 4.1 mg/kg bw per day (Pettersen & Turnier, 1995).

In a supplementary study of carcinogenicity designed to determine the incidence and onset of mammary tumours, groups of 80 ovariectomized or intact female Sprague-Dawley Crl:CD BR rats were fed diets containing atrazine (purity, 97.1%) at a concentration of 0, 25, 50, 70 or 400 ppm, equal to 0, 1.2, 2.5, 3.5 and 20.9 mg/kg bw per day in ovariectomized females and 0, 1.5, 3.1, 4.2 and 24.4 mg/kg bw per day in intact females, respectively. After 52 weeks of treatment, 20 rats in each group were killed and necropsied, and the surviving rats were killed after 104 weeks of treatment. Daily examinations for mortality, morbidity, and indications of toxic effects were performed. There were no clinical pathology investigations. Palpation for tissue masses in rats was performed before the initiation of dosing and weekly thereafter. Necropsies and complete histological examinations were performed on all rats. The study was conducted in compliance with GLP guidelines.

Survival and body-weight gain of ovariectomized rats was markedly better than that of intact rats, and there was no dose-related effect on survival among ovariectomized females. However, there was significantly impaired survival among intact females at the highest dose. Body-weight gain at the highest dose was impaired in intact and in ovariectomized rats, demonstrating a maximum tolerated dose (MTD) to be achieved.

In the ovariectomized rats, there were no treatment-related increases in mammary-gland proliferative changes and mammary tumours were not present in any of the rats. The lack of mammary tumours in ovariectomized rats provides evidence that the mode of action of atrazine is neither a direct genotoxic nor an estrogenic effect on the mammary gland. Rather, an indirect hormonally-mediated effect involving the ovary is implied.

In sexually intact rats, the incidence of palpable masses and of histological mammary neoplasia was higher than in ovariectomized females, and a statistically significant increased incidence of mammary neoplasia was seen at doses of 50 ppm and greater compared with the concurrent control group. When compared with the data for historical controls from the same laboratory (on the basis of 14 studies), only in the group at 400 ppm did incidences exceed the pooled incidence values for either fibroadenoma or carcinoma. An earlier onset and increased incidence of mammary carcinoma were observed only in the intact rats at 400 ppm (Table 9). The study authors therefore concluded that increases in mammary tumours at 50 and 70 ppm did not represent part of a carcinogenic dose–response trend.

However, the comparison with data for historical controls considers carcinoma and fibroadenoma in isolation but not as a combined analysis (i.e. all rats bearing mammary tumours). Also, the spacing of doses between the two intermediate doses (50 and 70 ppm) is small in terms of normal design for studies of carcinogenicity, and is possibly too small to prudently allow for dose–response differentiation. Were the groups at 50 and 70 ppm to be combined, this would result in a statistically significantly increased incidence of tumours, which would be part of a clear trend.

Table 9. Selected findings of a study of carcinogenicity in rats fed diets containing atrazine

Finding	Dietary concentration (ppm)				
	0	25	50	70	400
Intact females					
Body weight (g), week 52	412	428	414	419	390*
Survival, week 104	26/60	19/60	17/59	19/60	13*/60
No. of rats with palpable masses	29/80	34/80	43/80	39/80	46/80
Palpable masses; week of first observation	29	28	38	32	14
Mammary tumours, weeks 1–53:					
Fibroadenomas	0/22	2/22	2/23	2/23	1/25
Carcinomas	2/22	2/22	0/23	2/23	6/25
Total No. with mammary neoplasia	2/22	3/22	2/23	4/23	6/25
Mammary tumours, weeks 1–104:					
Fibroadenomas	16/80 (20%)	25/80 (31%)	33/78** (42%)	29/80* (36%)	25/80* (31%)
Adenomas	0/80	0/80	1/78 (1.3%)	0/80	0/80
Carcinomas	12/80 (15%)	18/80 (23%)	20/78 (26%)	14/80 (18%)	27/80** (34%)
Adenomas/carcinomas	12/80 (15%)	18/80 (23%)	21/78* (27%)	14/80 (18%)	27/80** (34%)
Total No. with mammary neoplasia	24/80 (30%)	34/80 (43%)	44/78** (56%)	38/80* (48%)	43/80** (54%)
Ovariectomized females					
Body weight (g), week 52	523	528	524	526	479*
Survival, week 104	44/60	42/60	46/59	45/60	44/60
No. of rats with palpable masses	3/80	2/80	2/80	2/80	6/80
Palpable masses; week of first observation	35	87	75	91	56
Total No. with mammary neoplasia	0/64	0/66	0/70	0/71	0/72

From Morseth (1996d, 1998)
* $p < 0.05$; ** $p < 0.01$.
Data for historical controls from 14 studies: adenomas, 1.3% (0–5.6%); carcinomas, 21.5% (6.0–32.8%); fibroadenomas, 47.8% (35.3–65.0%).

The NOAEL for mammary carcinogenicity was 25 ppm, equal to 1.5 mg/kg bw per day, on the basis of a statistically significant increase in mammary neoplasia in intact female rats at 50 ppm, equal to 3.1 mg/kg bw per day, and greater compared with the concurrent control group. The carcinogenic effect of atrazine was abolished in ovariectomized rats (Morseth, 1996d, 1998).

In a study of carcinogenicity reported in a published paper, groups of 53–56 male and 50–55 female Fischer 344 rats received diets containing atrazine (purity, 98.9%) for the lifespan of most of the rats (126 weeks in males, 123 weeks in females). Dietary concentrations of 500 and 1000 ppm were used at commencement and were reduced to 350 and 750 ppm after 8 weeks because of excessive retardation in body-weight gain. Histological examination was conducted on a full spectrum of tissues, except that in decedents, results were only considered if a satisfactory evaluation was possible. There was no clinical pathology, diet analysis or evidence of GLP quality-assurance auditing, and the level of detail presented in the paper would be inadequate, in isolation, for regulatory submission.

Body-weight gain was retarded by treatment in both groups of treated males and females. This however appeared to be dose-related only in females, and the growth curves of females were otherwise abnormal in that suppression of normal weight gain (unremarked and cause not obvious) would appear to have occurred between weeks 10 and 60. Water consumption was increased before the reduction in dose but was normal subsequently. The rate of mortality in both groups of treated males was less than in controls, hence more treated animals survived for longer than did controls. Because of the lifetime design of the study, a large number of the rats died during the study, with potential tissue loss to autolysis tending to be more in males in the control group due to the altered survival rate. Statistically significantly increased incidences were reported for benign mammary tumours (fibroma, fibroadenoma and adenoma) in males at the highest dose (1 out of 48, 1 out of 51 and 9 out of 53 at 0, 375 and 750 ppm, respectively), for uterine adenocarcinomas in females at the highest dose (6 out of 45, 8 out of 52 and 13 out of 45 at 0, 375 and 750 ppm, respectively), and for combined leukaemias and lymphomas in females at the highest dose (12 out of 44, 16 out of 52 and 22 out of 51 at 0, 375 and 750 ppm, respectively). However, clear interpretation of these results was compromised by the increased survival at the highest dose (also confirmed for females by additional analysis).

Although this study is inadequate to permit conclusions on the carcinogenicity of atrazine due to significant flaws in design and reporting, the study authors stated that the results are suggestive evidence for tumorigenic activity of atrazine in Fischer 344 rats (Pintér et al., 1990).

To conclude, the increased incidence of combined leukaemias and lymphomas is not indicative of a carcinogenic effect as the combining of these tumour types is inappropriate and neither tumour type displayed a significantly increased incidence when evaluated alone. Also, an additionally conducted survival-adjusted analysis of tumour prevalence did not indicate any treatment-related statistically significant differences in the incidence of benign, malignant nor combined mammary tumours for either males or females (Liu & Thakur, 1999).

In a supplementary study of carcinogenicity designed to investigate the effect of atrazine on the estrous cycle, selected hormone concentrations and the development of mammary, ovary, uterine and pituitary tumours, groups of 70 female Fischer 344 rats, CDF(F344)/CrlBR, were fed diets containing atrazine (purity, 97.0%) at a concentration of 0, 10, 70, 200 and 400 ppm, equivalent to 0, 0.5, 3.5, 10 and 20 mg/kg bw per day, for 104 weeks. Interim kills of 10 females per group were conducted at 1, 3, 9, 12, 15 and 18 months of the study, and all remaining survivors killed after 2 years. The terminations were conducted at proestrus as determined by vaginal smear, unless this phase was not reached within 7 days of the scheduled termination time-point. In order to avoid stress-related hormone changes, the rats were killed by decapitation without anaesthesia. Samples of blood were collected post mortem for hormone analysis. The pituitary, mammary glands, uterus and ovaries from all animals were examined microscopically. The study was conducted in compliance with GLP and OECD test guidelines.

There were no treatment-related effects on survival or on the incidence of clinical observations. A dose-related and statistically significant decrease in mean body-weight gain was observed at 200 ppm (3–10%) and 400 ppm (10–15%) at several time-points. There was no increase in mammary tumours or any other type of tumour at any dose. The NOAEL was 70 ppm, equivalent to 3.5 mg/kg bw per day, on the basis of decreased body-weight gain at 200 ppm and greater (Thakur, 1991b).

From the study described above (Thakur, 1991b), data on estrous cycles, vaginal cytology and selected serum hormone concentrations were also collected from rats killed at 1, 3, 9, 12, 15, 18 and 24 months. Results were evaluated as a function of treatment and as a function of treatment over time.

There were normal age-related changes in rats in the control group and in rats treated with atrazine, which included a shift in estrous cycle patterns toward an enhancement of percent of total days in

proestrus at the expense of days in estrus. In rats in the control group as well as treated rats, E2 concentrations increased during the first 9 months of the study and decreased thereafter. By contrast, progesterone concentrations rose in a much more prolonged pattern, with the highest mean for each group always ocurring at 15 or 18 months. There were no consistent treatment-related changes in serum hormone concentrations compared with controls for any of the hormones tested, nor were there differences from control age-related patterns in animals fed up to 400 ppm atrazine. The normally occurring senescence of reproductive cycling parameters was not affected by treatment (Eldridge et al., 1993b).

From rats in the study described above (Thakur, 1991b), the ovaries, uterus, vagina, mammary gland and pituitary were re-evaluated microscopically for specific indications of reproductive senescence, which might relate to the onset-time of hormonally-mediated mammary tumours.

Ovarian histomorphology showed that the great majority of rats in all groups, the control group and dosed groups, maintained corpora lutea throughout most of the study. Only at the final, 24-month, time-point were there dramatic decreases in corpora lutea numbers. At this time-point, the rats treated with atrazine did not show decreases in corpora lutea numbers that were any more severe than those observed in rats in the control group. The reduction in numbers of corpora lutea at this late time-point appeared to be a consequence of a natural progression of the rats from persistent diestrus into acyclicity. All rats in all groups receiving atrazine maintained moderate numbers of secondary, antral and atretic follicles throughout the study, including at the 24-month time-point. The data on ovarian histomorphology indicated that treatment with atrazine did not alter the number of rats in repetitive pseudopregnancy/persistent diestrus. Estrous cycling in F344 females treated with atrazine was not altered.

Mammary gland histomorphology showed that rats in all groups receiving atrazine displayed histomorphological alterations in the mammary gland that would be expected in normally ageing F344 female rats, i.e. there was some evidence of lobular/acinar development with secretory activity and occasional galactocoeles in all groups receiving atrazine at 15, 18 and 24 months.

The histomorphological alterations in the mammary gland that were seen are those that would be expected in normally ageing F344 female rats. Exposure to atrazine did not increase the severity of any histomorphological findings in the mammary gland or decrease their time of onset (McConnell, 1995).

In a study of carcinogenicity , which complied with GLP and EPA guidelines, groups of 60 male and 60 female Fischer 344 rats, Crl:CDF (F344), were fed diets containing atrazine (purity, 97.0%) at a concentration of 0, 10, 70, 200 and 400 ppm, equivalent to 0, 0.5, 3.5, 10 and 20 mg/kg bw per day, for 104 weeks.

Administration of atrazine did not affect survival nor were any clinical signs apparent. Body weight and body-weight gain were significantly reduced in males and females at 200 and 400 ppm throughout most of the study. Food consumption in the group of males at the highest dose was significantly reduced throughout the study, and significantly reduced in females at the highest dose for the first 13 weeks of exposure. Histopathologically, the incidence of pituitary tumours in the group at the highest dose was slightly lower in both sexes than that of the controls. The incidence of mammary tumours among females increased slightly with dose (7, 8, 12, 17 and 13% at 0, 10, 70, 200 and 400 ppm), but these values were within the range for historical controls and did not attain statistical significance. There were no other histological changes considered to be related to treatment. Atrazine was not carcinogenic in Fischer 344 rats in an adequately-conducted study. The NOAEL was 70 ppm, equivalent to 3.5 mg/kg bw per day, on the basis of decreased body-weight gain at 200 ppm and greater (Thakur, 1992b).

2.4 Genotoxicity

Atrazine was tested for possible mutagenic potential in nearly 100 tests conducted either by the sponsor or independently in external laboratories. These studies covered different end-points in both eukaryotes and prokaryotes in vivo and/or in vitro. A summary of selected studies conducted in vitro is presented in Table 10, and selected studies conducted in vivo in Table 11.

Brusick (1994) had reviewed a large number of reports and publications (non-plant studies) from the years 1977 to 1992 on the genetic toxicity of atrazine, the results of which were positive in six cases, using two approaches. One was the "expert judgement" in which conflicting results were resolved by thoroughly reviewing each study and critically assessing the detailed data. The second approach used a computer-assisted "weight-of-the-evidence" method. The conclusions reached about the genotoxicity of atrazine were "equivocal" using the first method and "negative" using the second. The weight-of-the-evidence (computer) model, which was originally developed by the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC), was considered by Brusick to be more relevant for assessing atrazine using such a large database. The author concluded also that the positive responses reported for atrazine in plant systems cannot be assumed to be relevant to effects in mammals, especially when differences in metabolite profiles between plants and mammals are known to exist.

In a later review of the genetic toxicity of atrazine by Brusick (2000), data from 21 additional publications from the years 1993 to 2000 were evaluated and summarized. These studies included both standard and non-standard assays. Of the 17 studies using conventional assays for genetic toxicology, 12 were reported as giving negative results while five gave positive results. On the basis of a critical assessment of these four in-vitro assays and one in-vivo assay reported in four papers (Guigas et al., 1993; Della Croce et al., 1996; Gebel et al., 1997; Lioi et al., 1998), it was evident that all had serious deficiencies and the positive responses were attributed to inappropriate study design or technique. Taking these limitations into consideration, the weight of evidence indicates that atrazine is not genotoxic.

Additional studies using novel, but not-yet-validated techniques including single-cell gel electrophoresis (Comet assay), flow cytometry, and differential gene expression, have reported positive findings. These techniques must be carefully evaluated, validated and confirmed by independent investigators before they can be used in decision-making regarding the genotoxic potential of any chemical including atrazine (Brusick, 2000).

2.5 Reproductive toxicity

(a) Multigeneration studies

In a two-generation study of reproductive toxicity , which complied with GLP and US EPA test guidelines, groups of 30 male and 30 female Sprague-Dawley rats were fed diets containing atrazine technical (purity, 97.6%) at a concentration of 0, 10, 50 and 500 ppm, for 10 weeks before mating and further during mating, gestation and rearing of offspring. Thirty male and 30 female F_1 pups from each group were selected and after 12 weeks exposure to the treated diets were mated to derive the F_2 generation. F_2 pups were killed 21 days after birth. Histology of reproductive organs and tissues was conducted from 30 rats of each sex from the control groups and groups at the highest dose of the F_0 and F_1, and five of each sex of the control group and group at the highest dose for the F_2 generations. The mean daily intakes at 0, 10, 50 and 500 ppm in the period before mating were 0, 0.7, 3.6 and 36.1 mg/kg bw per day for F_0 males and 0, 0.8, 4.0 and 40.7 mg/kg bw per day for F_0 females,

Table 10. Selected studies of genotoxicity with atrazine in vitro

End-point	Test system	Concentration or dose	Purity (%)	Results	References
Gene mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	50–10 000 µg/plate	98.8	Negative	Sutou et al. (1979)
Gene mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	10–810 µg/plate	NR	Negative	Arni (1978)
Gene mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	20–5 000 µg/plate	98.2	Negative	Deparade (1986)
Gene mutation	*S. typhimurium* TA97, TA98, TA100	2–2 157 µg/plate	> 99	Negative	Butler & Hoagland (1989)
Gene mutation	*S. typhimurium* TA98, TA100	0.86 mg/ml	NR	Negative	De Veer et al. (1994)
Gene mutation	*S. typhimurium* TA98, TA100	10–1 000 µg/plate	42.7[a]	Negative	Della Croce et al. (1996)
Gene mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	1–1 000 µg/plate	98.9	Negative	Ruiz & Marzin (1997)
Gene mutation	*E. coli* B/r WP2 Tyr⁻ Hcr⁻	50–5 000 µg/plate	98.8	Negative	Sutou et al. (1979)
Gene mutation	Chinese hamster V79 cells; HGPRT	1.25–10 mmol/l	NR	Negative	Adler (1980)
Gene mutation	CHO cells; HGPRT	0.023–0.23 µg/ml	NR	Positive	Guigas et al. (1993)
Chromosomal aberration	CHO cells	1.25–10 mmol/l	NR	Negative	Adler (1980)
Chromosomal aberration	Human lymphocytes	0.01–1 µg/ml	NR	Positive	Meisner et al. (1992)
Chromosomal aberration	Human lymphocytes	5–51 µmol/l	≥ 98	Positive	Lioi et al. (1998)
Chromosomal aberration	Human lymphocytes	5–100 µg/ml	98.4	Negative	Ribas et al. (1998)
Chromosomal aberration	Human lymphocytes	0.5–50 µg/ml	97.7	Negative	Kligerman et al. (2000a)
Micronucleus formation	Human lymphocytes	1–100 µg/ml	98.4	Negative	Surrallés et al. (1995)
Micronucleus formation	Human lymphocytes	5–200 µg/ml	98.4	Negative	Ribas et al. (1998)
DNA damage and repair (Rec assay)	*B. subtilis* H17/M45	100–10 000 µg/well	98.8	Negative	Sutou et al. (1979)
SOS chromotest	*E. coli* PQ37	NR	98.9	Negative	Ruiz & Marzin. (1997)
UDS	Rat primary hepatocytes	1.2–150 µg/ml	98.2	Negative	Puri (1984a)
UDS	Human fibroblasts CRL 1121	1.2–150 µg/ml	98.2	Negative	Puri (1984b)

End-point	Test system	Concentration or dose	Purity (%)	Results	References
UDS	Rat primary hepatocytes	15.5–1 670 µg/ml	97.1	Negative	Hertner (1992)
SCE	CHO cells	1.25–10 mmol/l	NR	Negative	Adler (1980)
SCE	CHO cells; HGPRT	0.023–0.23 µg/ml	NR	Negative	Guigas et al. (1993)
SCE	Chinese hamster V79 cells	0.86 mg/ml	NR	Negative	De Veer et al. (1994)
SCE	Human lymphocytes	5–50 µmol/l	98.7	Negative	Dunkelberg et al. (1994)
SCE	Human lymphocytes	5–51 µmol/l	≥ 98	Positive	Lioi et al. (1998)
SCE	Human lymphocytes	5–200 µg/ml	98.4	Negative	Ribas et al. (1998)
SCE	Human lymphocytes	0.5–50 µg/ml	97.7	Negative	Kligerman et al. (2000a)
Comet assay	Human lymphocytes	50–200 µg/ml –S9	98.4	Positive	Ribas et al. (1995)
		50–200 µg/ml +S9		Negative	
Micronucleus formation[b]	Human lymphocytes	1–100 µg/ml	98.4	Negative	Surrallés et al. (1995)
Mitotic gene conversion	*S. cerevisiae* D7	150–350 mmol/l (stationary phase)	42.7[a]	Negative	Della Croce et al. (1996)
		1–10 mmol/l (log phase)		Positive	

NR, not reported; SCE, sister-chromatid exchange; UDS, unscheduled DNA synthesis.
[a] Formulation tested.
[b] Used as biomarker of excision repair.

Table 11. Selected studies of genotoxicity with atrazine in vivo

End-point	Test object	Concentration or dose	Purity (%)	Results	References
Somatic cells					
Chromosomal aberration	Chinese hamster bone marrow	500 mg/kg bw; oral, single dose	NR	Negative	Basler & Röhrborn (1978)
Micronucleus formation	Mouse bone marrow	2000 mg/kg bw; oral, single dose	NR	Negative	Ehling (1979)
Chromosomal aberration	Mouse bone marrow	1500, 2000 mg/kg bw; oral, single dose	NR	Positive	Adler (1980)
Nucleus anomaly test	Chinese hamster bone marrow	282, 564, 1128 mg/kg bw per day; oral, on two consecutive days	NR	Negative	Hool (1981a)
Chromosomal aberration	Mouse bone marrow	6 mg/kg, intraperitoneal, single dose	NR	Negative	Chollet et al. (1982)
		1 ppm (drinking-water) for 7 weeks			
Chromosomal aberration	Mouse bone marrow	562.5, 1125, 2250 mg/kg bw; oral, single dose	98.2	Negative	Ceresa (1988b)
Chromosomal aberration	Mouse bone marrow	20 ppm (drinking-water) over 30 or 90 days	NR	Negative	Meisner et al. (1992)

End-point	Test object	Concentration or dose	Purity (%)	Results	References
Chromosomal aberration	Mouse bone marrow	Males: 900–1750 mg/kg bw; Females: 1400, 1750 mg/kg bw; oral, single dose	98.7	Negative in males Positive in females	Gebel et al. (1997)
Chromosomal aberration	Mouse bone marrow	125, 250, 500 mg/kg bw; intraperitoneal, two injections 24 h apart	97.7	Negative	Kligerman et al. (2000b)
DNA damage	Rat stomach, liver and kidney	875 mg/kg bw; oral, single dose 350 mg/kg bw per day; oral, 5 or 15 days	NR	Positive	Pino et al. (1988)
DNA damage	Rat lung	875 mg/kg bw; oral, single dose 350 mg/kg bw per day; oral, 5 or 15 days	NR	Negative	Pino et al. (1988)
DNA damage	Mouse blood leukocytes	125, 250, 500 mg/kg bw; intraperitoneal	97.7	Equivocal	Tennant et al. (2001)
Germ cells					
Chromosomal aberration	Mouse spermatogonia	444, 1332 mg/kg bw per day; oral, on five consecutive days	NR	Negative	Hool (1981b)
Chromosomal aberration	Mouse spermatogonia	6 mg/kg bw, intraperitoneal, single dose 1 ppm (drinking-water) for 7 weeks	NR	Negative	Chollet et al. (1982)
Chromosomal aberrations	Mouse spermatocytes	444, 1332 mg/kg bw; oral, five doses over 10 days	NR	Negative	Hool (1981c)
Dominant lethal mutation	Mouse spermiogenesis	1500, 2000 mg/kg bw; oral, single dose	NR	Positive	Adler (1980)
Dominant lethal mutation	Mouse spermiogenesis	444, 1332 mg/kg bw; oral, single dose	98.9	Negative	Hool (1981d)
Dominant lethal mutation	Mouse spermiogenesis	6 mg/kg, intraperitoneal, single dose	NR	Negative	Chollet et al. (1982)
Dominant lethal mutation	Mouse spermiogenesis	500, 1000, 2000, 2400 mg/kg bw; oral, single dose	97.1	Negative	Hertner (1993)
Sperm head morphology	Mouse sperm	600 mg/kg bw per day; intraperitoneal, on five consecutive days	97.2	Negative	Osterloh et al. (1983)

NR, not reported.

respectively, and 0, 0.7, 3.5 and 35.0 mg/kg bw per day for F_1 males and 0, 0.8, 3.8 and 37.5 mg/kg bw per day for F_1 females, respectively.

No treatment-related mortality or signs of toxicity were observed during the study. Parental body weights, body-weight gain, and food consumption were statistically significantly reduced at 500 ppm (the highest dose tested) in both sexes and both generations throughout the study. Compared with controls, body weights for F_0 males and females at the highest dose at 70 days into the study were decreased by 12% and 15%, respectively, while body weight of the F_1 generation for the same period was decreased by 15% and 13% for males and females, respectively.

Litter parameters, including litter size, sex ratio, and postnatal survival indices, of the F_1 and F_2 generations were not affected by treatment with atrazine at any dose. There were no clinical symptoms, necropsy findings or malformations that were considered to be related to treatment. Slightly lower body weights (8–10%) were noted in both generations of male pups at 500 ppm on postnatal day 21.

No treatment-related pathological or micropathological findings were noted in any of the reproductive organs of F_0 or F_1 generations. Relative testes weights were increased in F_1 and F_2 males at 500 ppm as a result of decreased terminal body weights.

The NOAEL for parental toxicity was 50 ppm, equal to 3.6 and 4.0 mg/kg bw per day in males and females, respectively, on the basis of decreased body-weight gains and food consumption at 500 ppm. The NOAEL for reproductive toxicity was 50 ppm on the basis of decreased body weights of male pups at 500 ppm on postnatal day 21 (Mainiero et al., 1987).

(b) Studies of developmental toxicity

Rats

In a study of prenatal developmental toxicity, which complied with GLP and US EPA test guidelines, groups of 27 pregnant female Crl:COBS CD(SD)BR rats were given atrazine (purity, 96.7%; suspended in 3% corn starch with 0.5% Tween 80) at a dose of 0, 10, 70 and 700 mg/kg bw per day by oral gavage on days 6 to 15 of gestation.

A total of 21 dams treated at 700 mg/kg bw per day died between days 13 and 20. All other dams survived the study. Treatment-related clinical signs were confined to the group at 700 mg/kg bw per day and included salivation, oral/nasal discharge, ptosis, swollen abdomen and blood on the vulva. Necropsy findings included enlargement of the stomach. A marked reduction in food intake was recorded for the group at 700 mg/kg bw per day and was associated with a marked loss of maternal body weight. In the group at 70 mg/kg bw per day, significant reductions of food consumption and body-weight gain were reported at days 6–7 or 6–10 of gestation, respectively.

Fetal toxicity, attributed to maternal toxicity, was observed in the groups at 70 and 700 mg/kg bw per day and manifested as a marked reduction of fetal weight at 700 mg/kg bw per day (no skeletal examination was performed in this group). A marginal increase in numbers of runted pups at all doses was within the range of data for historical controls. Skeletal variations in the group at 70 mg/kg bw per day included incomplete ossification of skull, teeth, metacarpals and hindpaw distal phalanges, and bipartite metacarpals. These findings can be considered to be developmental delays attributable to maternal toxicity, although it should be noted that maternal toxicity at 70 mg/kg bw per day was minimal and fetal weights were not different to those of the controls (Table 12).

There was no evidence of teratogenicity.

The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of decreased body-weight gain and food intake at 70 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 10 mg/kg bw per day on the basis of incomplete ossification at several sites at 70 mg/kg bw per day and greater (Infurna, 1984).

In a study of prenatal developmental toxicity, which complied with GLP and US EPA test guidelines, groups of 26 pregnant female Crl:COBS CD(SD)BR rats were given atrazine (purity, 97.6%; suspended in 3% corn starch with 0.5% Tween 80) at a dose of 0, 5, 25 and 100 mg/kg bw per day by oral gavage on days 6 to 15 of gestation.

One female at the highest dose died without obvious cause on day 20 of gestation. At the highest dose, an increased incidence of salivation was seen. Also at the highest dose, food consumption

Table 12. Relevant findings in a study of developmental toxicity in rats given atrazine by gavage

Finding	Dose (mg/kg bw per day)			
	0	10	70	700
Salivation (No. of rats with finding/No. of rats in group)	0/27	0/27	1/27	13/27**
Ptosis (No. of rats with finding/No. of rats in group)	0/27	0/27	1/27	11/27**
Abdominal area swollen (No. of rats with finding/ No. of rats in group)	0/27	0/27	1/27	8/27**
Blood on vulva (No. of rats with finding/No. of rats in group)	0/27	0/27	1/27	11/27**
Death (No. of rats with finding/No. of rats in group)	0/27	0/27	1/27	21/27**
Food consumption (g):				
Day 6	23	23	17**	13**
Day 7	23	22	20**	13**
Body-weight gain (g):				
Days 6–10	13	16*	6**	–1**
Days 0–20	61	59	53	–25**
No. of pregnant rats	24	23	25	26
No. of litters examined	23	23	25	5
Mean No. of resorptions	0.8	0.9	0.9	1.3
Postimplantation loss (%)	9.7	5.9	6.1	20.9
Mean No.of live fetuses	12.7	13.7	14.0	13.4
Mean fetal weights (g), males/females	3.4/3.3	3.6/3.3	3.4/3.2	1.9*/1.8*
Skeletal examinations (fetuses/litters)	203/23	217/23	244/25	—[a]
Skull not completely ossified (fetuses/litters)	23/9	7/4	47**/16**	—
Hyoid not ossified (fetuses/litters)	20/10	9/5	44**/12*	—
Teeth not ossified (fetuses/litters)	2/1	4/4	34**/10**	—
Forepaw:				
Metacarpals not ossified (fetuses/litters)	117/19	106/20	184*/23*	—
Metacarpals bipartite (fetuses/litters)	0	0	3*/3*	—
Hindpaw: distal phalanx not ossified (fetuses/litters)	0	0	6*/3*	—

From Infurna (1984)

[a] No skeletal examination was performed due to extremely reduced fetal weights and subsequent reduced ossification.

* $p < 0.05$; ** $p < 0.01$.

was significantly reduced on days 6–8 and 8–12 of gestation, while body weight was significantly reduced on days 8 to 16 of gestation. Corrected body-weight gain for the entire gestation period was reduced by 20%.

There were no treatment-related effects on any reproductive parameter examined, on fetal sex ratio, mean fetal weights or the incidences of gross, visceral and skeletal malformations. A significantly increased incidence of incomplete ossification of various skull bones (hyoid, interparietal, occipital and parietal bones) was observed at the highest dose. A significantly increased incidence of incomplete ossification of the interparietals was also observed in the groups at the intermediate and lowest dose, but there was no dose–response relationship when evaluated by litters (Table 13).

There was no evidence of teratogenicity.

The NOAEL for maternal toxicity was 25 mg/kg bw per day on the basis of decreased body-weight gain and food intake at 100 mg/kg bw per day. The NOAEL for developmental toxicity was

Table 13. Relevant findings of a study of developmental toxicity in rats given atrazine by gavage

Finding	Dose (mg/kg bw per day)			
	0	5	25	100
Salivation (No. of rats with finding/No of rats in group)	0/26	0/26	0/26	18/26
Food consumption (g):				
Days 6–8	24.40	23.52	23.02	16.98*
Days 8–12	25.13	24.18	23.73	21.89*
Body-weight gain (g):				
Days 6–8	7.54	6.92	6.48	–3.91*
Days 6–16	57.23	55.88	56.36	46.95*
No. of pregnant rats	26	25	25	22
No. of litters examined	26	25	24	21
Mean No. of resorptions	0.58	0.80	0.50	0.67
Postimplantation loss (%)	4.09	5.52	3.40	4.47
Mean No. of live fetuses	13.42	13.80	14.46	15.43*
Mean fetal weights (g), males/females	3.52/3.31	3.63/3.41	3.56/3.42	3.49/3.32
Hyoid not completely ossified (fetuses/litters)	20/10	26/9	27/13	36*/15*
Interparietal not completely ossified (fetuses/litters)	16/10	42*/15*	43*/14*	73*/20*
Occipitals not completely ossified (fetuses/litters)	14/10	26/13	22/10	35*/16*
Parietals not completely ossified (fetuses/litters)	4/3	9/6	7/3	14*/9*

From Giknis (1989)
* $p < 0.05$.

25 mg/kg bw per day on the basis of incomplete ossification of skull bones at 100 mg/kg bw per day (Giknis, 1989).

Rabbits

In a study of prenatal developmental toxicity, which complied with GLP and US EPA test guidelines, groups of 19 inseminated female New Zealand White rabbits were given atrazine (purity, 96.3%; suspended in 3% corn starch with 0.5% Tween 80) at a dose of 0, 1, 5 or 75 mg/kg bw per day by oral gavage on days 7 to 19 of gestation.

Three females at the lowest dose died during the study; one, possibly two, being the result of misdosing; one doe at 5 mg/kg bw per day and two does at 75 mg/kg bw per day were killed because they were aborting. At 75 mg/kg bw per day, all dams exhibited stool changes (little, none, and/or soft), and showed marked reductions in food consumption and body-weight loss, with rebound after cessation of dosing. The slight reductions in food intake and body-weight gain at 1 and 5 mg/kg bw per day were not considered to be adverse.

Embryotoxicity was evident at 75 mg/kg bw per day and included increased numbers of resorptions and postimplantation losses, and thus decreased numbers of viable fetuses (Table 14). Fetotoxicity was also observed at 75 mg/kg bw per day and included lower fetal weights and delayed ossification of forelimb metacarpals and middle phalanges, and hindlimb patellae, tali and middle phalanges. Treatment-related malformations were not observed.

There was no evidence of teratogenicity.

The NOAEL for maternal toxicity was 5 mg/kg bw per day on the basis of clinical signs, abortion, and decreased food intake and body-weight gain at 75 mg/kg bw per day. The NOAEL

Table 14. Relevant findings in a study of developmental toxicity in rabbits given atrazine by gavage

Finding	Dose (mg/kg bw per day)			
	0	1	5	75
Little, none and/or soft stool (No. of rabbits with finding/ No. of rabbits in group)	9/19	4/19	10/19	19/19**
Blood on vulva (No. of rabbits with finding/No. of rabbits in group)	0/19	1/19	0/19	4/19*
Abortion (No. of rabbits with finding/No. of rabbits in group)	0/19	(1)/19[a]	1/19	2/19
Death (No. of rabbits with finding/No. of rabbits in group)	0/19	3/19	0/19	0/19
Food consumption (g):				
Day 7	183	164	185	76**
Day 13	182	155*	163	1**
Day 17	182	157	134*	6**
Day 19	164	136	129*	14**
Body-weight change (g):	113	76	63	–522**
Days 7–14				
Days 14–19	120	105	45*	–204**
Days 0–29[b]	–73	–113	–143	–393**
No. of pregnant rabbits	16	17	16	18
No. of litters examined	16	14	15	15
Mean No. of resorptions	1.3	1.4	1.4	4.8**
Postimplantation loss (%)	12.0	11.4	13.0	42.6**
Mean No. live fetuses	8.8	8.9	9.1	5.9*
Mean fetal weights (g), males/females	46.1/44.0	44.0/43.3	43.2/43.1	35.7*/35.8*
Forepaw:				
Metacarpal not ossified (fetuses/litters)	1/1	0	1/1	7*/5*
Proximal phalanx not ossified (fetuses/litters)	0	0	1/1	14**/7**
Hindpaw:				
Patella not ossified (fetuses/litters)	5/5	9/4	12/5	35**/10**
Talus not ossified (fetuses/litters)	0	0	0	10**/5**
Middle phalanx not ossified (fetuses/litters)	0	0	2/1	4**/4**

From Arthur (1984)

[a] One dam found dead was possibly aborting.

[b] Terminal body weight minus uterus, placentas and fetuses.

* $p < 0.05$; ** $p < 0.01$.

for developmental toxicity was 5 mg/kg bw per day on the basis of increased resorptions, reduced litter size and incomplete ossification of appendicular skeletal elements at 75 mg/kg bw per day (Arthur, 1984).

2.6 Special studies

(a) Studies on metabolites

A number of metabolites of atrazine, such as the chlorometabolites (DEA, DIA, DACT) and hydroxyatrazine, can be found in drinking-water and in the diet.

Dealkylation reactions at the 4 and 6 positions resulting in the formation of either of the mono-dealkylated metabolites (desethyl-atrazine, desisopropyl-atrazine), which in turn can be further dealkylated to DACT, may occur in animals, plants and bacteria. Hydroxyatrazine is the major metabolite in plants, but is only a minor metabolite in animals in which the varying forms of the delkylated chlorometabolites dominate instead. The metabolism of atrazine to hydroxyatrazine in plants is a detoxification reaction as the phytotoxicity of hydroxyatrazine is greatly reduced compared with the parent compound.

A limited toxicology database is available for these four metabolites and the studies are summarized below.

(i) Deethyl-atrazine, DEA (G 30033)

DEA was of moderate acute oral toxicity in rats (LD_{50}, 1110 mg/kg bw). In short-term studies in rats given DEA at dietary concentrations of up to 1500 ppm, effects included reduced body-weight gain and food consumption, decreased erythrocyte parameters and lymphoid atrophy of the thymus. The overall NOAEL was 50 ppm, equal to 3.2 mg/kg bw per day. In a 13-week study in dogs given DEA at dietary concentrations of up to 1000 ppm, effects included reduced body weight and food consumption, decreased erythrocyte parameters and renal tubular epithelial hyperplasia/basophilia. The NOAEL was 100 ppm, equal to 3.7 mg/kg bw per day. DEA was not genotoxic in a battery of tests including assays for point mutation and DNA repair in vitro and testing for clastogenicity in vivo. In a study of prenatal developmental toxicity in rats, the NOAEL for maternal toxicity was 5 mg/kg bw per day on the basis of decreased body-weight gain and food intake at 25 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 25 mg/kg bw per day on the basis of increased incidences of fused sternebrae and incomplete ossification of the proximal phalanx of posterior digit 5 at 100 mg/kg bw per day. There was no evidence of teratogenicity. In a special study on the effects of DEA on pubertal development in male rats, atrazine equimolar doses of ≥ 25 mg/kg bw per day delayed preputial separation, with a NOAEL of 12.5 mg/kg bw per day.

In a study of acute oral toxicity, which complied with GLP and the US EPA test guidelines, groups of HSD:(SD) rats received DEA (purity, 95.7%; suspended in 2% carboxymethyl-cellulose) as a single dose at 250, 500, 2000, 3500 or 5050 mg/kg bw by gavage. Each group consisted of five males and five females with the exception of the group at the lowest dose, which consisted of five females. The rats were observed for clinical signs and mortality for 14 days. The LD_{50}s for males, females and both sexes combined were 1890, 669 and 1110 mg/kg bw, respectively (Kuhn, 1991c).

In a short-term study of oral toxicity, which complied with GLP and the test guidelines of OECD and US EPA, groups of 10 male and 10 female KFM-WIST rats were fed diets containing DEA (purity, 99.3%) at a concentration of 0, 50, 500 or 1500 ppm, equal to 0, 4.5, 49.3 and 145.9 mg/kg bw per day in males and 0, 4.6, 49.0 and 132.8 mg/kg bw per day in females, for 4 weeks.

Clinical signs of excitement were observed in the rats at the highest dose during the fourth week of treatment. There was a decrease in food consumption that was apparent at 1500 ppm with an average reduction over the 4-week treatment period of 24% in males and 19% in females. A dose-related reduction of body weight with statistically significant differences from the second week of treatment was observed for the males and females at the highest dose and for the males at the intermediate dose. At the end of the 4-week treatment, body weights were significantly decreased in both sexes at 500 ppm (17% in males, 9% in females) and at 1500 ppm (30% in males, 19% in females) when compared with rats in the control group. Slight changes in haematological and clinical biochemistry parameters were observed in the groups at the intermediate and highest dose. Thymus : body weight ratios were decreased in females at the lowest, intermediate and highest dose (15%, 19% and 26%,

respectively) and in males at the highest dose (33%). A significantly increased incidence of minimal to slight lymphoid atrophy of the thymus was observed in both sexes at 1500 ppm (the incidences at 0, 50, 500 and 1500 ppm were 1, 2, 2, and 9 in males and 2, 3, 5 and 10 in females, respectively).

The NOAEL was 50 ppm, equal to 4.5 and 4.6 mg/kg bw per day in males and females, respectively, on the basis of reduced body weights and lymphoid atrophy of the thymus at 500 ppm and greater (Duchosal et al., 1990b).

In a short-term study of oral toxicity, which complied with GLP and the test guidelines of OECD and US EPA, groups of 10 male and 10 female Tif:RAIf rats were fed diets containing DEA (purity, 95.7%) at a concentration of 0, 10, 50 or 500 ppm, equal to 0, 0.68, 3.20 and 35.2 mg/kg bw per day in males and 0, 0.73, 3.35 and 38.7 mg/kg bw per day in females, for 90 days.

No mortalities or clinical symptoms were observed and ophthalmological examination did not reveal treatment-related findings. Body-weight gains of males and females were reduced by 21% and 19% at 500 ppm. Food consumption was reduced by 10% and 7% in males and females of the same group, respectively. Haematology data revealed a slightly lower mean cell volume (MCV) and erythrocyte volume fraction and increased mean cell haemoglobin concentration (MCHC) values in females at 500 ppm. Alkaline phosphatase activity was increased in females at 500 ppm. A 12% increase in relative liver weight in females at the highest dose was without any histopathological correlates.

The NOAEL was 50 ppm, equal to 3.2 and 3.35 mg/kg bw per day in males and females, respectively, on the basis of reduced body weight and food efficiency at 500 ppm (Gerspach, 1991).

In a short-term study of oral toxicity, which complied with GLP and US EPA test guidelines, groups of four male and four female beagle dogs were fed diets containing DEA (purity, 95.7%) at a concentration of 0, 15, 100 or 1000 ppm, equal to 0, 0.56, 3.71 and 28.85 mg/kg bw per day in males and 0, 0.51, 3.88 and 32.18 mg/kg bw per day in females, for 13 weeks.

Treatment did not induce mortality or clinical signs of toxicity. At 1000 ppm, a slight body-weight loss was observed in males at days 7 to 42 and a decreased body-weight gain in females lasted the entire study period. Also, food consumption was 73% and 68% that of controls in males and females. Haematology revealed a slight reduction in erythrocyte parameters in dogs at 1000 ppm. No significant changes were recorded in clinical chemistry or urine analysis data.

Decreased uterus and thymus weights were observed in females receiving the highest dose and heart weight was decreased in males at the highest dose. Histopathology investigations confirmed these changes; females at the highest dose displayed thymic atrophy and anovulatory uterine atrophy that were considered to be secondary to reductions in food intake and body weights. Haemorrhagic inflammation with angiomatous hyperplasia in the right atrial wall of the heart was noted in one male at 1000 ppm (without any associated electrocardiographic changes), while the electrocardiographic findings (paroxysmal atrial fibrillation) in one female at 1000 ppm was not associated with histopathological changes in the heart. Mild renal tubular epithelial hyperplasia/basophilia was observed in three males and two females at 1000 ppm.

The NOAEL was 100 ppm, equal to 3.71 and 3.88 mg/kg bw per day in males and females, respectively, on the basis of reduced body weight and renal tubular hyperplasia at 1000 ppm (Rudzki et al., 1992).

In an assay for reverse mutation in bacteria, DEA (purity, 99.3%) did not induce gene mutations at the histidine locus of *S. typhimurium* and at the tryptophan locus in *E. coli* when tested at concentrations of up to 5000 μg/plate (Deparade, 1989).

In a test for DNA repair in vitro, DEA (purity, 99.3%) did not induce unscheduled DNA synthesis in primary rat hepatocytes exposed at concentrations of up to 1000 µg/ml (Geleick, 1991a).

In an assay for micronucleus formation in mouse bone marrow, DEA (purity, 99.3%) gave negative results for induction of micronuclei in the polychromatic erythroctes (PCE) of Tif:MAGF mice treated once orally at doses ranging from 120 to 480 mg/kg bw (Ogorek, 1991b).

In a study of prenatal developmental toxicity, which complied with GLP and the test guidelines of OECD and US EPA, groups of 24 mated female Tif:RAIf rats were given DEA (purity, 95.7%; suspended in 3% corn starch) at a dose of 0, 5, 25 or 100 mg/kg bw per day by oral gavage on days 6 to 15 of gestation.

All dams survived until the end of the experiment and no treatment-related clinical observations were made. Maternal toxicity was observed at 25 and 100 mg/kg bw per day in a dose-related manner as evidenced by effects on body weight/weight gain and food consumption. Body weights were significantly decreased at 100 mg/kg bw per day on days 7–20 of gestation. Body-weight gains were significantly decreased on days 6–11 of gestation at 25 and 100 mg/kg bw per day (83% and 41% of value for controls, respectively) and on days 11–16 of gestation at 100 mg/kg bw per day (87% of value for controls). Corrected body-weight gains were non-significantly decreased at 25 and 100 mg/kg bw per day (73% and 80% of value for controls, respectively). Food consumption was significantly decreased on days 6–11 of gestation at 25 and 100 mg/kg bw per day (91% and 70% of value for controls, respectively).

There were no treatment-related effects on any reproductive parameter examined, on fetal sex ratio, mean fetal weights or the incidences of gross, visceral and skeletal malformations. At 100 mg/kg bw per day, fetal and litter incidences of fused sternebrae 1 and 2 were significantly increased, as was the fetal incidence of poor ossification of the proximal phalanx of posterior digit 5 (Table 15).

There was no evidence of teratogenicity.

The NOAEL for maternal toxicity was 5 mg/kg bw per day on the basis of decreased body-weight gain and food intake at 25 mg/kg bw per day. The NOAEL for developmental toxicity was 25 mg/kg bw per day on the basis of increased incidences of fused sternebrae and incomplete ossification of the proximal phalanx of posterior digit 5 at 100 mg/kg bw per day (Marty, 1992b).

(ii) Deisopropyl-atrazine, DIA (G 28279)

DIA was of moderate acute oral toxicity in rats (LD_{50}, 1240 mg/kg bw). In short-term studies in rats given DIA at dietary concentrations of up to 2000 ppm, effects included reduced body-weight gain and food consumption, extramedullary haematopoiesis in liver and spleen, and histomorphological changes in adrenals, thyroid and pituitary. The overall NOAEL was 50 ppm, equal to 3.2 mg/kg bw per day. In a 14-week study in dogs given DIA at dietary concentrations of up to 1000 ppm, effects consisted of reduced body weight and food consumption. The NOAEL was 100 ppm, equal to 3.8 mg/kg bw per day. DIA was not genotoxic in a battery of tests including assays for point mutation and DNA repair in vitro and testing for clastogenicity in vivo. In a study of prenatal developmental toxicity in rats, the NOAEL for maternal toxicity was 5 mg/kg bw per day on the basis of decreased body-weight gain and food intake at 25 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 5 mg/kg bw per day on the basis of increased incidences of fused sternebrae at 25 mg/kg bw per day and greater. There was no evidence of teratogenicity. In a special study on the effects of DIA on pubertal development in male rats, atrazine equimolar doses of ≥ 25 mg/kg bw per day delayed the preputial separation, with a NOAEL of 12.5 mg/kg bw per day.

In a study of acute oral toxicity, which complied with GLP and the US EPA test guidelines, groups of HSD:(SD) rats received DIA (purity, not reported; suspended in 2% carboxymethyl-cellulose) as a

Table 15. Relevant findings in a study of prenatal developmental toxicity in rats given DEA by gavage

Finding	Dose (mg/kg bw per day)			
	0	5	25	100
Food consumption (g/day):				
Days 6–11	23	22	21*	16**
Days 11–16	26	25	26	24
Body-weight gain (g):				
Days 6–11	26.0	24.3	21.5*	10.7**
Days 11–16	39.7	39.8	37.3	34.5*
Net body-weight change (g) from day 6	37.1	31.9	27.2	29.5
No. of animals pregnant	23	23	22	24
Postimplantatation loss (mean)	0.6	0.8	0.9	1.1
Postimplantation loss (%)	4.0	5.1	6.3	8.8
Mean No. of live fetuses	14.2	14.5	14.2	12.6
Mean fetal weights (g), males/females	5.7/5.3	5.6/5.3	5.8/5.4	5.6/5.3
Skeletal examination (No. of fetuses/litters)	168/23	174/23	164/22	158/24
Fused sternebrae 1 and 2[a]	2/2	1/1	2/2	32**/14**
Total skeletal anomalies[a]	5/5	5/5	5/5	41/18**
Poster. digit 5, proximal phalanx; absent ossification[a]	16/11	23/12	8/6	28*/16
Total skeletal variations[a]	168/23	173/23	163/22	158/24

From Marty (1992b)
DEA, deethyl-atrazine.
[a] No. of fetuses/litters.
* $p < 0.05$; ** $p < 0.01$.

single dose at 250, 500, 2000, 3500 or 5050 mg/kg bw by gavage. Each group consisted of five males and five female with the exception of the group at the lowest dose that consisted of five females. The rats were observed for clinical signs and mortality for 14 days. The LD_{50}s for males, females and both sexes combined were 2290, 810 and 1240 mg/kg, respectively (Kuhn, 1991d).

In a short-term study of oral toxicity, which complied with GLP and the test guidelines of OECD and US EPA, groups of 10 male and 10 female KFM-WIST rats were fed diets containing DIA (purity, 97.4%) at a concentration of 0, 50, 500 or 2000 ppm, equal to 0, 4.6, 46.5 and 161.2 mg/kg bw per day in males and 0, 4.6, 49.7 and 164.7 mg/kg bw per day in females, for 4 weeks.

There was no mortality. Clinical signs of restlessness were observed in the rats at the highest dose during the fourth week of treatment. At 2000 ppm, food consumption was reduced by approximately 60% during the first week of treatment and by about 40% over the 4-week treatment period. Body weights were significantly reduced at 500 ppm from the third week and from the second week at 2000 ppm; after 4 weeks the body weights at 2000 ppm were reduced by 34% (males) and 28% (females) when compared with rats in the control group. Slight changes in haematological and clinical biochemistry parameters were observed in the groups at the intermediate and highest dose, with indication of a compensated haemolytic anaemia in the group at the highest dose. Absolute and relative thymus weights were reduced at 500 ppm in males and at 2000 ppm in both sexes. No treatment-related histopathological findings were noted in the groups at the lowest and intermediate dose. At 2000 ppm, mineralized deposits were noted in the renal pelvis of four males and two females, and a minimal erosion of the gastric mucosa was noted in one male.

The NOAEL was 50 ppm, equal to 4.6 mg/kg bw per day in males and females, on the basis of reduced body weights at 500 ppm and greater (Duchosal et al., 1990a).

In a short-term study of oral toxicity, which complied with GLP and the test guidelines of OECD and US EPA, groups of 10 male and 10 female Tif:RAIf rats were fed diets containing DIA (purity, 96.7%) at a concentration of 0, 10, 50 or 500 ppm, equal to 0, 0.60, 3.20 and 34.9 mg/kg bw per day in males and 0, 0.64, 3.34 and 37.5 mg/kg bw per day in females, for 13 weeks.

No mortalities or treatment-related clinical symptoms were observed during the study. At 500 ppm, body-weight gains of males and females were reduced by 16% and 21%, respectively, while food consumption of females was slightly reduced by 7% during weeks 1–7. There were no treatment-related changes in haematological or clinical chemistry parameters. A 13% increase in relative liver weights in females at 500 ppm was accompanied by extramedullary haematopoiesis in the liver and spleen. Histopathological findings in males at the highest dose included fatty changes in the adrenal cortex, hypertrophy of thyroid follicular epithelium and hypertrophy of thyroid-stimulating hormone (TSH)-producing cells in the pituitary gland.

The NOAEL was 50 ppm, equal to 3.2 and 3.34 mg/kg bw per day in males and females, respectively, on the basis of reduced body weight and histopathological changes in the liver, spleen, adrenals, thyroid and pituitary at 500 ppm (Schneider, 1992).

In a short-term study of oral toxicity, which complied with GLP and US EPA test guidelines, groups of four male and four female beagle dogs were fed diets containing DIA (purity, 96.7%) at a concentration of 0, 15, 100, 500 or 1000 ppm, equal to 0, 0.6, 3.8, 18.9 and 33.4 mg/kg bw per day in males and 0, 0.6, 3.8, 18.0 and 33.3 mg/kg bw per day in females, for 14 weeks.

No mortalities or treatment-related clinical symptoms were observed during the study. Males at 1000 pm and females at 500 and 1000 ppm exhibited significant decreases in food consumption and decreased body-weight gain relative to controls and to respective baseline values. Mean food efficiency was negative in males at 1000 ppm and in females at 500 and 1000 ppm. Haematology and clinical chemistry parameters were unaffected by treatment. Gross and histopathological examinations revealed no treatment-related findings. Absolute and relative (to brain) heart weights were significantly decreased in males at 500 and 1000 ppm, but there were no histopathological or functional (electrocardiography) correlates indicating myocardial toxicity.

The NOAEL was 100 ppm, equal to 3.8 mg/kg bw per day in males and females, on the basis of decreased body weight and heart weight at 500 ppm and greater (Thompson et al., 1992).

In an assay for reverse mutation in bacteria, DIA (purity, 97.4%) did not induce gene mutations at the histidine locus of *S. typhimurium* and at the tryptophan locus in *E. coli* when tested at concentrations of up to 5000 μg/plate (Deparade, 1990).

In a test for DNA repair in vitro, DIA (purity, 97.4%) did not induce unscheduled DNA synthesis in primary rat hepatocytes exposed at concentrations of up to 800 μg/ml (Geleick, 1991b).

In an assay for micronucleus formation in mouse bone marrow, DIA (purity, 97.4%) gave negative results for micronucleus formation in the PCE of Tif:MAGF mice treated once orally at doses ranging from 120 to 480 mg/kg bw (Ogorek, 1991a).

In a study of prenatal developmental toxicity, which complied with GLP and the test guidelines of OECD and US EPA, groups of 24 mated female Tif:RAIf rats were given DIA (purity, 97.4%; suspended in 3% corn starch) at a dose of 0, 5, 25 or 100 mg/kg bw per day by oral gavage on days 6 to 15 of gestation.

All dams survived until the end of the experiment and no treatment-related clinical symptoms were observed. Body-weight gains were decreased by 27% at 25 mg/kg bw per day and by 70% at 100 mg/kg bw per day during days 6–11 of gestation, while food consumption was decreased by 9% at 25 mg/kg bw per day and by 20% at 100 mg/kg bw per day during days 6–11 gestation.

There were no treatment-related effects on any reproductive parameter examined, on fetal sex ratio, mean fetal weights or the incidences of gross, visceral and skeletal malformations. At 50 and 100 mg/kg bw per day, fetal and litter incidences of fused sternebrae 1 and 2 were significantly increased (Table 16). Significantly increased incidences of incomplete or absent ossification were noted at 100 mg/kg bw per day, which included poor ossification of sternebra 2, absent ossification of proximal phalanx of posterior digit 2, 3, 4 and 5, and absent ossification of metatarsal 1 (Table 16)

There was no evidence of teratogenicity.

The NOAEL for maternal toxicity was 5 mg/kg bw per day, on the basis of decreased body-weight gain and food intake at 25 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 5 mg/kg bw per day, on the basis of increased incidences of fused sternebrae 1 and 2 at 25 mg/kg bw per day and greater (Marty, 1992a).

Table 16. Relevant findings in a study of prenatal developmental toxicity in rats given DIA by gavage

Finding	Dose (mg/kg bw per day)			
	0	5	25	100
Food consumption (g/day):				
Days 6–11	23	22	21**	16**
Days 11–16	26	25	25	23**
Body-weight gain (g), days 6–11	26.1	24.6	19.0	7.7**
Net weight change (g) from day 6	39.9	32.1	32.0	22.1**
No. of animals pregnant	23	21	24	24
Postimplantatation loss, mean	0.8	0.6	1.2	1.0
Postimplantation loss (%)	5.8	3.8	9.8	8.2
Mean No. of live fetuses	14.0	14.3	13.3	14.0
Mean fetal weights (g), males/females	5.6/5.3	5.6/5.3	5.7/5.3	5.5/5.2
Skeletal examination (No. of fetuses/litters)	160/22	155/21	165/22	172/23
Fused sternebrae 1 and 2[a]	0	0	9**/6*	29**/16**
Asymmetrically shaped sternebra 6[a]	0	0	1/1	4/3
Total skeletal anomalies[a]	2/2	4/4	14/11**	38/18**
Metatarsal 1; absent ossification[a]	14/9	6/6	22/9	34**/14
Posterior digit 2, proximal phalanx; absent ossification[a]	37/13	27/15	51/15	66**/18
Posterior digit 3, proximal phalanx; absent ossification[a]	23/11	12/9	29/14	42*/14
Posterior digit 4, proximal phalanx; absent ossification[a]	24/13	10*/8	28/13	43*/15
Posterior digit 5, proximal phalanx; absent ossification[a]	69/15	58/17	88/20	113**/22
Total skeletal variations[a]	160/22	155/21	164/22	172/23

From Marty (1992a)
DIA, deisopropyl-atrazine.
[a] No. of fetuses/litters.
* $p < 0.05$; ** $p < 0.01$.

(iii) Diaminochlorotriazine, DACT (G 28273)

DACT was of low acute oral toxicity in rats (LD_{50}, 2310 to 5460 mg/kg bw). In a short-term study in rats given DACT at dietary concentrations of up to 500 ppm, effects included reduced body-weight gain and food consumption and an increased number of females with shortened or prolonged estrous cycles and with persistent estrus or diestrus. The NOAEL was 10 ppm, equal to 0.7 mg/kg bw per day. In a 13/52-week study in dogs given DACT at dietary concentrations of up to 750–1500 ppm, effects included clinical signs and mortality, cardiac damage and failure, liver toxicity and decreased erythrocyte parameters. The NOAEL was 100 ppm, equal to 3.5 mg/kg bw per day. DACT was not genotoxic in a battery of tests, including in-vitro assays for point mutation and DNA repair and tests for clastogenicity in vivo. In a study of prenatal developmental toxicity in rats, the NOAEL for maternal toxicity was 2.5 mg/kg bw per day on the basis of decreased body-weight gain at 25 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 2.5 mg/kg bw per day on the basis of increased incidences of incompletely ossified interparietals or parietals and unossified hyoids at 25 mg/kg bw per day and greater. There was no evidence of teratogenicity. In special studies on the effects of DACT on pubertal development in male and female rats, atrazine equimolar doses of ≥ 12.5 or ≥ 50 mg/kg bw per day delayed preputial separation or vaginal opening, respectively, with NOAELs of 6.25 or 25 mg/kg bw per day, respectively.

In a study of acute oral toxicity, which complied with GLP and the US EPA test guidelines, Blu:(SD) rats received DACT (purity, not reported; 16.65% w/v suspension in corn oil) as a single dose at 2463, 3547 or 4256 mg/kg bw by gavage. The group at the lowest dose contained five females and the group at the highest dose contained five males; 10 male and 10 females were used in the group at the intermediate dose. The rats were observed for clinical signs and mortality for 14 days. The oral LD_{50}s for males, females and both sexes combined were 3690, 2360 and 2310 mg/kg bw, respectively (Mehta, 1980a).

In a study of acute oral toxicity, which complied with GLP and the US EPA test guidelines, Blu:(SD) rats received DACT (purity, not reported; 33.3% w/v suspension in corn oil) as a single dose at 2491, 3547, 5050, or 7189 mg/kg bw by gavage. The group at the lowest dose contained five males, and five males and five females were used in all other groups. The rats were observed for clinical signs and mortality for 14 days. The oral LD_{50}s for males, females and both sexes combined LD_{50} were 11 300, 5230 and 5460 mg/kg bw, respectively (Mehta, 1980b).

In a study of acute oral toxicity, which complied with GLP and the US EPA test guidelines, HSD:(SD) rats received DACT (purity, not reported; 40% w/v suspension in deionized water) as a single dose at 4000, 5050, or 5500 mg/kg bw. The groups at the lowest dose and the highest dose contained five females, and the group at the intermediate dose contained five males and five females. The rats were observed for clinical signs and mortality for 14 days. The LD_{50} was > 5050 mg/kg bw for males and > 5500 mg/kg bw for females (Kuhn, 1991b).

In a short-term study of oral toxicity , which complied with GLP and the test guidelines of OECD and US EPA, groups of 15 male and 15 female Crl:CD(SD)BR rats were fed diets containing DACT (purity, 98.2%) at a concentration of 0, 10, 100, 250 or 500ppm, equal to 0, 0.7, 6.7, 16.7 and 34.1 mg/kg bw per day in males and 0, 0.7, 7.6, 19.7 and 40.2 mg/kg bw per day in females, for 90 days. In addition to the standard examinations, the estrous cycles and hormone concentrations of female rats were evaluated.

No mortalities or treatment-related clinical symptoms were observed during the study. There was a statistically significant decrease in body-weight gain in males at 500 ppm and in females at

250 and 500 ppm. Body-weight gain at week 12 was decreased by 19% in males at 500 ppm and by 15% and 17% in females at 250 and 500 ppm, respectively. Food consumption was generally comparable between all groups. There were no biologically significant effects on haematology, clinical chemistry and urine analysis at any dose. Organ-weight changes were unremarkable and tended to be associated with body-weight gain reductions. Macro- and micropathological examinations revealed no treatment-related findings.

At dietary concentrations of 100 ppm or greater, the proportion of rats exhibiting normal, 4- to 5-day estrous cycles tended to be reduced, while the incidence of persistent estrus tended to be increased, with the consequence of a reduction of the mean number of estrous cycles during the observation period. These effects, which were more pronounced on days 70–85 than during days 42–56, were statistically significant only at dietary concentrations of 250 ppm or greater after 70 days of treatment (Table 17). A more exact determination of effects at lower doses was precluded by the greater variability between individual rats in data on estrous cycle in the treated groups. There were no treatment-related effects on plasma concentrations of E2, progesterone, prolactin and corticosterone.

The NOAEL was 100 ppm, equal to 7.6 mg/kg bw per day in females, on the basis of decreased body-weight gain and significant effects on the estrous cycle at 250 ppm and greater (Pettersen et al., 1991; Terranova, 1991).

In a combined short-term and long-term study of oral toxicity, which complied with GLP and US EPA test guidelines, groups of 8–10 male and 8–10 female beagle dogs were fed diets containing DACT (purity, 98.7%) at a concentration of 0, 5, 100 or 1500 ppm for 13 or 52 weeks. Because of severe toxicity at the highest dose, which was evident after 6 weeks of treatment, the diet of the group of dogs at the highest dose was chanegd to one containing DACT at 750 ppm. Females tolerated this dose and received diet at 750 ppm until termination at 13 or 52 weeks. Since males continued to exhibit signs of toxicity at 750 ppm, they were fed untreated diet for weeks 9–13. At 13 weeks, two to six dogs per group were killed for interim study. Two females at the highest dose were then placed

Table 17. Data on the estrous cycle in rats given DACT in a short-term study of oral toxicity

Parameter	Dietary concentration (ppm)				
	0	10	100	250	500
Days 42–56					
Four- to five-day cycles[a]	14/15	12/15	9/15	8/15	8/15
Persistent estrus[a]	0/15	1/15	3/15	5/15	4/15
Persistent diestrus[a]	2/15	2/15	3/15	4/15	1/15
Mean No. of cycles (days)	2.60 ± 0.51	2.40 ± 0.74	2.07 ± 0.88	1.87 ± 0.83	2.00 ± 1.00
Days 70–85					
Four- to five-day cycles[a]	15/15	13/15	14/15	7/15*	6/15*
Persistent estrus[a]	0/15	1/15	2/15	8/15*	4/15
Persistent diestrus[a]	0/15	3/15	1/15	3/15	5/15
Mean No. of cycles (days)	2.80 ± 0.41	2.33 ± 0.90	2.47 ± 0.83	1.80 ± 1.01**	1.67 ± 1.11**

From Terranova (1991)
DACT, diaminochlorotriazine.
[a] Total may not equal 15 since a rat might display a regular 4- to 5-day cycle followed by persistent estrus or persistent diestrus.
* $p < 0.05$; ** $p < 0.01$.

on control diet for a 39-week recovery period, while four males at the highest dose were placed again on a diet containing DACT at 750 ppm until termination at 52 weeks. The mean daily intakes at 0, 5, 100 or 1500/750 ppm were equal to doses of 0, 0.2, 3.5 and 23.8 mg/kg bw per day for males and 0, 0.2, 3.3 and 29.9 mg/kg bw per day for females, respectively.

Five males and two females in the group at the highest dose were kiled in a moribund condition, mainly during the first 9 weeks. Clinical observation revealed tremors in all dogs at the highest dose during weeks 5–15. Additionally, inactivity, paleness, abdominal distension and emaciation were noted. Dogs that were killed moribund displayed inappetence, hypothermia, laboured breathing, hunched posture, and abnormal gait.

In the group at the highest dose, treatment-related physical examination findings consistent with impaired heart function occurred from week 6 onwards and included irregular (rapid) heart rate, pericardial thrill, pulse deficit, abdominal ascites and emaciation. Electrocardiographic abnormalities (atrial fibrillation) ocurred in three males and two females at week 5, the first electrocardiographic evaluation during the treatment period, and were diagnosed in a total of six males and four females in the group at the highest dose. Ophthalmological examinations revealed no treatment-related findings.

Dogs in the group at the highest dose lost weight during the first 6 weeks of treatment. After the concentration of DACT in the diet was reduced to 750 ppm, the dogs maintained their body weight. Body-weight gain of females in the group improved after cessation of test article treatment and was greater than the body-weight gain of females in the control group for the same period. Food consumption was decreased in males at the highest dose during the first 6 months of the study, except during the period when the dogs were fed the control diet. In females at the highest dose, the reduction of food consumption was not as pronounced as in males and was comparable to that of controls from week 18 onwards.

Moderate anaemia accompanied by an increased number of reticulocytes indicating an increased erythropoiesis was noted in dogs receiving the highest dose. The decrease of erythrocyte counts, erythrocyte volume fraction and haemoglobin concentration was significant in females at weeks 13 and 26. By week 52 the number of reticulocytes was within the normal range and the erythrocyte parameters had returned to nearly normal levels. Decreased calcium and increased lactate dehydrogenase and albumin concentrations were observed in the group at the highest dose. There were no effects of treatment on urinary parameters.

Mean absolute and relative liver, spleen and kidney weights were increased in dogs at the highest dose when compared with those of the controls at 13 and 52 weeks. At necropsy, heart and liver lesions were observed in dogs at the highest dose. Accumulations of fluids in the abdominal or thoracic cavity or the pericardium were secondary to the impaired heart function. Histopathology revealed chronic myocarditis in several dogs at the highest dose. The right atrium was most often affected, although the ventricles and papillary muscles were also affected in several animals. Males were more severely affected than females. Liver lesions included centrilobular fibrosis/atrophy, bile stasis, necrosis, haemorrhage, haemosiderosis and inflammation. Hyperplasia of the bone marrow, thymus atrophy and hypospermatogenesis of the testes were recorded at the interim kill, primarily in male dogs receiving the highest dose.

The effects observed in females at the highest dose were reversible 3 months after the cessation of treatment. The two females in the recovery group did not exhibit any clinical, electrocardiographic, gross or microscopic evidence of cardiac abnormalities. Likewise, no effects on haematological or biochemistry parameters were recorded at termination.

The NOAEL was 100 ppm, equal to 3.5 and 3.3 mg/kg bw per day in males and females, respectively, on the basis of clinical, electrocardiographic, gross and microscopic evidence of impaired heart function at 1500/750 ppm (Thompson et al., 1990).

In an assay for reverse mutation in bacteria, DACT (purity, 97%) did not induce gene mutations at the histidine locus of *S. typhimurium* when tested at concentrations of up to 5000 µg/plate (Deparade, 1987).

In two tests for DNA repair in vitro, DACT (purity, 97%) did not induce unscheduled DNA synthesis in primary rat hepatocytes exposed at concentrations of up to 400 µg/ml (Hertner, 1988b) and in human fibroblasts exposed at concentrations of up to 600 µg/ml (Meyer, 1987).

In an assay for micronucleus formation in mouse bone marrow , DACT (purity, 97%) gave negative results for micronucleus formation in the PCE of Tif:MAGF mice treated once orally at doses ranging from 1250 to 5000 mg/kg bw (Strasser, 1988).

In a study of prenatal developmental toxicity, which complied with GLP and the US EPA test guidelines, groups of 26 mated female Crl:COBS CD(SD)BR rats were given DACT (purity, 98.7%; suspended in 3% corn starch) at a dose of 0, 2.5, 25, 75 or 150 mg/kg bw per day by oral gavage on days 6 to 15 of gestation.

No mortalities or treatment-related clinical observations were noted. Food consumption was significantly decreased throughout the treatment period at 150 mg/kg bw per day and during days 6–8 of gestation at 75 mg/kg bw per day, while a non-significant decrease was noted at 25 mg/kg bw per day during the first 3 days of treatment. Body-weight loss was observed during the first 3 days of treatment (days 6–8 of gestation) in females at 75 and 150 mg/kg bw per day, and body weight was significantly decreased in the dams at the highest dose during the rest of the treatment period. Total (−33%) and corrected (−43%) body-weight gain (days 0–20 of gestation) was also significantly decreased at the same dose. A tendency towards a reduced body-weight gain was observed at 75 mg/kg bw per day for days 6–16 of gestation (–28%) and for days 6–8 of gestation (–32%) at 25 mg/kg bw per day. These decreases were of transient nature; total and corrected body-weight gains in the group at 25 mg/kg bw per day were comparable to those of rats in the control group.

The number of corpora lutea and implantation sites was comparable between all groups. The number of resorptions was significantly increased in the group at the highest dose. Fetal body weights were significantly decreased in the groups at 75 and 150 mg/kg bw per day.

No treatment-related malformations were observed, although incidental malformations included an umbilical hernia observed in one fetus at the lowest dose and one fetus at the highest dose; one fetus at the lowest dose had a protruding tongue, one fetus at 75 mg/kg bw per day had a filamentous tail, and one fetus at the highest dose was acaudate. Visceral malformations were restricted to a single fetus at 25 mg/kg bw per day. A significantly increased incidence of visceral variations was observed in fetuses at the highest dose (pitted kidneys and absent renal papillae). No skeletal malformations were observed at any dose. There was a dose-related increase of incomplete ossification of several bones at 75 and 150 mg/kg bw per day and in three skull bones (interparietal, parietals; hyoid [unossified]) at 25 mg/kg bw per day and greater (Table 18).

There was no evidence of teratogenicity.

The NOAEL for maternal toxicity was 2.5 mg/kg bw per day on the basis of decreased body-weight gain during the initial 3 days of treatment at 25 mg/kg bw per day. The NOAEL for developmental toxicity was 2.5 mg/kg bw per day on the basis of increased incidences of incompletely ossified interparietals or parietals and unossified hyoids at 25 mg/kg bw per day and greater (Hummel et al., 1989).

(iv) Hydroxyatrazine (G 34048)

Hydroxyatrazine was of low acute oral toxicity in rats (LD_{50}, > 5050 mg/kg bw). In short-term studies in rats given hydroxyatrazine at dietary concentrations of up to 750 ppm, effects included

Table 18. Relevant findings in a study of prenatal developmental toxicity in rats given DACT by gavage

Finding	Dose (mg/kg bw per day)				
	0	2.5	25	75	150
Food consumption (g/day):					
Days 6–8	22.95	22.80	20.62	18.48*	11.98*
Days 8–12	22.54	24.73	23.70	21.85	15.63*
Body-weight gain (g):					
Days 6–8	11.68	11.39	7.92	−1.64*	−11.26*
Days 8–12	21.18	23.00	22.20	17.12	5.70*
No. of pregnant rats	22	23	25	25	23
Mean No. of resorptions	0.77	0.48	1.04	0.84	2.61*
Postimplantatation loss (mean)	0.77	0.48	1.04	0.84	2.70*
Postimplantation loss (%)	5.56	3.59	7.15	6.16	18.86*
Mean No. of live fetuses	13.18	12.61	13.20	13.60	11.26
Mean fetal weights (g), males/females	3.45/3.29	3.45/3.32	3.43/3.29	3.14*/3.03*	2.79*/2.68*
Visceral examination (No. of fetuses/litters)	141/22	140/23	160/25	166/25	126/23
Renal papilla absent (No. of fetuses/litters)	5/3	7/6	8/5	12/8	28*/11*
Kidneys pitted (No. of fetuses/litters)	0	0	0	0	6*/3*
Skeletal examination (No. of fetuses/litters)	149/22	150/23	170/25	174/25	133/23
Hyoid not ossified (No. of fetuses/litters)	7/5	7/5	26*/10*	49*/16*	53*/15*
Interparietal not completely ossified (No. of fetuses/litters)	27/10	28/11	60*/20*	92*/23*	86*/21*
Parietals not completely ossified (No. of fetuses/litters)	5/3	14/5	18*/10*	20*/10*	20*/8*

From Hummel et al. (1989)
DACT, diaminochlorotriazine.
* $p < 0.05$; ** $p < 0.01$;

reduced body-weight gain, increased water consumption, changes in clinical chemistry and urine analysis parameters as well as macroscopic and microscopic lesions in the kidney, due to the low solubility of hydroxyatrazine in water resulting in crystal formation and consequent inflammatory response. The overall NOAEL was 100 ppm, equal to 6.3 mg/kg bw per day. In a 13-week study in dogs given hydroxyatrazine at dietary concentrations of up to 6000 ppm, effects included reduced body-weight gain and food consumption, changes in clinical chemistry and urine analysis parameters and macroscopic and microscopic lesions in the kidney. The NOAEL was 150 ppm, equal to 5.8 mg/kg bw per day. In a 2-year study of toxicity and carcinogenicity in rats given hydroxyatrazine at dietary concentrations of up to 400 ppm, effects included clinical signs and increased mortality, reduced body-weight gain and food consumption, increased water consumption, changes in haematological, clinical chemistry and urine analysis parameters and macroscopic and microscopic lesions in the kidney. The NOAEL was 25 ppm, equal to 1 mg/kg bw per day. There was no evidence of carcinogenicity. Hydroxyatrazine was not genotoxic in a battery of tests including assays for point mutation and DNA repair in vitro and tests for clastogenicity in vivo. In a study of prenatal developmental toxicity in rats, reduced food consumption and body-weight gain in dams and increased incidences of incompletely ossified hyoid and interparietal bones and not ossified forepaw metacarpals and proximal phalanges in fetuses were seen at 125 mg/kg bw per day, and the NOAEL was 25 mg/kg bw per day for both maternal and developmental toxicity. In a special study on the effects of hydroxyatrazine

on pubertal development in female rats, atrazine equimolar doses of up to 200 mg/kg bw per day did not significantly delay vaginal opening.

In a study of acute oral toxicity, which complied with GLP and the US EPA test guidelines, a group of five male and five female HSD:(SD) rats received hydroxyatrazine (purity, 97.1%; suspended in 2% carboxymethyl-cellulose) as a single dose at 5050 mg/kg bw by gavage. The rats were observed for clinical signs and mortality for 14 days. The oral LD_{50} was > 5050 mg/kg bw (Kuhn, 1991e).

In a short-term study of oral toxicity, which complied with GLP and US EPA test guidelines, groups of 10 male and 10 female Sprague-Dawley rats were fed diets containing hydroxyatrazine (purity, not reported) at a concentration of 0, 10, 250, 500 or 750 ppm, equal to 0, 0.92, 22.5, 44.4 and 67.3 mg/kg bw per day in males and 0, 1.0, 25.2, 47.3 and 67.4 mg/kg bw per day in females, for at least 29 days.

No mortalities were observed during the study. An increased incidence of diarrhoea was observed in males at 250 ppm and greater. Body weight and body-weight gain were decreased in males at 500 ppm and greater and in females at 750 ppm, and food consumption was reduced in males at 250 ppm and greater and in females at 500 ppm and greater. Some changes in haematological and clinical chemistry parameters were observed in both sexes at 500 ppm and greater. At necropsy, an increased incidence of mottled and rough kidneys was seen in males at 500 ppm and greater and in females at 750 ppm.

The NOAEL was 10 ppm, equal to 0.9 and 1.0 mg/kg bw per day in males and females, respectively, on the basis of increased incidence of diarrhoea and reduced food consumption at 250 ppm and greater (Hazelette & Arthur, 1989a).

In a short-term study of oral toxicity, which complied with GLP and US EPA test guidelines, groups of 15 male and 15 female Sprague-Dawley (Crl:CD Br) rats were fed diets containing hydroxyatrazine (purity, 97.1%) at a concentration of 0, 10, 100, 300, or 600 ppm, equal to 0, 0.64, 6.3, 18.89 and 37.47 mg/kg bw per day in males and 0, 0.75, 7.35, 22.73 and 45.64 mg/kg bw per day in females, for 13 weeks.

No mortality occurred throughout the study and no treatment-related clinical symptoms were observed. Body-weight gain was decreased by about 12.5% in both sexes at 600 ppm, while food consumption was slightly decreased in males at 600 ppm. An increase in water consumption was observed in both sexes at 600 ppm. Slight decreases in erythrocyte parameters (erythrocyte count, haemoglobin concentration and erythrocyte volume fraction) and increases in serum urea nitrogen, creatinine, sodium and chloride concentrations were seen at 600 ppm, while increased chloride concentrations were also observed in females at 300 ppm. Mean urine volume was increased in males at 300 ppm and in both sexes at 600 ppm, and mean specific gravity in urine was decreased in females at 600 ppm.

At necropsy, mean absolute and relative kidney weights were increased in both sexes at 600 ppm (absolute weights, +33.7 and +33.2%; relative organ-to-body weights, +44.3 and +45.1% in males and females, respectively). Rough or pitted kidneys, with or without pale and tan discoloration, were noted in all males and in 14 out of 15 females at 600 ppm. Similar findings were noted in four males and two females at 300 ppm. Treatment-related histopathological findings were restricted to the kidneys at 300 ppm and greater. All rats at the highest dose had marked tubular dilatation and basophilia, extensive chronic hyperplastic inflammation in the interstices, and cellular casts. Anisotropic crystals, later identified as hydroxyatrazine, were noted in the papillary tubules of 11 males and 13 females of the group at the highest dose. Less severe lesions (minimal tubular dilatation and tubular

basophilia, minimal subacute interstitial inflammation) were observed in 7 males and 11 females at 300 ppm.

The NOAEL was 100 ppm, equal to 6.3 and 7.35 mg/kg bw per day in males and females, respectively, on the basis of kidney toxicity (tubular and interstitial chronic nephropathy) at 300 ppm and greater (Rudzki et al., 1989).

In a short-term study of oral toxicity, which complied with GLP and US EPA test guidelines, groups of four male and four female beagle dogs were fed diets containing hydroxyatrazine (purity, 97.1%) at a concentration of 0, 15, 150, 1500, or 6000 ppm, equal to 0, 0.6, 5.8, 59.6, and 247.7 mg/kg bw per day in males and 0, 0.6, 6.2, 63.9, and 222.1 mg/kg bw per day in females, for 13 weeks.

There were no mortalities. A treatment-related reduction in body-weight gain (−29% in males, -31-37% in females), accompanied by some initial reduction in food consumption was observed at 1500 ppm and greater. Treatment-related clinical chemistry changes were restricted to females at 6000 ppm that displayed increased blood urea nitrogen (BUN) and/or creatinine concentrations. Urine analysis performed at day 85 revealed an increased urine volume (about twofold) and a decreased specific gravity at 1500 ppm and greater.

There were no treatment-related effects on organ weights, although at necropsy, pitted or rough kidneys were observed in three out of four males and one out of four females at 1500 ppm and four out of four males and two out of four females at 6000 ppm. Treatment-related histopathological lesions were restricted to the kidneys at 1500 ppm and greater. Minimal to marked multifocal, chronic nephropathy characterized by tubular dilation, atrophy and basophilia was observed, often in the presence of a prominent chronic interstitial fibrosis and lymphocytic infiltration. The kidney lesions appeared predominantly in the cortex, while medullary areas were occasionally involved. Intratubular crystalline casts were observed in the renal papilla in all males at 1500 ppm and greater and in three out of four females in each group at 1500 and 6000 ppm.

The NOAEL was 150 ppm, equal to 5.8 and 6.2 mg/kg bw per day in males and females, respectively, on the basis of decreased body-weight gain and kidney toxicity (tubular and interstitial chronic nephropathy) at 1500 ppm and greater (Chau et al., 1990).

In a combined long-term study of toxicity and carcinogenicity, which complied with GLP and the test guidelines of OECD and US EPA, groups of 80 male and 80 female (control and highest dose) or 70 male and 70 female (all other groups) Crl:CD(SD)BR rats were fed diets containing hydroxyatrazine (purity, 97.1%) at a concentration of 0, 10, 25, 200, or 400 ppm, equal to 0, 0.388, 0.962, 7.75 and 17.4 mg/kg bw per day in males and 0, 0.475, 1.17, 9.53 and 22.3 mg/kg bw per day in females, for 24 months. Ten males and 10 females (intermediate dose) or 20 males and 20 females (control and highest dose) were scheduled for interim kill at 12 months.

Mortality attributable to severe renal failure was markedly increased at 400 ppm (survival rate by week 52, 96%, 94%, 94%, 94% and 75% in males; 98%, 97%, 97%, 97%, and 76% in females at 0, 10, 25, 200 and 400 ppm, respectively) and, thus, rats at the highest dose were killed after 18 months. Treatment-related clinical signs were limited to the group at 400 ppm and included emaciation, polyuria, general pallor, piloerection and tremors. Body weight and body-weight gain were significantly decreased at 400 ppm throughout the study. Food consumption was decreased at 200 ppm and greater, while food efficiency was decreased only at 400 ppm. Water consumption was increased at 200 ppm and greater, but only for the first year.

Treatment-related changes in haematology parameters (decreases in erythrocyte count, haemoglobin concentration, erythrocyte volume fraction), clinical chemistry parameters (increased calcium, phosphorus, gamma-glutamyl transferase, BUN and creatinine concentrations; decreased glucose, total protein and albumin plasma concentrations) and urine analysis

parameters (decreased pH, specific gravity and colour intensity; increased urine volume; presence of crystalline hydroxyatrazine sediments) were observed at 400 ppm.

Necropsy of rats at interim and decedent rats at 400 ppm revealed discoloration and enlargement of renal lymph nodes and calculi, cysts, dilated pelvis and rough pitted surface of the kidney. Additionally, in males calculi and thickened walls were recorded in the urinary bladder. Absolute and relative kidney weights were increased in males (+19% and +50%) and females (+10% and +51%) at 400 ppm at 12 months only.

Treatment-related histopathological changes in the kidneys were noted at 200 ppm and greater at all killing intervals and included the deposition of crystalline material within collecting ducts, renal pelvises and occasional distal tubules (summarized in Table 19 as "dilatation with crystal deposits"). Tubules and collecting ducts that contained the crystalline material were dilated and either devoid of epithelium or lined by hyperplastic tubular epithelium. The tubular changes were often accompanied by acute intratubular inflammatory infiltration and by thickening and fibrosis of the papillary interstitium. In kidneys that contained pelvic aggregates of the crystalline material, multifocal transitional cell erosion and/or ulceration of the renal transitional epithelium was noted.

In the renal papillae, swelling and increased eosinophilia of interstitial cells, which was accompanied by the interstitial accumulation of a hyaline basophilic material (acidic sulfated mucosubstances that make up the ground substance of the interstitial matrix) was significantly increased in incidence/severity in males at 200 ppm and greater and in females at at 25 ppm and greater. However, the toxicological significance of the minimal to moderate accumulation of this matrix in females at 25 ppm, in the absence of any other signs of renal damage or impaired renal function, was highly questionable.

In males at 400 ppm and in females at 200 ppm and greater, papillary lesions were accompanied by cortical changes consistent with chronic progressive nephropathy. These consisted of thickening of tubular and glomerular basement membranes, tubular dilatation with accumulation of proteinaceous material, chronic interstitial nephritis, hyaline droplet accumulation within proximal tubular cells, pronounced glomerulosclerosis and infrequent tubular epithelial basophilia or hyperplasia.

In both sexes at 400 ppm, nephropathy was often accompanied by mineralization of renal (tubular epithelia and basement membranes) as well as extrarenal (e.g. aorta, heart, and lungs) tissues (metastatic mineralization).

There was no increase in the incidence of any tumour or decrease in any tumour-onset time that was attributable to treatment.

Table 19. Relevant histopathological findings in the kidney of rats fed diets containing hydroxyatrazine in a long-term study of toxicity

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	10	25	200	400	0	10	25	200	400
Kidney, No. examined	79	69	70	70	80	79	70	68	69	80
Dilatation with crystal deposits	0	0	0	5**	79**	0	0	0	15**	78**
Nephropathy, progressive	75	67	64	65	80**	36	32	34	50**	79**
Papilla, accumulation, interstitial, matrix	4	3	2	32**		17	10	26*	26	0**
Papilla, fibrosis, interstitial	1	2	1	11**	80**	0	0	0	20**	79**
Pelvis, dilatation with crystal deposits	0	0	0	5	60**	0	0	0	9*	40**

From Chow & Hart (1995)
* $p < 0.05$; ** $p < 0.01$.

Hydroxyatrazine was not carcinogenic in rats. The NOAEL was 25 ppm, equal to 0.96 and 1.2 mg/kg bw per day in males and females, respectively, on the basis of kidney toxicity at 200 ppm and greater (Chow & Hart, 1995).

In an assay for reverse mutation in bacteria, hydroxyatrazine (purity, 99%) did not induce gene mutations at the histidine locus of *S. typhimurium* when tested at concentrations of up to 5000 µg/plate (Deparade, 1988).

In two tests for DNA repair in vitro, hydroxyatrazine (purity, 96–99%) did not induce unscheduled DNA synthesis in primary rat hepatocytes exposed at concentrations of up to 1500 µg/ml (Hertner, 1988a) and in human fibroblasts exposed at concentrations of up to 1500 µg/ml (Meyer, 1988).

In an assay for micronucleus formation in mouse bone marrow, hydroxyatrazine (purity, 99%) did not induce micronucleus formation in the PCE of Tif:MAGF mice treated once orally at doses ranging from 1250 to 5000 mg/kg bw (Ceresa, 1988a).

In a study of prenatal developmental toxicity, which complied with GLP and US EPA test guidelines, groups of 26 pregnant female Crl:COBS CD(SD)BR rats were given hydroxyatrazine (purity, 96.7%; suspended in 3% corn starch with 0.5% Tween 80) at a dose of 0, 5, 25 or 125 mg/kg bw per day by oral gavage on days 6 to 15 of gestation.

No mortalities or treatment-related clinical observations were noted. Food consumption was significantly decreased at 125 mg/kg bw per day during days 8–12 of gestation (−12.1%) and during the entire dosing period (−8.4%). Body-weight gain at 125 mg/kg bw per day was decreased by about 24% during days 8–12 of gestation, while corrected body-weight gain was decreased by about 11%.

There were no treatment-related effects on any reproductive parameter examined. Fetal body weights were slightly, but significantly lower at 125 mg/kg bw per day (males, −3.9%; females, −5.0%). There were no treatment-related external, visceral or skeletal malformations. At 125 mg/kg bw per day, there were significantly increased fetal and litter incidences of not completely ossified hyoids, not completely ossified interparietals, and not ossified forepaw metacarpals and proximal phalanges (Table 20).

There was no evidence of teratogenicity.

The NOAEL for maternal toxicity was 25 mg/kg bw per day on the basis of decreased food consumption and body-weight gain at 125 mg/kg bw per day. The NOAEL for developmental toxicity was 25 mg/kg bw per day on the basis of increased incidences of incompletely ossified hyoid and interparietal bones and not ossified forepaw metacarpals and proximal phalanges at 125 mg/kg bw per day (Lindsay et al., 1989).

(b) Studies on site and mechanism of action in the central nervous system

In a in-vitro study on the mechanism by which chlorotriazines interfere with hypothalamic control of the gonadotrophin releasing hormone (GnRH) release and luteinizing hormone (LH) surge, the ability of atrazine and its metabolites DIA, DEA and DACT to interact with gamma-aminobutyric acid A-type ($GABA_A$) receptors in rat cortical membranes was examined by measuring their effects on binding of the following prototypical ligands to their recognition sites on $GABA_A$ receptors: [^{3}H] muscimol, which binds to the GABA binding site; [^{3}H]Ro15-4513, which binds to the benzodiazepine site on the $GABA_A$ receptor; and [^{35}S]*tert*-butylbicyclophosphorothionate (TBPS), which binds to the picrotoxin binding site in the chloride channel of $GABA_A$ receptors.

Atrazine significantly inhibited the binding of [^{3}H]Ro15-4513 at concentrations of 30 µmol/l and greater, and the half-maximal inhibitory concentration (IC_{50}) was calculated to be 305 µmol/l. The chlorotriazine metabolites, however, were without significant effect on Ro15-4513-binding when

Table 20. Relevant findings in a study of prenatal developmental toxicity in rats given hydroxyatrazine by gavage

Finding	Dose (mg/kg bw per day)			
	0	5	25	100
Food consumption (g/day), days 8–12	23.4	23.7	22.6	20.6*
Total food consumption (g), days 6–16	229.4	236.7	223.3	210.1*
Body-weight gain (g), days 8–12	23.5	20.9	21.1	18.0
Net body-weight change (g), days 0–20	77.2	81.9	71.1	68.6
No. of rats pregnant	25	23	23	22
Postimplantatation loss (mean)	0.5	1.1	1.0	0.7
Postimplantation loss (%)	5.6	7.4	11.6	4.9
Mean No. of live fetuses	13.40	13.30	12.30	14.14
Mean fetal weights (g), males/females	3.61/3.43	3.71/3.51	3.55/3.35	3.47*/3.26*
Skeletal examination (No. of fetuses/litters)	177/25	159/23	147/22	160/22
Hyoid not completely ossified (No. of fetuses/litters)	11/5	26/9	14/8	27*/12*
Interparietal not completely ossified (No. of fetuses/litters)	35/14	57/19	42/14	70*/20*
Metacarpal not ossified (No. of fetuses/litters)	67/21	58/18	61/19	96*/21*
Proximal phalange not ossified (No. of fetuses/litters)	169/24	153/23	139/22	159*/22*

From Lindsay et al. (1989)
* $p < 0.05$; ** $p < 0.01$.\

compared with controls. All substances were without effect on [^{3}H]muscimol or [^{35}S]TBPS binding. The results suggested that atrazine modulates benzodiazepine, but not the muscimol (GABA receptor site) or TBPS (chloride channel), binding sites on $GABA_A$ receptors (Shafer et al., 1999).

In a study on the effect of chlorotriazines on catecholamine metabolism in vitro using pheochromocytoma (PC12) cells, intracellular norepinephrine (NE) and dopamine (DA) concentrations and spontaneous NE release were measured after treatment with atrazine (0, 12.5, 25, 50, 100 and 200 µmol/l) for 6, 12, 18, 24 and 48 h.

Intracellular DA concentration was significantly decreased at 12.5 µmol/l and greater, while intracellular NE concentration was significantly decreased at 100 µmol/l and greater. Similarly, there was a dose-dependent inhibition of NE release at 50 µmol/l and greater. The $GABA_A$-receptor agonist, muscimol (at 0, 0.01, 0.1, and 1.0 µmol/l) had no effect on either the release or on intracellular catecholamine concentrations from 6 h until 24 h of treatment. Cell viability was somewhat lower at 100–200 µmol/l, but the reduction in viability was significant only at 200 µmol/l at 24 h. The data indicated that atrazine inhibits the cellular synthesis of DA mediated by the tyrosine hydroxylase, and NE mediated by dopamine beta-hydroxylase, resulting in an inhibition of NE release. Thus, atrazine presumably acts at the enzymatic steps or sites of DA biosynthesis to modulate monoaminergic activity in PC12 cells (Das et al., 2000).

In a subsequent study on the effect of chlorotriazine metabolites on catecholamine metabolism in vitro using PC12 cells, intracellular NE and DA concentrations and spontaneous NE release were measured after treatment with hydroxyatrazine (0–400 µmol/l), DEA (0–200 µmol/l), DIA (0–200 µmol/l) and DACT (0–160 µmol/l) for 3–24 h.

Hydroxyatrazine significantly decreased intracellular DA and NE concentrations in a dose- and time-dependent manner, and caused also a significant inhibition of NE release from cells. In contrast,

DEA and DIA significantly increased intracellular DA concentrations at 50 μmol/l and greater from 12 to 24 h. Intracellular NE was significantly reduced at 50 μmol/l and greater of DEA at 24 h, while DIA had no effect. NE release was decreased at 100 μmol/l and greater for both DEA and DIA. DACT significantly increased intracellular DA and NE concentrations at 160 μmol/l, while NE release was increased at 40 μmol/l and greater. Cell viability was reduced by 10–12% in the presence of hydroxyatrazine at 200–400 μmol/l, while for the other metabolites, viability was reduced by only 2–5% at the highest concentrations. The data suggested that the catecholamine neurons may be a target for the chlorotriazines and/or their metabolites, that the metabolites produce a unique pattern of catecholamine response, and that all of the changes were seen within the same range of doses (Das et al., 2001).

In a subsequent study on mechanisms responsible for chlorotriazine-induced alterations in catecholamines in PC12 cells in vitro, the effect of atrazine (100 μmol/l for 1–36 h) on the protein expression of the enzymes responsible for synthesis of DA—tyrosine hydroxylase (TH)—and NA—dopamine-β-hydroxylase (DβH)—was determined.

Atrazine decreased intracellular DA and NE concentration and NE release, and the protein expression of TH and DβH. Siumultaneous exposure to the essential cofactors for TH (iron and tetrahydrobiopterine) was ineffective in altering cellular DA. Agents known to enhance TH and DβH transcription, phosphorylation or activity (e.g. 8-bromo cAMP, forskolin or dexamethasone) reversed the inhibitory effects of atrazine on NE. The data indicated that atrazine affects DA and NE synthesis, probably via an alteration of the enzymes TH and DβH (Das et al., 2003).

In a study on the effects of atrazine on the brain monoamine systems, groups of male Long-Evans (LE) rats received diets containing atrazine (purity, 98%) at a dose of 0, 5 or 10 mg/kg bw per day for 6 months, and locomotor activity (at 2, 3 and 6 months and 2 weeks after cessation of exposure), monoamine levels (in hypothalamus, prefrontal cortex, striatum, nucleus accumbens) and the numbers of TH-positive and TH-negative dopaminergic neurons (in substatia nigra pars compacta, ventral tegmental area) were determined. At 10 mg/kg bw per day, rats exhibited an enhanced locomotor activity response to a *d*-amphetamine challenge (evaluated at 2 months only), while increased locomotor activity was present at 3 and 6 months and at termination (2 weeks after cessation of exposure). Also at termination, the levels of various monoamines were decreased (e.g. DA, −20% in striatum; serotonin, −15% in hypothalamus; NE, −20% in prefrontal cortex), and the numbers of TH-positive and TH-negative dopaminergic neurons were reduced in both dopaminergic tracts (9–13%) at 10 mg/kg bw per day. Acute exposures of male rats to atrazine given intraperitoneally at doses of 100 and 200 mg/kg bw reduced basal and potassium-evoked striatal release of DA (Rodriguez et al., 2005).

In studies conducted in vivo to further examine effects of atrazine on the concentration of DA and NE in three different hypothalamic nuclei, administration of atrazine to intact female rats at doses of 25 and 75 mg/kg bw per day by gavage during one complete 4-day estrous cycle resulted in an increased DA concentration in the median eminence region (presumably the tuberoinfundibular dopaminergic neurons) and the medial preoptic area, when compared with controls, while NE concentration was not different in any of the three nuclei. Treatment at doses of 25 and 75 mg/kg bw per day also resulted in an increased concentration of GnRH in the median eminence when compared with controls (Cooper et al., 2007).

(c) Studies on effects on estrous cycle or LH surge

Rats

In a study on the effects of atrazine on ovarian function in the rat, female LE hooded and Sprague-Dawley rats aged approximately 15 weeks were given atrazine (purity, 97.1%) at a dose of

0, 75, 150 or 300 mg/kg bw per day by gavage for 21 days. Only rats that displayed regular 4-day estrous cycles for 2 weeks were used in the study. Blood for serum hormone concentrations (E2, progesterone) was taken from rats that displayed a pattern of vaginal diestrus for 10 days. After the 21-day treatment period, all females were ovariectomized (the day of ovariectomy was selected by using the vaginal smear pattern) and the ovaries were examined microscopically.

In both strains, dosing at 75 mg/kg bw per day disrupted the 4-day ovarian cycle; however, no distinct alteration (i.e. irregular cycles but not persistent estrus or diestrus) was apparent at this dose. At 150 mg/kg bw per day, atrazine induced repetitive pseudopregnancies in females of both strains. The highest dose tested (300 mg/kg bw per day) also induced repetitive pseudopregnancies in the Sprague-Dawley females, while the ovaries of the LE hooded females appeared regressed and the smear cytology was indicative of the anestrous condition.

A NOAEL was not identified; however, the doses employed were in excess of those used in long-term feeding studies in which an early onset of mammary gland tumours was noted. These data demonstrate that atrazine can disrupt ovarian function and bring about major changes in the endocrine profile of the female rat (Cooper et al., 1996).

In a pilot study designed to determine the validity of a proposed protocol for testing the effect of exposure to atrazine on the proestrous afternoon LH surge, E2 as estradiol benzoate was given to 70 ovariectomized female Sprague-Dawley rats (Crl:CD BR) through a subcutaneously surgically implanted capsule. Serum concentrations of LH, E2, and prolactin were measured 3 days later at 2-h intervals. The results showed that nearly all of the rats had E2 concentrations within the desired range of 75–150 pg/ml in the hour preceeding and during the rise of LH. There was a peak in LH secretion at 16:00 biological time, while prolaction showed a peak at 14:00 biological time and a return to baseline values by 24:00 biological time. The results were consistent with numerous published reports of LH and prolactin surges in intact cycling female rats, thus demonstrating the validity of the experimental method (Morseth, 1996a).

In a study of method validation designed to determine the optimum experimental methods to be used for testing the integrity of the LH surge in atrazine-treated female rats, E2 (as estradiol benzoate) was given to 20 ovariectomized female Sprague-Dawley (Crl:CD BR) rats through a subcutaneously surgically implanted capsule. Ten rats were used as a control group for the vehicle (0.5% aqueous carboxymethyl-cellulose), and ten rats were given atrazine (purity, 97.1%) by oral gavage for 3 days, beginning the day after surgery. The rats were subsequently bled at designated intervals (11:00, 13:00, 15:00, 18:00 and 22:00 biological time), and serum LH and prolactin concentrations were measured. Results indicated that the LH surge was attenuated in rats treated with atrazine at 15:00, the time range in which the peak LH surge is expected to occur in young intact rats. Prolactin concentrations rose over the course of the day, but failed to return to the expected baseline level late in the day in the control group and in the rats treated with atrazine, which was considered to be a response to the stress of repeated jugular bleeding (Morseth, 1996b).

In a study designed to evaluate the effects of atrazine on the pre-ovulatory LH surge and on the estrous cycle, groups of 90 female Sprague-Dawley (Crl:CD BR) rats received diets containing atrazine (purity, 97.1%) at a concentration of 0, 25, 50 or 400 ppm (equal to 0, 1.8, 3.65 and 29.44 mg/kg bw per day) for 26 weeks. The study was conducted in compliance with GLP guidelines. Vaginal smears were evaluated for 2-week periods each 4 weeks. Ten days before beng killed, the rats were ovariectomized, and 3 days before being killed a capsule releasing E2 was implanted subcutaneously. The rats were killed by decapitation in groups of 10 or 15 at designated intervals (11:00, 14:00, 16:00, 18:00, 20:00 and 23:00 biological time), and blood samples were analysed for LH, prolactin and E2. Serial blood samples were drawn without anaesthesia at the same time-point from an additional group of 10 rats and

analysed for LH. Rats were necropsied and histology (limited to mammary tissue, uterus, vagina and ovaries by the study plan) was not reported. Rats showing abnormal E2 data were excluded from the results.

There were no compound-related effects in mortality or clinical signs. Body weight, body-weight gain and food consumption were significantly decreased in rats at the highest dose tested compared with controls.

The percentage of days in estrus was significantly increased during the 21–22 and 25–26-week periods at 400 ppm. The percentage of days in estrus was also increased during the 21–22 and 25–26-week periods at 50 ppm, but the increase was only significant for the 21–22-week period (Table 21).

All the rats evaluated had E2 concentrations that were within the acceptable range to prime the LH surge in the hour preceding and during the rise of LH. The baseline (11:00) LH values were similar among groups. In non-repeat bled rats, LH surges comparable to those in controls were observed at 25 and 50 ppm, while rats at 400 ppm failed to have an LH surge, with LH values never rising above baseline. In repeat-bled rats, the LH surge was also severely attenuated at 400 ppm and less so at 50 ppm (maximum increase over baseline was 157% compared with maximum increase over baseline of of 273% in controls). Prolactin concentrations increased over the course of the sampling period in all groups, with peak values noted at 14:00 and 16:00, and began to return to their baseline values by 20:00 biological time. Treatment with atrazine had no effect on prolactin concentrations.

Most of the rats at termination had distended uteri with fluid-filled lumen, a condition commonly seen in ovarietomized, E2-stimulated rats. Selected tissues were saved for histopathology, but the absence of the histopathology report was not considered to be of significance when interpreting the results.

Table 21. Selected findings in a study designed to evaluate the effects of atrazine on the pre-ovulatory LH surge and on the estrous cycle in rats

Finding	Dietary concentration (ppm)			
	0	25	50	400
Body-weight gain (g), weeks 1–26	133	138	131	114*
Food consumption (g), weeks 1–25	3438	3462	3455	3309*
Percentage of days in diestrus/estrus				
Weeks 1–2	58/22	57/22	56/22	61/21
Weeks 13–14	49/31	53/28	49/31	44/40*
Weeks 17–18	47/34	49/33	47/36	41/45*
Weeks 21–22	51/32	43/41	39**/45*	37**/51**
Weeks 25–26	40/47	42/48	34/54	29*/63*
LH (pg/ml), non-repeat bled rats:				
11:00	1900	1816	1581	1863
18:00	3456	3235	3175	1358*
20:00	2327	2249	1899	1308*
LH (pg/ml), repeat-bled rats:	909	1075	972	1005
11:00				
18:00	3336	3631	2500	858*
20:00	3388	2510	2409	1042*

From Morseth (1996c)
LH, luteinizing hormone.
* $p < 0.05$; ** $p < 0.01$.

The NOAEL was 25 ppm, equal to 1.8 mg/kg bw per day, on the basis of estrous cycle alterations and attenuation of the LH surge at 50 ppm (Morseth, 1996c).

In a study on the effects of atrazine on the hypothalamic control of pituitary-ovarian function in the rat, the estrogen-induced surges of LH and prolactin were examined in ovariectomized Sprague-Dawley and LE rats treated with atrazine (purity, 97.1%) by gavage for 1, 3 or 21 days. Rats treated for 1 day were ovariectomized and received a subcutaneous estrogen implant on day 0, and then atrazine at a dose of 0, 50, 100, 200 or 300 mg/kg bw on day 3, and were killed at 0, 1, 3 or 6 h after treatment. Rats treated for 3 days were ovariectomized and received a subcutaneous estrogen implant on day 0, and then atrazine at a dose of 0, 50, 100, 200 or 300 mg/kg bw per day on days 1–3, and were killed at 0, 1, 3 or 6 h after treatment on day 3. Rats treated for 21 days were ovariectomized on day 0, and then received atrazine at a dose of 0, 75, 150 or 300 mg/kg bw per day on days 1–21, followed by a subcutaneous estrogen implant on day 21 and were killed on day 24 at the time of expected peak LH concentration.

Atrazine at the highest single dose (300 mg/kg bw) significantly suppressed the LH and prolactin surge in ovariectomized LE rats, but not in Sprague-Dawley rats. Treatment with atrazine on three consecutive days suppressed the estrogen-induced LH and prolactin surges in ovariectomized LE females in a dose-dependent manner at 50 mg/kg bw and greater, but this same treatment was without effect on serum LH and prolactin in Sprague-Dawley females. Also in LE females, exposure to atrazine at a dose of 50 mg/kg bw and greater inhibited the decrease in pituitary prolactin concentration that was observed in rats in the control group. After 21 days of treatment with atrazine, there was a significant dose-dependent suppression of serum LH and prolactin in both strains at all doses, while the concentration of prolactin in the pituitary was significantly increased in both strains at all doses.

In a further experiment conducted to determine the effect of a single dose of atrazine on ovulation and ovarian cycling, intact female LE rats displaying regular 4-day estrous cycles received atrazine at a dose of 0, 75, 150 or 300 mg/kg bw on the day of vaginal proestrus, and vaginal smears were examined for 3 weeks in half of the rats in each group, while in the remaining rats, oocytes were collected and quantified on the day of vaginal estrus. Atrazine administered at the highest dose of 300 mg/kg bw induced a pseudopregnancy in seven of nine females, but was without effect on ovulation.

Three further experiments were performed to determine whether the brain, pituitary or both organs were target sites for atrazine. These included examination of the ability of: (a) the pituitary lactotrophs to secrete prolactin, using hypophysectomized females bearing pituitary autotransplants (ectopic pituitaries) in female LE rats receiving atrazine as a single dose at 300 mg/kg bw; (b) the synthetic GnRH to induce LH secretion in ovariectomized LE rats treated with atrazine at a dose of 300 mg/kg bw for 3 days; and (c) atrazine (administered in vivo or in vitro) to suppress LH and prolactin secretion from pituitaries, using a flow-through perfusion procedure. The results indicated that: (a) the secretion of prolactin by the pituitary was not altered by atrazine if the gland was removed from the influence of central nervous sytem factors; (b) the effect of atrazine on LH secretion was not a result of a direct impairment of LH release from the pituitary; and (c) direct exposure of the pituitary had no effect on the release of LH and prolactin.

In summary, the experiments demonstrated a clear effect of atrazine on the estrogen-induced LH and prolactin surge in female LE and Sprague-Dawley rats. LE rats appeared to be more sensitive to the hormone-suppressive effects of atrazine than Sprague-Dawley rats. The results support the hypothesis that the effects of atrazine on LH and prolactin secretion are mediated via a hypothalamic site of action (Cooper et al., 2000).

In a study designed to compare the effects of atrazine and its metabolite DACT on the the pre-ovulatory LH surge, groups of 20 female Sprague-Dawley [Crl:CD BR] rats were given atrazine (purity, 97.1%) or DACT (purity, 96.8%) at a dose of 2,5, 5, 40 or 200 mg/kg bw per day by oral

gavage for 4 weeks; a group of 40 females served as a control group for the vehicle (0.5% carboxymethyl cellulose). The study was conducted in compliance with GLP guidelines. On day 22, all rats were ovariectomized, and on day 28, a capsule releasing E2 was implanted subcutaneously. On day 31, all rats were dosed at about 06:30 (10:30 biological time), and serial blood samples were collected from each rat at designated time-points (13:00, 16:00, 18:00, 20:00, 22:00 and 24:00 biological time) for analysis of LH. The maximum LH amplitude (LH_{max}), the time to maximum concentration of LH (T_{max}), and the area under the LH curve (AUC) were evaluated. After the final blood sample, the rats were killed and mammary tissues, uterus, vagina and pituitary were examined and preserved. Administration of both atrazine and DACT was associated with body-weight losses during the first week of treatment at doses of 40 mg/kg bw per day and greater, while body-weight gain during the study was decreased at doses of 40 mg/kg bw per day or greater for DACT and 200 mg/kg bw per day for atrazine, respectively. Both LH_{max} and AUC were significantly decreased in rats given DACT at 200 mg/kg bw per day, but not in rats given atrazine. There was no effect of treatment on T_{max} for either substance (Minnema, 2001).

In a subsequent study designed to compare the effects of atrazine and its metabolite DACT on the the pre-ovulatory LH surge, groups of 16 (dose groups) or 32 (control group) female Sprague-Dawley [Crl:CD BR] rats received diets containing atrazine (purity, 97.1%) at a concentration of 0, 25, 50, 70 or 400 ppm (equal to 0, 1.8, 3.4, 4.9 or 28.2–29.1 mg/kg bw per day) or DACT (purity, 96.8%) at atrazine molar equivalent concentrations of 0, 17, 34, 48 or 270 ppm (equal to 0, 1.2, 2.4, 3.4 or 18.8–19.7 mg/kg bw per day), respectively. Sixteen rats in each treatment group and 32 rats in the control group were designated for assessment of the plasma LH surge during weeks 30–31 (after at least 29 weeks of treatment). Fifty rats in the control group and 20 rats in each of the groups at the highest dose were designated for histopathology examination after 52 weeks of treatment. The study was conducted in compliance with GLP guidelines. During weeks 30 and 31, all surviving rats designated for plasma LH-surge assessment were ovariectomized. Six days later, a silastic E2 capsule was implanted subcutaneously; the LH surge was examined 3 days later. For the LH-surge measurement, blood samples were collected from each rat at designated time-points (13:00, 16:00, 18:00, 20:00, 22:00 and 24:00 biological time). At the highest doses of atrazine or DACT, the mean body-weight gains were 72% or 84% of those in the control group after 29 weeks of treatment, respectively, and 65% or 95% of those in the control group after 52 weeks of treatment, respectively. Exposure of rats to DACT at the highest dose resulted in a significant reduction in the estrogen-induced LH surge, while there was no such effect for atrazine.

The NOAEL for effects on LH was 400 ppm (equal to 29.1 mg/kg bw per day) for atrazine and 48 ppm (equal to 3.4 mg/kg bw per day) for DACT (Sielken & Holden, 2002).

On the basis of the analysis of the vaginal smears (percentage days in diestrus, percentage days in diestrous blocks, percentage days in estrus, and percentage days in estrous blocks), treatment with atrazine or DACT did not affect the estrous cycle. Microscopic examination of the female reproductive organs revealed no effects associated with treatment with atrazine or DACT, although a non-significant increase in the percentage of pituitary adenomas was noted at the highest doses of atrazine and DACT when compared with controls (19% and 22% vs 8%, respectively). Statistically significant increases in the incidences of mammary carcinoma and mammary fibroadenoma-carcinoma were noted at the highest dose of DACT (17% and 23%, respectively), when compared with those for the controls (5% and 7%, respectively).

Overall, the NOAEL for atrazine was 70 ppm, equal to 4.9 mg/kg bw per day, on the basis of decreased body-weight gain at 400 ppm. The NOAEL for DACT was 48 ppm, equal to 3.4 mg/kg bw per day, on the basis of decreased body weight, attenuation of the LH surge and increased incidences of mammary carcinoma and mammary fibroadenoma-carcinoma at 270 ppm (Minnema, 2002; Sielken & Holden, 2002).

In a study on the effect of atrazine on LH secretion in the intact proestrus female rat, LE rats displaying regular 4-day estrous cycles reveived atrazine (purity, not reported) at a dose of 0, 6.25, 12.5 or 25 mg/kg bw per day by gavage through a full ovarian cycle, beginning at vaginal estrus. The rats were maintained on a 14-h light : 10-h dark schedule (lights on at 05:00) and dosing was done at 12:00. The last dose was administered on the day of vaginal proestrus and groups were killed at 2-h intervals until lights out (12:00, 14:00, 16:00, 18:00 and 20:00). Atrazine, at all doses administered, resulted in a significant decrease in peak LH concentration when determined at 18:00. However, the effect was of a similar extent at all doses tested, and the study authors believed that the flat dose–response relationship reflected the effect of atrazine on LH within this range of dosing (Cooper et al., 2007).

Monkeys

In a study on the effect of atrazine on pituitary hormone secretion in a non-human primate model, groups of six female ovariectomized Rhesus monkeys received atrazine (purity, 97.2%; in 0.5% carboxymethyl cellulose) at a dose of 0 or 25 mg/kg bw per day by oral gavage for 30 days, and were then kept an additional 60 days for recovery. All monkeys received a subcutaneous injection of estradiol benzoate (330 µg) on day 5 before treatment, on days 5 and 26 of treatment and on day 26 of recovery. Blood samples for hormone analysis (LH, FSH, prolactin, progesterone, E2, cortisol) were collected from each monkey approximately 12 h after administration of E2 (as estradiol benzoate), and then at 6-h intervals (up to 102 h after administration of E2).

No treatment-related clinical signs were observed. Body weight was not affected by treatment except in one monkey treated with atrazine that lost 1 kg (19%) of its initial body weight over the course of the study. This monkey displayed a marked suppression of LH and FSH surges after 5 or 26 days of treatment, which was confounded with the effects of other non-specific stressors. In the remaining monkeys, no treatment-related effects on concentrations of LH, FSH, E2, progesterone or prolactin were observed. However, a non-specific stress response in treated monkeys appeared to be associated with intolerance to high doses of atrazine. This resulted in the inability to acclimatize to experimental conditions as demonstrated by smaller reductions in cortisol in monkeys treated with atrazine when compared with controls. In conclusion, the primate model for assessing effects on LH secretion is limited by intra- and inter-animal variability in the normal response to estrogen-induced LH secretion, which is confounded by apparent stress effects related to the required experimental design (Osterburg & Breckenridge, 2004; Simpkins & Eldridge, 2004).

(d) Studies on reproductive and developmental effects

In a study designed to examine the effects of atrazine on suckling-induced prolactin release in Wistar rats, dams were given atrazine (purity, 98%) at a dose of 0, 6.25, 12.5, 25, or 50 mg/kg bw twice per day by gavage on postnatal days 1–4, or the DA-receptor agonist bromocriptine (BROM, which is known to suppress the release of prolactin) twice per day by subcutaneous injection at a dose of 0.052, 0.104, 0.208, or 0.417 mg/kg bw. Serum prolactin concentrations were measured on postnatal day 3 using a serial sampling technique and in-dwelling cardiac catheters. The study hypothesis was that early lactational exposure to agents that suppress the suckling-induced release of prolactin would lead to a disruption in the development of the tuberoinfundibular neurons in the pups (presumably due to the lack of prolactin derived from the dam's milk), with the consequence of impaired regulation of prolactin secretion, hyperprolactinaemia before puberty and prostatitis in the adult male offspring.

A significant rise in the release of prolactin in serum was noted in all females in the control group within 10 min of the initiation of suckling. At 50 mg/kg bw, atrazine inhibited the suckling-induced release of prolactin in all females, while atrazine at a dose of 25 and 12.5 mg/kg bw inhibited this release in some dams but had no discernible effect in others. Give twice per day at a at

a dose of 6.25 mg/kg bw (i.e. 12.5 mg/kg bw per day), atrazine was without effect. BROM, used as a positive control, also inhibited the suckling-induced release of prolactin at doses of 0.104 to 0.417 mg/kg bw, with no effect at 0.052 mg/kg bw. To examine the effect of postnatal exposure to atrazine and BROM on the incidence and severity of inflammation of the lateral prostate of the offspring, adult males were examined at 90 and 120 days. While no effect was noted at age 90 days, by 120 days, the incidence and severity of prostate inflammation were both increased in offspring of dams treated with atrazine at 25 and 50 mg/kg bw. Atrazine at a dose of 12.5 mg/kg bw and BROM at a dose of 0.208, or 0.417 mg/kg bw increased the incidence, but not the severity, of prostatitis. Combined treatment with ovine prolactin and atrazine at 25 or 50 mg/kg bw on postnatal day 1–4 reduced the incidence of inflammation observed at 120 days, indicating that this increase in inflammation, seen after atrazine alone, resulted from the suppression of prolactin in the dam.

To determine whether there was a critical period for these effects, dams were given atrazine at a dose of 25 or 50 mg/kg bw twice per day on postnatal days 6–9 and postnatal days 11–14. Inflammation was increased in offspring from dams treated on postnatal days 6–9, but this increase was not statistically significant. Dosing on postnatal day 11–14 was without effect. These data demonstrated that atrazine suppresses the suckling-induced release of prolactin and that this suppression results in lateral prostate inflammation in the offspring. The critical period for this effect is postnatal days 1–9 (Stoker et al., 1999).

In a study on the effects of atrazine on implantation and embryo viability during early gestation in rats, groups of rats of one of four strains (Holtzman; Sprague-Dawley; LE; Fischer 344, F344) were given atrazine (purity, 97.1%) at a dose of 0, 50, 100 or 200 mg/kg bw per day by gavage during days 1–8 of gestation during either the light or dark period of a 14 : 10 light : dark cycle. All rats were necropsied on day 8 or 9 of pregnancy. At a dose of 200 mg/kg bw per day, atrazine reduced body-weight gain in all except one group (F344 diurnal dosing), and nocturnal dosing resulted in significant effects on body-weight gain at lower doses than did diurnal dosing. F344 rats showed a significant increase in pre-implantation loss after nocturnal dosing at 100 and 200 mg/kg bw per day, with no effect in other strains, while Holtzman rats showed a significant increase in postimplantation loss after diurnal and nocturnal dosing at 100 and 200 mg/kg bw per day, with no effect in other strains. Only in Holtzman rats was there a significant decrease in serum progesterone concentrations at 100 and/or 200 mg/kg bw per day in both intervals of dosing, while serum E2 concentration was significantly increased only in Sprague-Dawley rats at 200 mg/kg bw per day and by diurnal dosing. A significant reduction in serum LH concentration was seen in several groups, but there was no effect in Sprague-Dawley rats. In conclusion, F344 rats were most susceptible to the pre-implantation effects of atrazine, while Holtzman rats appeared to be most sensitive to the postimplantation effects. LE and Sprague-Dawley rats were least sensitive to the effects of atrazine during very early pregnancy (Cummings et al., 2000).

In a study on the effects of atrazine on the early postimplantation phase of pregnancy, F344, Sprague-Dawley and LE rats were given atrazine (purity, 97.1%) at a dose of 0, 25, 50, 100 or 200 mg/kg bw per day by gavage on days 6 to 10 of gestation. The dams were allowed to deliver and litters were examined postnatally.

Significant maternal body-weight losses on days 6–7 of gestation were seen in F344 rats at a dose of 50 mg/kg bw per day and greater, in Sprague-Dawley rats at a dose of 25 mg/kg bw per day and greater and in LE rats at a dose of 100 mg/kg bw per day and greater. The F344 strain was the most sensitive to effects on pregnancy, showing full-litter resorption at a dose of 50 mg/kg bw per day and greater. In surviving F344 litters, prenatal loss was increased at 200 mg/kg bw per day. In Sprague-Dawley and LE rats, full-litter resorption occurred only at 200 mg/kg bw per day (Table 22).

Delayed parturition was seen at a dose of 100 mg/kg bw per day and greater in F344 and Sprague-Dawley rats.

In F344 rats given atrazine at at a dose of 200 mg/kg bw per day on days 11 to 15 of gestation (after the LH-dependent period of pregnancy), no full-litter resorptions were seen. These findings suggest that the induction of full-litter resorption by atrazine is maternally mediated, and consistent with loss of LH support of the corpora lutea (Narotsky et al., 2001).

(e) Studies on female pubertal development

In a study on the effects of atrazine on pubertal development and thyroid function in rats, groups of 15 female Wistar rats were given atrazine (purity, 97.1%) at a dose of 0, 12.5, 25, 50, 100 or 200 mg/kg bw per day by gavage on postnatal days 22 to 41. An additional control group was included that was pair-fed with the group at 200 mg/kg bw per day in order to detect any effects caused by the reduced food consumption observed at this dose.

Body weight on postnatal day 41 was reduced by 11.6% at 200 mg/kg bw per day, but at 50 and 100 mg/kg bw per day was not different from that of the controls. Adrenal, kidney, pituitary, ovary, and uterine weights were also reduced at 200 mg/kg bw per day. The day of vaginal opening was significantly delayed by 3.4, 4.5 or more than 6.8 days at 50, 100, and 200 mg/kg bw per day, respectively. Although body weight in the pair-fed controls was reduced to the same extent as at 200 mg/kg bw per day, vaginal opening was not significantly delayed. Serum triiodothyronine (T3), thyroxine (T4), and TSH concentrations were unaltered by atrazine, which was consistent with the fact that no histological changes were observed in the thyroid.

Estrous cyclicity was monitored in a second group of females from the day of vaginal opening to postnatal day 49. The number of females displaying regular 4- or 5-day estrous cycles during the first 15-day interval after vaginal opening was decreased at 100 and 200 mg/kg bw per day and in the pair-fed controls. Irregular cycles were characterized by extended periods of diestrus. By the end of the second 15-day interval (postnatal days 57–71), no effects on estrous cyclicity were observed.

The data indicated that atrazine can delay the onset of puberty and alter estrous cyclicity in the female Wistar rat. Reduced food consumption and body weight did not account for the delay in vaginal opening, because this effect was not observed in the pair-fed controls. In addition, no effect on estrous cyclicity was observed at 100 mg/kg bw per day where no significant reduction in body weight was observed.

The NOAEL was 25 mg/kg bw per day, on the basis of delayed vaginal opening at 50 mg/kg bw per day and greater (Laws et al., 2000).

Table 22. Summary of ED_{10} estimates and benchmark doses for full-litter resorption in rats given atrazine by gavage

Strain	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)	Dams with full-litter resorption at LOAEL (%)	ED_{10} (mg/kg bw per day)	Benchmark dose (mg/kg bw per day)
Fischer 344	25	50	36	15.1	11.7
Sprague-Dawley	100	200	67	170.3	97.9
Long-Evans	100	200	67	170.6	103.0

From Narotsky et al. (2001)
ED10, estimated dose that causes full litter resorption in 10% of the dams; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level.

In a study on the effects of atrazine on the sexual maturation of female rats, groups of 8–10 female Wistar and Sprague-Dawley rats were given atrazine (purity, 98.2%) at a dose of 0, 10, 30 or 100 mg/kg bw per day by gavage from postnatal day 21 until postnatal day 43 (Wistar rats) or postnatal day 46 (Sprague-Dawley rats). A separate group of Wistar rats was given the GnRH agonist, antarelix (ANT), which is known to block the release of LH from the pituitary and delay vaginal opening. Uterine weights were determined at postnatal days 30, 33, 43 (Wistar rats) and 46 (Sprague-Dawley rats), and the time of vaginal opening was assessed.

In rats exposed to ANT, uterine growth and vaginal opening were completely prevented. In Wistar rats exposed to atrazine at 100 mg/kg bw per day, vaginal opening was significantly delayed (by 3 days), while uterine growth was delayed at postnatal days 30 and 33, but this growth inhibition had been overcome by postnatal day 43. In Sprague-Dawley rats exposed to atrazine at 30 and 100 mg/kg bw per day, vaginal opening was significantly delayed (by 2.5 and 3 days, respectively), while uterine weights were unaffected at postnatal day 46.

The NOAEL was 10 mg/kg bw per day in Sprague-Dawley rats and 30 mg/kg bw per day in Wistar rats on the basis of delayed vaginal opening at 30 or 100 mg/kg bw per day, respectively (Ashby et al., 2002).

In a study on the effects of atrazine metabolites on pubertal development and thyroid function in female rats, groups of 15 female Wistar rats were given DACT (purity, 96.8%) at a dose of 0, 16.7, 33.8, 67.5 or 135 mg/kg bw per day or hydroxyatrazine (purity, 97.1%) at a dose of 0, 22.8, 45.7, 91.5 or 183 mg/kg bw per day by gavage on postnatal days 22–41. These doses were equivalent to atrazine equimolar doses of 0, 25, 50, 100 and 200 mg/kg bw per day.

The females were monitored daily for vaginal opening and killed on postnatal day 41. DACT significantly delayed vaginal opening by 3.2, 4.8, and 7.6 days at doses of 33.8, 67.5, and 135 mg/kg bw per day, respectively. The NOAEL for DACT of 16.7 mg/kg bw per day was identical to the equimolar NOAEL of 25 mg/kg bw per day for atrazine. For hydroxyatrazine, no significant delays in pubertal development were observed in two separate studies with doses ranging up to 183 mg/kg bw per day, identical to the equimolar NOAEL for atrazine of 200 mg/kg bw per day. No significant or dose-related effects were observed on serum thyroid hormone concentrations (T3, T4 and TSH) or thyroid histopathology (Laws et al., 2003).

(f) Studies on male pubertal development

In two separate studies, the effects of atrazine on pubertal development in male rats were investigated. In the first study, groups of 9–12 male Sprague-Dawley rats were given atrazine (purity, 96.1%) at a dose of 0, 1, 2.5, 5, 10, 25, 50, 100 or 200 mg/kg bw per day on postnatal days 22–47. Since the first study showed that atrazine significantly suppressed body-weight gain at doses of 100 mg/kg bw per day and greater, a second study was performed. One group received atrazine at a dose of 100 mg/kg bw per day, another group received the vehicle (0.5% carboxymethylcellulose) and was fed the mean daily intake of food consumed by the atrazine-treated group on the previous day, and a further group received the vehicle and was fed ad libitum.

There was no effect on any of the measured variables (body weight, food consumption, day of preputial separation, serum testosterone, serum LH, intratesticular testosterone, ventral prostate weight, and seminal vesicle weight) at doses of up to 50 mg/kg bw per day, while doses of 100 mg/kg bw per day or more significantly reduced body-weight development (–9% and −21% at 100 and 200 mg/kg bw per day, respectively). In the second study in the additional pair-fed control group, body-weight was 10% lower than that of the control group fed ad libitum and was comparable to that of the group at 100 mg/kg bw per day. Lower values for serum and intratesticular testosterone concentrations were seen at doses of 100 mg/kg bw per day and greater; similar changes were also

observed in the pair-fed group. Reduced ventral prostate and seminal vesicle weights (both organs being androgen-dependent) paralleled the lower testosterone concentrations. Again, decreased ventral prostate and seminal vesicle weights were noted in pair-fed rats. Additionally, lower LH concentrations were observed in groups treated with atrazine at 100 mg/kg bw per day and greater, as well as in pair-fed rats. A delay in preputial separation by about 3 and 4 days was observed at doses of 100 and 200 mg/kg bw per day, respectively (Trentacoste et al., 2001).

In a study on the effects of atrazine on pubertal development and thyroid function in male rats, male Wistar rats were given atrazine (purity, 97.1%) at a dose of 0, 6.25 (preputial separation study only) 12.5, 25, 50, 100, 150, or 200 mg/kg bw per day by gavage on postnatal days 23–53. An additional control group was included that was pair-fed with the group at 200 mg/kg bw per day in order to detect any effects caused by the reduced food consumption observed at this dose. Preputial separation was monitored from postnatal day 33 onwards. The majority of the males were killed on postnatal day 53. The pituitary, testes, ventral and lateral prostates, epididymides, and seminal vesicles with coagulating gland were removed and weighed. Blood was analysed for TSH, T4, T3, LH, prolactin, E2 and estrone. Additionally, concentrations of LH and prolactin were determined in the anterior pituitaries. A subgroup of males in the control group and males at the highest dose was killed on day 45 as described in the previous study for determination of LH receptors as well as serum and intratesticular testosterone content. Finally, another subgroup of rats was killed on postnatal day 120 to examine the reversibility of the effects.

Body weights at 200 mg/kg bw per day were significantly decreased (−17% at postnatal day 53) and returned to normal at postnatal day 120, while body weights of pair-fed rats were decreased to a comparable extent (−14% at postnatal day 53). A dose-dependent decrease in ventral prostate weights was observed with atrazine at doses of 50 mg/kg bw per day and greater and in the pair-fed group; this effect was still seen at postnatal day 120 at 200 mg/kg bw per day. Seminal vesicle and epididymal weights were decreased on postnatal day 53 at 200 mg/kg bw per day and in the pair-fed group. The latter effects were no longer seen at postnatal day 120. There was no effect of treatment on testes weight.

Preputial separation was significantly delayed at 12.5, 50, 100, 150, and 200 mg/kg bw per day (by 2.3, 1.7, 1.7, 1.7, and 3 days, respectively), while there was no such effect in a separate group at a dose of 6.25 mg/kg per day. Preputial separation was also delayed (by 2 days) in the pair-fed control group, although significantly less than in the group at 200 mg/kg bw per day.

Serum testosterone concentrations were decreased at doses of 25 mg/kg bw per day and greater at postnatal day 53 and at 200 mg/kg bw per day at postnatal day 45; however, the decreases were not statistically significant. Intratesticular testosterone concentration was significantly lower at 200 mg/kg bw per day on postnatal day 45, but not on postnatal day 53. Testosterone concentrations in the pair-fed rats were not significantly different to those in rats in the control group that had access to food ad libitum. There was a dose-dependent increase in serum E2 and estrone concentrations on postnatal day 53; however, this increase was statistically significant only at 200 mg/kg bw per day, while concentrations in the pair-fed group were comparable to those in the control group that had access to food ad libitum.

There was a significant trend for a dose-dependent decrease in serum LH concentrations on postnatal day 53; however, mean LH concentrations were not significantly different from those of the controls. Prolactin was reduced at doses of 150 mg/kg bw per day and greater, but the differences failed to reach statistical significance. The number of LH receptors in the testes was not altered by treatment. Serum LH and prolactin concentrations were comparable in the pair-fed group and the rats in the control group that had access to food ad libitum. Serum T3 concentration was significantly increased at 200 mg/kg bw per day, while serum TSH and T4 concentrations were unaffected by treatment. Serum TSH, T3, T4, LH and prolactin concentrations in the pair-fed group were comparable to

those in the control group that had access to food ad libitum. There were no histopathological lesions observed in the thyroid gland of rats at the highest dose.

The results indicated that atrazine delays puberty and the development of the reproductive tract in the male rat. The mode of action appears to be in altering the secretion of steroids, probably due to disruption of control of pituitary function by the central nervous sysytem.

The NOAEL was 6.25 mg/kg bw per day on the basis of delayed preputial separation at 12.5 mg/kg bw per day and greater (Stoker et al., 2000).

In a subsequent study on the effects of atrazine metabolites on pubertal development and thyroid function in male rats, groups of 8–18 (dose groups) or 38 (control group) male Wistar rats were given DEA at a dose of 10.8, 21.7, 43,4, 86.8 or 173.9 mg/kg bw per day, or DIA at a dose of 10.4, 20.8, 40.1, 80.3 or 160.9 mg/kg bw per day, or DACT (purity, 97–98%) at a dose of 4.4, 8.4, 16.9, 33.8, 84.3 or 135.3 mg/kg bw per day by gavage on postnatal days 23–53. These doses were equivalent to atrazine equimolar doses of 0, 6.25 (DACT only), 12.5, 25, 50, 100 and 200 mg/kg bw per day.

Preputial separation was significantly delayed by treatment with DEA (atrazine equimolar dose, 25, 100 and 200 mg/kg bw per day), DIA (atrazine equimolar dose, 25, 100 and 200 mg/kg bw per day) and DACT (atrazine equimolar dose, 12.5–200 mg/kg bw per day). When the rats were killed on postnatal day 53, treatment with DEA (atrazine equimolar dose, 100 and 200 mg/kg bw per day), DIA (atrazine equimolar dose, 50–200 mg/kg bw per day) or DACT (atrazine equimolar dose, 200 mg/kg bw per day) caused a significant reduction in ventral protate weight, while only the highest doses of DEA and DIA resulted in a significant decrease in the lateral prostate weight. Seminal vesicle weight was reduced by DEA (atrazine equimolar dose, 25, 100 and 200 mg/kg bw per day), DIA (atrazine equimolar dose, 100 and 200 mg/kg bw per day) and DACT (atrazine equimolar dose, 100 and 200 mg/kg bw per day). Epididymal weights were reduced in the groups receiving DEA (atrazine equimolar dose, 200 mg/kg bw per day), DIA (atrazine equimolar dose, 200 mg/kg bw per day) and DACT (atrazine equimolar dose, 100 and 200 mg/kg bw per day). Serum testosterone concentration was reduced only in rats receiving DIA at the two higher doses. Serum estrone concentration was increased in the groups receiving DACT at the two higher doses, while serum E2 concentration was not changed relative to controls in any group. No differences were observed in any of the measures of thyroid activity.

The results indicated that the three chlorinated metabolites of atrazine delay puberty in a manner similar to atrazine, by affecting the control of the pituitary/gonadal axis by the central nervous system and subsequent development of the reproductive tract. The NOAELs for DEA, DIA, and DACT (expressed in atrazine equimolar doses) were 12.5, 12.5 and 6.25 mg/kg bw per day, respectively, on the basis of delayed preputial separation (Stoker et al., 2002).

(g) Studies on development of the mammary gland

In a study on the effects on development of the mammary gland in rats exposed in utero to atrazine, groups of 20 timed-pregnant LE rats were given atrazine (purity, 97.1%) at a dose of 0 (vehicle control) or 100 mg/kg bw per day by gavage on days 15–19 of gestation. On postnatal day 1, half of all litters were cross-fostered, creating four treatment groups: control–control, atrazine–control, control–atrazine, and atrazine–atrazine (dam–milk source, respectively). A significant delay in vaginal opening and increase in body weight at vaginal opening was seen only in the litters receiving milk from dams exposed to atrazine. However, mammary glands of female offspring (two per dam) in all groups exposed to atrazine (atrazine–control, control–atrazine, and atrazine–atrazine) displayed significant delays in epithelial development. These changes were detected as early as postnatal day 4 and stunted development was evident until postnatal day 40. Further, at all developmental stages

examined, offspring in the atrazine–atrazine group exhibited the least developed glands. These delays in pubertal end-points did not appear to be related to body weight or concentrations of endocrine hormone (Rayner et al., 2004).

In a subsequent study conducted to determine whether fetal development of the mammary gland was sensitive to treatment with atrazine during specific periods of development, timed-pregnant LE rats (n = 8 per group per block) were given atrazine (purity, 97.1%) at a dose of 0 or 100 mg/kg bw per day by gavage for 3- or 7-day intervals during gestation (days 13–15, 15–17, 17–19, or 13–19 of gestation), and their offspring were evaluated for changes. Mammary glands taken from pups exposed prenatally to atrazine displayed significant delays in epithelial development as early as postnatal day 4 compared with controls, and showed continued developmental delays at later time-points that varied by time of exposure. However, the most persistent and severe delays were seen in the groups exposed to atrazine during days 17–19 and 13–19 of gestation, which demonstrated statistically similar levels of growth retardation (Rayner et al., 2005).

In a study on development of the mammary gland in rats exposed in utero to atrazine, groups of seven or eight timed-pregnant LE rats were given atrazine (purity, 97.1%) at a dose of 0 or 100 mg/kg bw per day by gavage on days 15–19 of gestation. Delayed development of the mammary gland was detected in the pups on postnatal day 4 and persisted to postnatal day 66. Immunohistochemistry of mammary sections from postnatal day 41 demonstrated increased levels of staining for the estrogen receptor (ER) in the gland stroma, the epithelium and the stroma surrounding the epithelial layer of the terminal end buds of pups exposed to atrazine. Also, the level of progesterone-receptor staining was higher in the ductal epithelium and fat cell nuclei of rats exposed to atrazine in utero (Moon et al., 2007).

In a study on development of the mammary gland in rats exposed in utero to a mixture of atrazine metabolites containing atrazine, hydroxyatrazine, DACT, DEA and DIA, groups of six timed-pregnant LE rats were given the metabolite mixture at a dose of 0.09, 0.87 or 8.73 mg/kg bw per day by gavage on days 15–19 of gestation, using groups given atrazine at a dose of 0 or 100 mg/kg bw per day as negative and positive controls, respectively. Exposure to the metabolite mixture had no statistically significant effect on body-weight gain in dams during the dosing period, weight loss in pups on postnatal day 4, or pubertal timing, being effects that are seen with atrazine alone. However, as with atrazine, development of the mammary gland was delayed, when evaluated by whole-mount analysis, as early as postnatal day 4 in all treatment groups (Enoch et al., 2007).

Although alterations were seen in the development of the mammary gland after administration of atrazine alone or in combination with its metabolites (DACT, DEA, DIA, hydroxyatrazine), it is uncertain whether this morphological observation leads to an adverse consequence or a functionally relevant toxic effect. The scoring system for the observations of the mammary gland is not a validated and standardized procedure and it was not reported whether the scoring was done blind. Furthermore, the dose–response relationship appears flat or variable in the study in which a mixture of atrazine and its metabolites was administered. Because no data on the individual substances were generated, it is unclear what type of interactions may be occurring between atrazine and its metabolites in the mixture. Further work is needed to clarify and repeat these observations before concluding that exposure to a mixture of atrazine and its metabolites can cause alterations in development of the mammary gland at doses as low as 0.09 mg/kg bw per day, which would lead to an adverse public health consequence.

(h) Studies on estrogenic and anti-estrogenic potential

In tests for estrogenic bioactivity, groups of five or six ovariectomized adult female Sprague-Dawley rats were given atrazine (purity, 97.7%) or its metabolite DACT (purity, 98.2%) at an oral dose of 20, 100 or 300 mg/kg bw per day for three consecutive days. Uterine weight did not increase statistically significantly. At the highest dose of atrazine or or DACT, loss of body weight was seen. When the test substances were administered concomitantly with subcutaneous injections of E2 at a dose of 2 μg per rat, statistically significant decreases in uterine weight were seen with both atrazine and DACT at doses of 100 mg/kg bw per day and greater.

In further tests by the same research group, immature female Sprague-Dawley rats (age 23 days) were given atrazine or DACT at a dose of 1, 10, 50, 100 or 300 mg/kg bw per day by gavage for two consecutive days, and received E2 at a dose of 0.15 μg per rat by subcutaneous injection on the second day. Thymidine incorporation into uterine DNA was significantly reduced at doses of 50 mg/kg bw per day and greater.

Again, in further tests by the same research group, ovariectomized adult female Sprague-Dawley rats were given atrazine or DACT at a dose of 50 or 300 mg/kg bw per day by gavage for two consecutive days, and received E2 at a dose of 1 μg per rat by subcutaneous injection each day. Progesterone receptor-binding capacity in cytosol fractions from uteri was significantly reduced at 300 mg/kg bw per day. Uterine progesterone receptor levels were not stimulated in rats that received atrazine or DACT at doses of up to 300 mg/kg bw per day without E2 injections.

The results indicated that atrazine and DACT possess no intrinsic estrogenic activity, but that they are capable of weak inhibition of estrogen-stimulated responses in the rat uterus (Eldridge et al., 1994; Tennant et al., 1994a).

In a study on the interaction with ER binding, competitive co-incubation of atrazine (purity, 96.9%) or DACT (purity, 98.2%) and radiolabeled estrogen with rat uterine cytosol containing ER failed to demonstrate significant displacement of estrogen binding by atrazine. However, when cytosols were pre-incubated with atrazine, and tracer was added to the chilled incubation medium, there was a significant reduction of [^{3}H]E2 binding. Competition was very weak, with IC_{50} estimates of 20 μmol/l for atrazine and 100 μmol/l for DACT, which were four to five orders of magnitude greater than the approximate IC_{50} of E2.

Ex-vivo results in the same report indicated that the uterine ER-binding capacity was reduced by approximately 30% when ovariectomized adult female Sprague-Dawley rats were given atrazine or DACT at a dose of 300 mg/kg bw per day by gavage for two consecutive days; a dose of 50 mg/kg bw per day had no effect.

The result confirmed that atrazine and DACT may be capable of a very weak interaction with estrogen receptors, but only at extremely high concentrations (Tennant et al., 1994b).

The potential estrogenic activities of atrazine were investigated in vivo using the immature female Sprague-Dawley rat uterus and in vitro using the estrogen-responsive MCF7 human breast cancer cell line and the estrogen-dependent recombinant yeast strain PL3. Rats that were dosed with atrazine only at 50, 150, or 300 mg/kg bw per day for three consecutive days did not exhibit any significant increases in uterine wet weight, although decreases in cytosolic progesterone-receptor binding levels and uterine peroxidase activity were observed. 17β-estradiol (E2)-induced increases in uterine wet weight were not (statistically) significantly affected by co-treatment with atrazine; however, some dose-independent decreases in E2-induced cytosolic progesterone-receptor binding and uterine peroxidase activity were observed. In vitro, atrazine did not affect basal or E2-induced MCF7 cell proliferation or the formation of nuclear progesterone receptor–DNA complexes as determined by gel electrophoretic mobility shift assays. In addition, atrazine did not display agonist activity or

antagonize E2-induced luciferase activity in MCF7 cells transiently transfected with a Gal4-human estrogen receptor chimera (Gal4-HEGO) and a Gal4-regulated luciferase reporter gene (17m5-G-Luc). Moreover, the estrogen-dependent PL3 yeast strain was not capable of growth on minimal media supplemented with atrazine in place of E2. Collectively, the results indicated that atrazine did not exhibit estrogenic activity; however, antiestrogenic activity via competition for the estrogen receptor cannot be excluded (Connor et al., 1996, 1998).

In a study to investigate the effects of chlorotriazines on the E2 (as estradiol benzoate)/progesterone-induced LH surge and to determine whether such changes correlate with impaired estrogen receptor (ER) function, adult ovariectomized female Sprague-Dawley rats were given atrazine (purity, 97.1%) at a dose of 0, 30, 100 or 300 mg/kg bw per day or DACT (purity, 96.8%) at a dose of 0 or 77 mg/kg bw per day (a dose that achieved an AUC equivalent to dosing with atrazine at 300 mg/kg bw per day) by oral gavage for five consecutive days. Atrazine caused a dose-dependent suppression of the E2/progesterone-induced LH surge (dosing at 300 mg/kg bw per day completely blocked the LH surge), while DACT to a lesser degree suppressed total plasma LH and peak LH-surge concentrations in E2/progesterone-primed rats by 60% and 58%, respectively. Treatment with DACT also decreased the release of LH from the pituitary in response to exogenous GnRH by 47% compared with the controls. Total plasma LH secretion was reduced by 37% compared with controls, suggesting that, in addition to potential hypothalamic dysfunction, pituitary function is also altered. To further investigate the mechanism by which hypothalamic function might be altered, potential anti-estrogenicity of atrazine and DACT were assessed by evaluating ER function in treated rats. Using an assay for receptor binding in vitro, atrazine (but not DACT) inhibited binding of [^{3}H]E2 to ER at concentrations between 10^{-4} and 10^{-3} mol/l. In contrast, in female rats given atrazine under dosing conditions that suppressed the LH surge, the levels of unoccupied ER and the estrogen-induced up-regulation of progesterone receptor mRNA were not altered. The results indicated that although atrazine is capable of binding to ER in vitro, the suppression of LH after treatment with high doses of atrazine was not due to alterations of hypothalamic ER function (McMullin et al., 2004).

(i) Studies on aromatase activity or gene expression

Studies In vitro have indicated that atrazine (purity, not reported) in 0.1% dimethyl sulfoxide (DMSO) increased aromatase (CYP19) activity and expression in the human adrenocorticocarcinoma cell line H295R and in the human placental choriocarcinoma cell line JEG3, but not in the human breast-cancer cell line MCF7 or the rat Leydig-cell cancer cell line R2C (Sanderson et al., 2000, 2001; Heneweer et al., 2004). In the majority of these studies, atrazine induced aromatase activity to an apparent maximum of about 2- to 3-fold and increased CYP mRNA levels between 1.5- and 2-fold at concentrations of 30 μmol/l (6.5 ppm). DEA and DIA gave weaker responses than did atrazine, but DACT, which is the major metabolite of atrazine in rats, mice and Rhesus monkeys, had no effect, and neither did hydroxyatrazine, which is the major plant metabolite (Sanderson et al., 2001). In KGN human ovary granulosa-like carcinoma cells, atrazine had no effect on aromatase activity at concentrations of up to the maximum tested, 50 μmol/l (Ohno et al., 2004).

Atrazine significantly inhibited phosphodiesterase activity in vitro in homogenates of bovine and swine tissues (Roberge et al., 2004, 2006). Inhibition of phosphodiesterase activity resulted in elevated concentrations of cAMP, increased CYP19 mRNA levels and increased aromatase activity (Sanderson et al., 2002). However, atrazine increased aromatase expression only in cell and tissue types that use the steroidogenic factor 1-dependent aromatase promoter II, e.g. the human adrenocorticocarcinoma cell line H295R, while KGN human ovary granulosa-like carcinoma cells were not responsive (Fan et al., 2007).

Although it has been reported that atrazine induces aromatase (CYP19) mRNA and aromatase activity in certain human cell lines, their biological significance remains in question because of the complex, tissue-specific manner through which aromatase is regulated. The aromatase enzyme expressed in all tissue types is identical, but the promoters, signalling pathway, and proteins involved in initiation of transcription vary between tissue types (Simpson et al., 2002). Also, the results of studies in rats did not support any atrazine-induced upregulation of aromatase expression in brain, testes, or mammary gland.

In a study on development of the mammary gland in vivo that has been described above, in the offspring of rats exposed to atrazine on days 15–19 of gestation the mammary gland expressed significantly less aromatase than did controls at postnatal day 33, while no significant differences were observed between groups at postnatal day 40 (Rayner et al., 2004).

In studies on the effects of atrazine on steroidogenesis, male Wistar rats aged 60 days were given atrazine (purity, 97.1%) at a dose of 0, 50 or 200 mg/kg bw per day by gavage once per day for 1, 4 or 21 days. The rats were killed at 3, 6 or 24 h after the single dose, or 3 h after the last repeat dose (4 or 21 days). Serum estrone, E2, testosterone, androstenedione, progesterone, corticosterone, and hypothalamic and testicular CYP19 mRNA were measured for each time-point. After one dose of atrazine at 200 mg/kg bw, concentrations of progesterone and corticosterone were increased at 3 h, and corticosterone and estrone were elevated at 24 h; androstenedione and testosterone were increased at 6 h for both doses. After 4 or 21 days of treatment at 200 mg/kg bw, concentrations of corticosterone, estrone and E2 were elevated, but androstenedione and testosterone remained at concentrations similar to those of the controls. To determine the effect of atrazine on adrenal steroidogenesis, castrated males treated with atrazine were examined. Despite reduced androstenedione and testosterone concentrations in castrated 200 mg/kg males, increased serum corticosterone, estrone and E2 mirrored previous results. Elevated progesterone was also observed. No change in aromatase CYP19 mRNA was detected by real-time reverse transcription polymerase chain reaction (RT-PCR) in the testicular or hypothalamic tissues at any time-point. These data suggested that elevated concentrations of estrone and E2 were not strictly caused by increased testicular steroidogenesis, altered aromatase mRNA, or substrate availability, but did not rule out a change in steroid metabolism or elimination. Although no CYP19 mRNA was detected in the adrenals, a stress-induced adrenal response may be partially responsible for the increase in steroids. However, data from an assay with minced testes in vitro showed an increase in testosterone after a 4 h exposure to atrazine. Together, these data demonstrated that atrazine can alter the steroidogenic pathway in male Wistar rats (Modic, 2004; Modic et al., 2004).

(j) Studies on adrenal steroidogenesis

In a study to evaluate adenocorticotropic hormone (ACTH), corticosterone, progesterone and testosterone after a single dose of atrazine, male Wistar rats (age 60 days) were acclimatized to dosing by gavage (with methylcellulose) for 7 days. On day 8, the rats were divided into groups of 10, given a single dose of atrazine at 0, 5, 50, 100, or 200 mg/kg bw by gavag, and killed 5, 15, 30, 60 or 180 min later. A dose-dependent increase in plasma ACTH was observed 15 and 30 min after treatment; maximal concentrations of ACTH were observed at 15 min with increases of 2.5, 4.9 and 9.6-fold in the groups at 50, 100 and 200 mg/kg bw, respectively. Dose-dependent increases in serum corticosterone and progesterone concentrations were observed at 15 and 30 min in the groups at 50, 100, and 200 mg/kg bw with the maximal responses (increases of 9–12-fold) occurring at 30 min after dosing. Corticosterone and progesterone concentrations remained elevated in males at 200 mg/kg bw for 180 and 60 min, respectively. Increased serum testosterone concentration was observed at 30 min in the groups at 100 and 200 mg/kg bw, and at 60 min in the groups at 50–200 mg/kg bw. Thus, the atrazine-induced

increase in steroidogenesis is the result of increased ACTH secretion, either through a direct effect on the pituitary or through the release of corticotropin-releasing factor (CRF) in the central nervous system (Laws et al., 2006).

(k) Studies of immunotoxicity

The potential immunotoxicity of atrazine has been evaluated in a variety of mammalian and non-mammalian animal models (Table 23). Considered overall, the reports indicate that modulation of the immune system occurs after exposure to atrazine, albeit at doses greater than those known to disrupt neuroendocrine function and suppress LH and prolactin release.

Table 23. Selected published studies of immunotoxicity with atrazine

Test system	Dose/concentration	Finding	Comment	Reference
Lymphatic response in chicks (age 3 days)	150 ppm in feed for 21 days	Increased thymus and bursa weight that correlated with glycogen content.	All parameters returned to control levels by day 21.	Giurgea & Koszta (1979)
E. coli challenge test in Wistar rats	2 or 150 mg/kg bw per day for 60 days	Stimulated immunological reactions (antibody titre, gamma-globulin concentration, leukocyte count)	Inconsistent dose–response relationship; NOAEL could not be identified..	Giurgea et al. (1981)
Mouse leukocyte immunosuppression and phagocytic impairment	Single oral doses at 27.3, 109.4, 437.5 and 875.0 mg/kg bw	Transient and reversible suppression of humoral-mediated and cell-mediated responses; activated macrophage phagocytic activity.	Absence of a dose–response relationship; authors did not attribute the changes to a direct effect of atrazine on immune system	Fournier et al. (1992)
Haematopoietic system (progenitor cells in bone marrow) in atrazine-treated mice	Single intraperitoneal dose at 58.65 mg/kg bw	CFU-S and CFU-GM in bone marrow and reticulocyte count in blood reduced for 6–8 days after treatment; leukocyte count unaffected by treatment.	Transient response to a single high dose. NOAEL not identified.	Mencoboni et al. (1992)
Long-term exposure of laboratory and wild mice	10 ppb in water for 22–103 days	No effect on body weight, spleen weight or lymphocyte plaque-forming ability when challenged with foreign protein	Atrazine in combination with other chemicals not considered here	Porter et al. (1999)
Effect on cytokine production in vitro in mononuclear cells from humans	0.03, 0.3 and 3 µmol/l (6.5, 65 and 647 ppb) in 1% DMSO	Cytokine (IFN-γ, IL-5, TNF-α) production significantly reduced by up to 50–70% at ≥ 0.3 µmol/l	Effect of 1% DMSO on cellular uptake of atrazine unknown	Hooghe et al. (2000)
Cytokine production in vitro in mononuclear cells from humans	3 µmol/l (0.65 ppm) in 1% DMSO	Cytokine (IFN-γ, IL-5, TNF-α) production reduced by up to 40–60%; no effect on IL-8.	Authors concluded that the effect was not mediated via the glucocorticoid receptor.	Devos et al. (2003
Exposure of sheep leukocytes in vitro	10^{-6} – 10^{-1} mol/l (0.2–21 570 ppm) in 1% DMSO; added to leukocyte suspensions in 1% volumes	Decreased lymphocyte activation with PHA at concentrations of 10^{-1} – 10^{-2} mol/l (i.e. concentration in leukocyte suspensions: 22–216 ppm)	Limited biological relevance because concentration was at or above the limit of solubility for atrazine in water (33 ppm)	Pistl et al. (2003)

Immunological effects in B6C3F$_1$ mice	Single intraperitoneal. dose at 100, 200 or 300 mg/kg bw	Decrease in percentage of $CD4^+CD8^+$ cells in thymus and $B220^+$ cells in spleen; decreased splenic NK cell activity; decreased IgG1 and IgG2a response to KLH	Non-specific stress, as indicated by plasma corticosterone AUC, was predictive of the immune response	Pruett et al. (2003)
Study of developmental immunotoxicity in Sprague-Dawley rats	Oral gavage at 35 mg/kg bw per day from day 10 of gestation to postnatal day 23	Decrease in pup survival (postnatal days 2–14) and body weight (males, postnatal day 7). Primary antibody (IgM) response to SE and delayed-type hypersensitivity response to BSA decreased in males only.	Effects in males only, may be related to increased pup mortality or reduced pup body weight; NOAEL not identified.	Rooney et al. (2003)
Effect on human NK cell cytotoxic function (tumour-cell lysis) in vitro	10 µmol/l (2.2 ppm)	Decreased cytotoxic function of NK cells (by 63–83%) and of T/NK cells (by 61–65%) after 24 h and 6-day incubation	An effect attributable to high concentration in vitro	Whalen et al. (2003)
Cytokine production in vitro in mononuclear cells from humans	3 µmol/l (0.65 ppm)	Cytokine (IFN-γ, IL-5) production reduced by 12.3 and 14.8%, respectively; no effect on TNF-α, IL-4, IL-6 and IL-13.	Minimum effect that is unlikely to impact the allergic response; NOAEL not identified.	Devos et al. (2004)
Immunological effects in juvenile male C57BL/6 mice (age 1 month)	0, 5, 25, 125 or 250 mg/kg bw per day for 14 days	Decrease of thymus and spleen weights and organ cellularity at ≥ 25 mg/kg; transient changes in thymic and splenic subpopulations at ≥ 5 or ≥ 25 mg/kg, respectively.	Most effects no longer present at 7 weeks after treatment; the decreases of thymic T-cell populations at ≥ 5 mg/kg bw per day (at day 1 only) are not predictive of impaired function (i.e. not considered to be an adverse effect).	Filipov et al. (2005)
Immunological effects in female B6C3F$_1$ mice (age 4–6 weeks)	0, 25, 250 or 500 mg/kg bw per day for 14 days	Decrease of thymus and spleen weights, total spleen cell numbers and fixed macrophage function; increased number of splenic CD8+ T cells, increased cytotoxic T cell and mixed leukocyte responses, reduced host resistance to tumour challenge	Spleen weight reduced at ≥ 25 mg/kg bw per day Cytotoxic T-cell response increased at ≥ 25 mg/kg bw per day	Karrow et al. (2005)
Immunological effects in female B6C3F$_1$ mice	0, 75, 150, 225 or 300 mg/kg by intraperitoneal injection.	Effects on spleen and thymus of all stressors. Corticosterone comparable to atrazine	See Pruett et al. (2003)	Schwab et al. (2005)
Immunological effects in offspring of Balb/c mice exposed on day 10 of gestation to postnatal day 11	0.7 mg/dam per day, equal to 23–35 mg/kg bw per day (released from a subcutaneous implanted capsule)	Increase of HKSP-specific IgM-secreting B cells; increase in both T cell proliferation and cytolytic activity	Effects observed only in males; no changes in the number of CD8+ T-cell, CD4+ T-cell or B220+ B-cell subpopulations	Rowe et al. (2006)

BSA, bovine serum albumin; CFU-GM, colony-forming unit-granulocyte-macrophage; CFU-S, colony-forming unit-spleen; DMSO, dimethyl sulfoxide; IFN, interferon; IL, interleukin; KLH, keyhole limpet haemocyanin; NK, natural killer; PHA, phytohaemagglutinin; SE, sheep erythrocytes; TNF, tumour necrosis factor;

The immune system of adult mice appears to be relatively insensitive to atrazine. For example, exposure of female B6C3F$_1$ mice to 250 or 500 mg atrazine/kg bw per day for 14 days did not affect antibody synthesis, natural killer (NK) cell activity or lymphocyte proliferation, but did decrease body and lymphoid organ weights and shifted percentages of lymphocyte subpopulations (National Toxicology Program, 1994). The number of spleen cells producing antibody was increased by 35% in groups of mice exposed to atrazine at the lowest dose (25 mg/kg bw per day), but was similar to control values at higher doses. The authors dismissed this result as biologically irrelevant, because the antibody titre was not significantly decreased at this dose.

However, a recent peer-reviewed version of the original 1994 NTP report by Munson et al. (Karrow et al., 2005) concluded that the data at the lowest dose may represent an actual increase in numbers of antibody-producing cells in the spleen. Whether or not this type of enhancement is beneficial or detrimental to the individual has yet to be determined. With atrazine at the highest dose tested (500 mg/kg bw per day), resistance to an natural killer cell-dependent tumour-cell challenge was decreased, but resistance to bacterial challenge (*Listeria monocytogenes*) was not affected. However, animals at the highest dose experienced a 3% loss of body weight during dosing, vs a 10% body-weight gain in controls, indicating marked overt toxicity at the highest dose, thus calling into question the biological relevance of the observed immune effects at 500 mg/kg bw per day. Furthermore, given the lack of effects on natural killer cell activity, even at the highest dose, it is difficult to conclude that immune suppression was directly responsible for the apparent increased susceptibility to a tumour-cell challenge.

In a separate study, the antibody response of C57Bl/6 female mice was suppressed 7 days after a single exposure to a wide range of atrazine doses (27.3–875 mg/kg bw). However, suppression was not dose–responsive, and no dose-related pattern of suppression or recovery was apparent 2, 3 or 6 weeks after exposure (Fournier et al., 1992). Although the relatively short half-life of atrazine may explain why these effects were not persistent, it does not provide a satisfactory explanation for the lack of a dose–response relationship. Chemicals that are immunotoxic in adult animals generally induce similar effects, but at lower doses or for a prolonged period of time, if exposure occurs during development and maturation of the immune system (Luebke et al., 2006).

The effects of exposure to atrazine during immune system ontogeny were therefore evaluated in rats and mice. In replicate studies, exposure to atrazine at a dose of 35 mg/kg bw per day from day 10 of gestation until postnatal day 23 was found to suppress cellular and antibody-mediated immune function in male, but not female rats (Rooney et al., 2003). However, preliminary data from recent studies of dose–response did not corroborate suppressed function in developmentally exposed rats. The basis for this discrepancy is as yet unknown (Robert W. Luebke, personal communication). Studies of developmental exposure in mice indicated that the percentage of antibody-producing cells in the spleens of immunized male offspring of mice implanted with time-release atrazine pellets between days 10 and 12 of gestation was increased by approximately 33%; female offspring were not affected (Rowe et al., 2006). Daily doses were in the same range as those used by Rooney et al. (2003). The results for studies of developmental exposure results suggest that the immature immune system of males may be more sensitive to the effects of atrazine than that of the adult. Nevertheless, studies corroborating previous findings and those that incorporate a range of doses will be necessary to adequately determine NOAELs and LOAEL for immune function, and the relative sensitivity of the immune and endocrine systems in developing rodents.

3. Observations in humans

A large number of studies have been published on the association between exposure to triazines, including atrazine, and cancer epidemiology. Concerning the studies reported until 1999, three

independent reviews (Neuberger, 1996; Sathiakumar & Delzell, 1997; IARC, 1999) have identified ten case–control studies and two published cohort studies of workers exposed to triazines at manufacturing plants.

Neuberger (1996) reported that of the ten case–control studies published, six of which considered atrazine, none indicated any statistically significant association between atrazine and cancer. Two studies indicated marginally significant associations between triazines and cancer (odds ratio, OR, 1.6; 95% confidence interval, CI, 1.0–2.6; and OR, 2.7; 90% CI, 1.0–6.9). The author concluded:

> …on the basis of the data to date… there is no convincing evidence of a causal association between atrazine and/or triazine(s) and colon cancer, soft tissue sarcoma, Hodgkin's disease, multiple myeloma, or leukaemia… There is a suggestion of a possible association between atrazine and/or triazine(s) with ovarian cancer and non-Hodgkin's lymphoma. However, the ovarian cancer study needs to be replicated and the NHL studies fall short of providing conclusive evidence of risk because the results could be due to chance, bias, or confounding.

Sathiakumar & Delzell (1997) assessed the relation between triazines and non-Hodgkin lymphoma in four independent population-based case–control studies, reporting OR of between 1.2 and 2.5, and concluded that these weak statistical associations may have been produced by chance and/or confounding by other agricultural exposures. Furthermore, a pooled analysis of three case–control studies and the combined analysis of two retrospective follow-up studies did not demonstrate the types of dose–response relationship or induction-time patterns that would be expected if triazines were causal factors. The authors concluded that the available epidemiological studies, singly and collectively, did not provide any consistent, convincing evidence of a causal relationship between exposure to triazine herbicides and cancer in humans.

The International Agency for Research on Cancer (IARC, 1999) summarized their evaluation of data on human carcinogenicity as follows:

> A combined analysis of results of two cohort studies of agricultural chemical production workers in the United States showed decreased mortality from cancers at all sites combined among the subset of workers who had had definite or probable exposures to triazine. Site-specific analyses in this subset of workers yielded no significant findings; a non-significant increase in the number of deaths from non-Hodgkin's lymphoma was seen, but was on the basis of very few observed cases.
>
> A pooled analysis of the results of three population-based case–control studies of men in Kansas, eastern Nebraska and Iowa-Minnesota, United States, in which the risk for non-Hodgkin's lymphoma in relation to exposure to atrazine and other herbicides on farms was evaluated, showed a significant association; however, the association was weaker when adjustment was made for reported use of phenoxyacetic acid herbicides or organophosphate insecticides. In all these studies, the farmers tended to have an increased risk for non-Hodgkin's lymphoma, but the excess could not be attributed to atrazine
>
> Less information was available to evaluate the association between exposure to atrazine and other cancers of the lymphatic and haematopoietic tissues. One study of Hodgkin's disease in Kansas, one study of leukaemia in Iowa-Minnesota and one study of multiple myeloma from Iowa gave no indication of excess risk among persons handling triazine herbicides.
>
> In a population-based study in Italy, definite exposure to triazines was associated with a two to threefold increase of borderline significance in the risk for ovarian cancer. The study was small (65 cases, 126 controls), and potential confounding by exposure to other herbicides was not controlled in the analysis.

Therefore, on the basis of the findings described above the IARC (1999) concluded: "There is inadequate evidence in humans for the carcinogenicity of atrazine."

In a later review of cancer epidemiology after exposure to atrazine for the United States EPA (Blondell & Dellarco, 2003), four studies concerning prostate cancer were evaluated: a nested case–control study among workers in a plant manufacturing triazine in Louisiana (Hessel et al., 2004), the Agricultural Health Study which is a prospective cohort study of 55 332 male pesticide applicators from Iowa and North Carolina (Alavanja et al., 2003), an ecological study conducted in California (Mills, 1998), and a nested case–control study in Californian farm workers exposed to simazine (a triazine derivative similar to atrazine) and several other pesticides (Mills & Yang, 2003).

In the studies in California, a borderline statistically significant correlation was found between use of atrazine and prostate cancer in black males, but not among Hispanic, white, or Asian males (Mills, 1998), and a borderline significant association was found between high use of simazine and prostate cancer (Mills & Yang, 2003). However, both studies suffered from aggregation bias because there was no or only a crude measure of exposure and the results should thus not be considered for reaching conclusions about causation.

In the nested case–control study (Hessel et al., 2004), an elevated incidence of prostate cancer was found in active employees who received intensive screening for prostate specific antigen (PSA), but there was no increase in the incidence of advanced tumours or mortality, and proximity to atrazine-manufacturing plants did not appear to be correlated with risk. Thus, the increase in incidence of prostate cancer was probably attributable to increased detection because of the intensive screening programme for PSA (MacLennan et al., 2002).

The largest and most reliable study (Alavanja et al., 2003) showed no association of atrazine exposure with prostate cancer in cohort analysis of pesticide applicators. The overall conclusion of the reviewers was that studies in manufacturing and farming populations do not support a finding that atrazine is a likely cause of prostate cancer.

In a study of workers in a plant manufacturing triazine in Louisiana (MacLennan et al., 2003), a borderline significant result was found for non-Hodgkin lymphoma on the basis of 4 observed deaths vs 1.1 deaths expected. The study authors noted, however, that "one of the decedents whose death certificate included a diagnosis of non-Hodgkin lymphoma had medical records including a biopsy report that indicated a diagnosis of poorly differentiated nasopharyngeal cancer. This case was not removed from our analysis." This acknowledgment of bias on the basis of a misclassified case means that the borderline statistically significant finding would no longer be significant if the case were excluded. Therefore, the overall conclusion of the reviewers was that this evidence is not sufficient to support a finding that atrazine is a likely cause of non-Hodgkin's lymphoma.

Additional evaluations of the cancer incidences in the Agricultural Health Study did not find any clear associations between exposure to atrazine and any cancer analysed (Rusiecki et al., 2004). However, the authors pointed out that further studies were warranted for tumour types for which there was a suggestion of trend (lung, bladder, non-Hodgkin lymphoma, and multiple myeloma). It should be noted that the neuroendocrine mode of action of atrazine cannot account for the biological plausibility of these tumours.

An evaluation of risk of breast cancer among farmers' wives in the Agricultural Health Study (30 454 participants with no history of breast cancer before cohort enrolment in 1993–1997) did not find any association of increased incidence of breast cancer with the use of atrazine; however, reduced risk of breast cancer among postmenopausal women were linked to their use of atrazine (relative risk, RR, 0.4; 95% CI, 0.1–1.0) (Engel et al., 2005).

The incidence of cancer among pesticide applicators exposed to cyanazine (a triazine derivative similar to atrazine) in the Agricultural Health Study has also been unremarkable (Lynch et al., 2006).

Current evidence was not persuasive as to an association between ovarian cancer and exposure to triazine. In a population-based case–control study of incident cases (n = 256) and control subjects

selected by random digit-dialled techniques (n = 1122) assessing whether there was an increased risk of ovarian cancer associated with occupational exposure to triazine herbicides, no evidence of a dose–response relationship for triazines and ovarian cancer was found (Young et al., 2005).

In an ecological study using regression analysis to evaluate the incidence of breast cancer in Hispanic females in California (23 513 cases diagnosed with breast cancer during the years 1988–1999) at the county level as a function of use of organochlorine and triazine pesticides, no significant associations were found for atrazine and simazine (Mills & Yang, 2006).

In another large population-based study (using 3275 incident cases of breast cancer in women aged 20–79 years from 1987 to 2000 and living in rural areas of Wisconsin, and 3669 matched controls), there was no increased risk of breast cancer for women exposed to atrazine at concentrations of 1.0–2.9 ppb in drinking-water (OR, 1.1; 95% CI, 0.9–1.4) when compared with women with the lowest exposure to atrazine (< 0.15 ppb). Evaluation of a possible risk for women exposed to atrazine at concentrations at or above the statutory action levels of ≥ 3 ppb (OR, 1.3; 95% CI, 0.3–5.0) was limited by the small numbers in this category (McElroy et al., 2007).

Comments

Biochemical aspects

After oral administration to rats, ^{14}C-labelled atrazine was rapidly and almost completely absorbed, independent of dose and sex. Radioactivity was widely distributed throughout the body. Excretion was more than 93% of the administered dose within 7 days, primarily via the urine (approximately 73%) and to a lesser extent via the faeces (approximately 20%; approximately 7% via bile), with more than 50% being excreted within the first 24 h. The elimination half-life of radiolabel from the whole body was 31.3 h in rats; this prolonged half-life was caused by covalent binding of atrazine to cysteine sulfhydryl groups in the β-chain of rodent haemoglobin. Seven days after administration of a single low dose (1 mg/kg bw), tissue residues represented 6.5–7.5% of the dose, with the highest concentrations in erythrocytes (≤ 0.63 ppm), liver (≤ 0.50 ppm) and kidneys (≤ 0.26 ppm). Atrazine was extensively metabolized; more than 25 metabolites have been identified in rats. The major metabolic pathways were stepwise dealkylation via either DIA or DEA to DACT, the major metabolite. Dechlorination involving conjugation with glutathione was a minor pathway. The biotransformation of atrazine in rats and humans was qualitatively similar.

Toxicological data

Atrazine was of low acute toxicity in rats exposed orally (LD_{50}, 1870–3090 mg/kg bw), dermally (LD_{50}, > 2000 mg/kg bw) or by inhalation (LC_{50}, > 5.8 mg/l). Atrazine was not a skin irritant or an eye irritant in rabbits. Although spray dilutions of atrazine did not appear to be sensitizing in humans, atrazine was a skin sensitizer in tests in guinea-pigs (Magnusson & Kligman, Maurer optimization test).

In short-term studies of toxicity in rats, dogs and rabbits, the consistent toxic effects noted across species included reduced body-weight gain and food intake and a slight decrease in erythrocyte parameters. Also in rats, liver weights and splenic haemosiderin deposition were increased, while in dogs there was marked cardiac toxicity.

In a 90-day study of toxicity in rats, the NOAEL was 50 ppm, equal to 3.3 mg/kg bw per day, on the basis of decreased body-weight gain and increased splenic haemosiderin deposition at 500 ppm.

In a 52-week study of toxicity in dogs, the NOAEL was 150 ppm, equal to 5 mg/kg bw per day, on the basis of decreased body-weight gain and marked cardiac toxicity at 1000 ppm, equal to 33.7 mg/kg bw per day.

In a 25-day study in rabbits treated dermally, the NOAEL for systemic toxicity was 100 mg/kg bw per day on the basis of decreased body-weight gain and food intake, a slight reduction in erythrocyte parameters and increased spleen weight at 1000 mg/kg bw per day.

Atrazine was tested for genotoxicity in a large number of studies covering an adequate range of end-points, including assays for gene mutation in bacteria and eukaryotic cells in vitro, for DNA damage and repair in bacteria and mammalian cells (rat hepatocytes, human fibroblasts) in vitro, and for chromosomal aberration in vitro and in somatic and germ cells in vivo. Mostly negative results were obtained in standard assays. In a few published studies, positive responses were reported. However, a number of reviews by national and international agencies (United States Environmental Protection Agency, European Union, International Agency for Research on Cancer) have concluded that, on the basis of the weight of evidence, atrazine is not genotoxic.

The Meeting agreed that it is unlikely that atrazine is genotoxic.

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. As in short-term studies, reduced body-weight gain and food intake and a decrease in erythrocyte parameters were noted consistently. Additionally, reduced survival of females and cardiovascular effects (atrial thrombi) in both sexes were observed in mice at high doses.

In three studies of carcinogenicity in mice, no treatment-related carcinogenic effects were observed at dietary concentrations of up to 3000 ppm, equal to about 386 and 483 mg/kg bw per day in males and females, respectively. Overall, the NOAEL was 10 ppm, equal to 1.2 mg/kg bw per day, on the basis of lower body weight/body-weight gain at 300 ppm, equal to 38.4 mg/kg bw per day, and greater.

In two studies of carcinogenicity in F344 rats fed diets containing atazine at concentrations of up to 400 ppm, equivalent to about 20 mg/kg bw per day, there was no effect at any dose on the onset or incidence of tumours. The NOAEL was 70 ppm, equivalent to about 3.5 mg/kg bw per day, on the basis of decreased body weight at concentrations of 200 ppm and greater. In a non-guideline study of carcinogenicity in F344 rats, there was a significant increase in the incidence of benign mammary tumours in males and in uterine adenocarcinomas in females at the highest dose of 750 ppm, equivalent to about 38 mg/kg bw per day; however, interpretation of the result was limited by increased survival at the highest dose, and a survival-adjusted analysis of tumour prevalence did not indicate any significant increase in the incidence of benign, malignant or combined mammary tumours.

In seven studies of carcinogenicity in Sprague-Dawley rats fed diets containing atrazine at concentrations of up to 1000 ppm (equal to about 42 and 65 mg/kg bw per day in males and females, respectively), an increased incidence of mammary tumours (adenomas, carcinomas, fibroadenomas) with or without an earlier onset (relative to controls) was observed in four studies, while in two studies there was an earlier onset of mammary tumours without any increase in their overall lifetime incidence. An earlier onset of pituitary tumours was also observed in one study, with no increase in incidence at term. Overall, the NOAEL for mammary carcinogenicity was 25 ppm, equal to 1.5 mg/kg bw per day, on the basis of a statistically significant increased incidence in mammary tumours at 50 ppm, equal to 3.1 mg/kg bw per day.

In a study of carcinogenicity in ovariectomized Sprague-Dawley rats, neither increases in mammary-gland proliferative changes nor mammary tumours were seen at dietary concentrations of up to 400 ppm (equal to about 21 mg/kg bw per day), suggesting that the carcinogenic mode of action of atrazine in Sprague-Dawley rats is related to ovarian function.

In a mechanistic 6-month study in Sprague-Dawley rats, attenuation of the LH surge and subsequent disruption of the estrous cycle (characterized by an increase in days in estrus) were observed at $\geq$ 50 ppm (equal to 3.65 mg/kg bw per day), with a NOAEL of 25 ppm (equal to 1.8 mg/kg bw per day). The NOAEL and LOAEL for these effects were comparable to those identified in the studies of carcinogenicity. The effects on the LH surge and disruption of the estrous cycle were further

supported by a number of short-term mechanistic studies. Additional experiments suggested that the effects of atrazine on LH and prolactin secretion are mediated via a hypothalamic site of action.

The postulated mode of action for atrazine-induced mammary tumours in female Sprague-Dawley rats involved disruption of the hypothalamic–pituitary–ovary axis. Atrazine modifies catecholamine function and the regulation of GnRH pulsatility in the rat hypothalamus, with the consequence that the pulse of LH released from the pituitary gland is of insufficient amplitude or duration to trigger the ovulation. The failure to ovulate results in persistent secretion of estrogen, which provides a feedback to the pituitary leading to increased secretion of prolactin. As a result, atrazine accelerates the normal reproductive ageing process in female Sprague-Dawley rats whereby reproductive senescence is characterized by persistent exposure to estrogen and prolactin. In contrast, women respond to reduced levels of LH by reductions in estrogen concentrations. Thus, the Meeting considered that the mode of carcinogenic action in certain susceptible rat strains is not relevant for risk assessment in humans.

Investigations of other modes of action did not provide any evidence that atrazine had intrinsic estrogenic activity or that it increased aromatase activity in vivo.

The Meeting concluded that atrazine is not likely to pose a carcinogenic risk to humans.

Although carcinogenicity in humans was not a concern owing to the rat-specific mode of action, alterations in neurotransmitter and neuropeptide function regulating LH and secretion of prolactin may potentially induce adverse effects during critical periods of development (as found in special studies showing pregnancy loss, delayed puberty in males and females, and decreased suckling-induced prolactin release in lactating dams). Unlike the carcinogenic effects, the developmental effects do not appear to be specific to certain strains of rats and the Meeting therefore considered these effects to be relevant for risk assessment in humans.

In special studies of reproductive toxicity, exposure of rats during early pregnancy (i.e. the LH-dependent period) caused increased pre- or postimplantation losses, including full-litter resorptions. Effects were seen at doses of 50 mg/kg bw per day and greater after treatment on days 6–10 of gestation, with a NOAEL of 25 mg/kg bw per day. In contrast, exposure on days 11–15 of gestation (after the LH-dependent period of pregnancy) at a dose of 200 mg/kg bw per day did not induce full-litter resorptions.

Suppression of the suckling-induced release of prolactin in lactating rats was seen with atrazine at doses of 25 mg/kg bw per day and greater, with a NOAEL of 12.5 mg/kg bw per day. Treatment of lactating rats on postnatal days 1–4 affected the development of tuberoinfundibular dopaminergic neurons in the pups (presumably due to the lack of prolactin derived from the dam's milk), with the consequence of impaired regulation of prolactin secretion, hyperprolactinaemia before puberty and prostatitis in the adult male offspring.

A delay in sexual development was observed in female rats after exposure on postnatal days 21–46 at doses of 30 mg/kg bw per day and greater, with a NOAEL of 10 mg/kg bw per day, and in male rats after exposure on postnatal days 23–53 at doses of 12.5 mg/kg bw per day and greater, with a NOAEL of 6.25 mg/kg bw per day.

In a standard two-generation study of reproduction (conducted according to earlier guidelines, which did not include end-points such as estrous cyclicity and sexual development) in rats, there was no effect on fertility at 500 ppm, the highest dose tested. The NOAEL for parental toxicity was 50 ppm, equal to 3.6 mg/kg bw per day, on the basis of decreased body-weight gains and food consumption at 500 ppm, equal to 36.1 mg/kg bw per day. The NOAEL for reproductive toxicity was 50 ppm on the basis of decreased body weights of male pups at postnatal day 21 at 500 ppm.

In two studies of prenatal developmental toxicity in rats given atrazine on days 6–15 of gestation, the NOAELs for maternal toxicity were 10 or 25 mg/kg bw per day on the basis of decreased body-weight gain and food intake at 70 or 100 mg/kg bw per day, respectively. The NOAELs for

developmental toxicity were 10 or 25 mg/kg bw per day on the basis of incomplete ossification at several sites at 70 or 100 mg/kg bw per day, respectively. In a study of prenatal developmental toxicity in rabbits given atrazine on days 7–19 of gestation, the NOAEL for maternal toxicity was 5 mg/kg bw per day on the basis of clinical signs, abortion and decreased food intake and body-weight gain at 75 mg/kg bw per day. The NOAEL for developmental toxicity was 5 mg/kg bw per day on the basis of increased resorptions, reduced litter size and incomplete ossification at 75 mg/kg bw per day. In rats and rabbits, the developmental effects were observed only at maternally toxic doses.

The Meeting concluded that atrazine was not teratogenic.

Studies using a variety of test systems in vitro and in vivo indicated that modulation of the immune system occurs after exposure to atrazine. However, effects suggestive of impaired function of the immune system were only observed at doses greater than those shown to affect neuroendocrine function, leading to disruption of the estrous cycle or developmental effects.

A range of epidemiological studies (including cohort studies, case–control studies, and ecological or correlational studies) assessed possible relationships between atrazine or other triazine herbicides and cancer in humans. For some cancer types, such as prostate or ovarian cancer and non-Hodgkin's lymphoma, the increased risks reported in single studies could either be explained by the methodology used, or had not been confirmed in more reliable studies. Thus, the weight of evidence from the epidemiological studies did not support a causal association between exposure to atrazine and the occurrence of cancer in humans.

The Meeting concluded that the existing database on atrazine is adequate to characterize the potential hazards to fetuses, infants and children.

Metabolites of atrazine

The toxicity profiles and mode of action of the chloro-*s*-triazine metabolites were similar to those of atrazine; the potency of these metabolites appeared to be similar to that of the parent compound with regard to their neuroendocrine-disrupting properties.

Like atrazine, the chloro-*s*-triazine metabolites were of moderate or low acute oral toxicity in rats; LD_{50}s were 1110, 1240 and 2310–5460 mg/kg bw for DEA, DIA and DACT, respectively.

Like atrazine, its chloro-*s*-triazine metabolites delayed sexual development of male rats exposed on postnatal days 23–53 at atrazine molar equivalent doses of 25 mg/kg bw per day and greater (DEA, DIA) and 12.5 mg/kg bw per day and greater (DACT), with NOAELs of 12.5 and 6.25 mg/kg bw per day, respectively. Exposure of female rats to DACT on postnatal days 22–41 delayed sexual development at atrazine molar equivalent doses of 50 mg/kg bw per day and greater, and the NOAEL was 25 mg/kg bw per day. Doses at which these effects occurred were similar to those observed for parent atrazine.

In short-term feeding studies in rats, the main effects of the chlorinated metabolites were similar to those of atrazine and included reduced body-weight gain and decreased erythrocyte parameters, and also for DACT-induced disruption of the estrous cycle. The NOAELs were 50 ppm (equal to 3.2 mg/kg bw per day) for DEA and DIA, and 100 ppm (equal to 7.6 mg/kg bw per day) for DACT.

In a 29/52-week study with DACT in Sprague-Dawley rats, effects comparable to those observed with atrazine (attenuation of the LH surge, increased incidences of mammary tumours) were seen at 270 ppm; the NOAEL was 48 ppm, equal to 3.4 mg/kg bw per day. No long-term studies were performed with DEA or DIA.

In short-term feeding studies in dogs, the main effects of the chlorinated metabolites were similar to those of atrazine and included reduced body-weight gain and decreased erythrocyte parameters, while DEA and DACT showed cardiac toxicity. The NOAELs were 100 ppm, equal to 3.7, 3.8 and 3.5 mg/kg bw per day, for DEA, DIA and DACT, respectively.

DEA, DIA and DACT did not show genotoxicity in an adequate range of tests in vitro and in vivo.

In studies of prenatal developmental toxicity in rats, the chlorinated metabolites induced increased incidences of fused sternebrae and/or incomplete ossification at doses of 25 to 100 mg/kg bw per day; the NOAELs for developmental toxicity were 25, 5 and 2.5 mg/kg bw per day for DEA, DIA and DACT, respectively. The effects were seen only at doses that also produced maternal toxicity.

The metabolite hydroxyatrazine does not have the same mode of action or toxicity profile as atrazine and its chlorometabolites. The main effect of hydroxyatrazine was kidney toxicity (owing to its low solubility in water, resulting in crystal formation and a subsequent inflammatory response), and there was no evidence that hydroxyatrazine has neuroendocrine-disrupting properties. Also, the acute oral toxicity of hydroxyatrazine in rats (LD_{50}, > 5050 mg/kg bw) was lower than that of atrazine or its chlorometabolites.

In short-term feeding studies, the main effects of hydroxyatrazine in rats included reduced body-weight gain, increased water consumption, changes in clinical chemistry and urine analysis parameters, and kidney lesions. The overall NOAEL was 100 ppm, equal to 6.3 mg/kg bw per day. In dogs, effects included reduced body-weight gain and food consumption, changes in clinical chemistry and urine analysis parameters, and kidney lesions; the NOAEL was 150 ppm, equal to 5.8 mg/kg bw per day.

In a 2-year study of toxicity and carcinogenicity in rats, the effects of hydroxyatrazine included clinical signs and increased mortality, reduced body-weight gain and food consumption, increased water consumption, changes in haematological, clinical chemistry and urine analysis parameters, and kidney lesions. The NOAEL was 25 ppm, equal to 1.0 mg/kg bw per day. There was no evidence of carcinogenicity.

Hydroxyatrazine did not show genotoxicity in an adequate range of tests in vitro and in vivo.

In a study of prenatal developmental toxicity in rats, the effects of hydroxyatrazine consisted of reduced food consumption and body-weight gain in dams and increased incidences of incomplete and absent ossification in fetuses at 125 mg/kg bw per day; the NOAEL was 25 mg/kg bw per day for maternal and developmental toxicity. Exposure of female rats on postnatal days 22–41 at atrazine molar equivalent doses of up to 200 mg/kg bw per day did not delay sexual development.

Toxicological evaluation

Drinking-water may contain metabolites of atrazine as well as atrazine itself. The chloro-*s*-triazine metabolites DEA, DIA and DACT share the same mode of action as atrazine and have a similar toxicological profile and hence the Meeting decided to establish a group ADI and acute reference dose (ARfD). Hydroxyatrazine, the plant and soil degradate, was not included because its mode of action and toxicological profile are different to those of atrazine and its chloro-*s*-triazine metabolites.

The Meeting established a group ADI of 0–0.02 mg/kg bw on the basis of the NOAEL for atrazine of 1.8 mg/kg bw per day identified on the basis of LH-surge suppression and subsequent disruption of the estrous cycle seen at 3.6 mg/kg bw per day in a 6-month study in rats, and using a safety factor of 100. The Meeting considered that this NOAEL was protective for the consequences of neuroendocrine and other adverse effects caused by prolonged exposure to atrazine and its chloro-*s*-triazine metabolites.

The Meeting established a group ARfD of 0.1 mg/kg bw on the basis of the NOAEL for atrazine of 12.5 mg/kg bw per day identified on the basis of impaired suckling-induced prolactin secretion in dams and subsequent alterations in development of the central nervous system and prolactin

regulation in male offspring in a special 4-day study in rats, and using a safety factor of 100. This ARfD was supported by the results of other studies of developmental toxicity with atrazine and its chlorometabolites, from which overall NOAELs/LOAELs of 25/50 mg/kg bw per day in rats and 5/75 mg/kg bw per day in rabbits were identified on the basis of effects that might occur after a single exposure (i.e. postimplantation loss, fused sternebrae). The study in rabbits (in which there was a 15-fold difference between NOAEL and LOAEL) was not selected as the basis for the ARfD, because examination of the studies in rats indicated that the dose selected for the ARfD would be adequately protective for these end-points in rabbits.

For hydroxyatrazine, the Meeting established an ADI of 0–0.04 mg/kg bw on the basis of the NOAEL of 1.0 mg/kg bw per day identified on the basis of kidney toxicity (caused by low solubility in water resulting in crystal formation and a subsequent inflammatory response) at 7.8 mg/kg bw per day in a 24-month study in rats, and using a safety factor of 25. A modified safety factor on the basis of kinetic considerations was deemed appropriate since the critical effect of hydroxyatrazine is dependent on its physicochemical properties and the interspecies variability for such effects is lower than for AUC-dependent effects.

The Meeting concluded that it was not necessary to establish an ARfD for hydroxyatrazine in view of its low acute toxicity, the absence of relevant developmental toxicity that could be a consequence of acute exposure, and the absence of any other toxicological effects that would be likely to be elicited by a single dose.

Levels relevant to risk assessment

(a) Atrazine

Species	Study	Effect	NOAEL	LOAEL
Mouse	Long-term studies of carcinogenicity[a,d]	Toxicity	10 ppm, equal to 1.2 mg/kg bw per day	300 ppm, equal to 38.4 mg/kg bw per day
		Carcinogenicity	3000 ppm, equal to 385.7 mg/kg bw per day[c]	—
Rat	Thirteen-week study of toxicity[a]	Toxicity	50 ppm, equal to 3.3 mg/kg bw per day	500 ppm, equal to 34.0 mg/kg bw per day
	Two-year studies of toxicity and carcinogenicity[a,d] (Sprague-Dawley rats)	Toxicity	70 ppm, equal to 2.6 mg/kg bw per day	500 ppm, equal to 19.9 mg/kg bw per day
		Carcinogenicity	25 ppm, equal to 1.5 mg/kg bw per day	50 ppm, equal to 3.1 mg/kg bw per day[e]
	Two-year studies of toxicity and carcinogenicity[a,d] (Fischer 344 rats)	Toxicity	70 ppm, equal to 3.5 mg/kg bw per day	200 ppm, equal to 10 mg/kg bw per day
		Carcinogenicity	400 ppm, equal to 20 mg/kg bw per day[c]	—
	Multigeneration study of reproductive toxicity[a]	Fertility	500 ppm, equal to 36.1 mg/kg bw per day[c]	—
		Parental toxicity	50 ppm, equal to 3.6 mg/kg bw per day	500 ppm, equal to 36.1 mg/kg bw per day
		Offspring toxicity	50 ppm, equal to 3.6 mg/kg bw per day	500 ppm, equal to 36.1 mg/kg bw per day
	Developmental toxicity[b,d]	Maternal toxicity	10 mg/kg bw per day	70 mg/kg bw per day
		Embryo/fetotoxicity	10 mg/kg bw per day	70 mg/kg bw per day

	Special 6-month study[a]	Endocrine disruption (LH surge)	25 ppm, equal to 1.8 mg/kg bw per day	50 ppm, equal to 3.65 mg/kg bw per day
	Special 4-day study[b]	Endocrine disruption (prolactin release)	12.5 mg/kg bw per day	25 mg/kg bw per day
	Special 5-day study[b]	Postimplantation loss	25 mg/kg bw per day	50 mg/kg bw per day
	Special 25-day study[b]	Female pubertal delay	10 mg/kg bw per day	30 mg/kg bw per day
	Special 30-day study[b]	Male pubertal delay	6.25 mg/kg bw per day	12.5 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	5 mg/kg bw per day	75 mg/kg bw per day
		Embryo/fetotoxicity	5 mg/kg bw per day	75 mg/kg bw per day
Dog	One-year study of toxicity[a]	Toxicity	150 ppm, equal to 5 mg/kg bw per day	1000 ppm, equal to 33.7 mg/kg bw per day

[a] Dietary administration.
[b] Gavage administration.
[c] Highest dose tested.
[d] Results of two or more studies combined.
[e] Mammary gland tumours—not relevant to humans.

(b) Deethyl-atrazine (DEA)

Species	Study	Effect	NOAEL	LOAEL
Rat	Thirteen-week study of toxicity[a]	Toxicity	50 ppm, equal to 3.2 mg/kg bw per day	500 ppm, equal to 35.2 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	5 mg/kg bw per day	25 mg/kg bw per day
		Embryo- and feto-toxicity	25 mg/kg bw per day	100 mg/kg bw per day
	Special 30-day study[b]	Male pubertal delay	12.5 mg/kg bw per day[c]	25 mg/kg bw per day[c]
Dog	Thirteen-week study of toxicity[a]	Toxicity	100 ppm, equal to 3.7 mg/kg bw per day	1000 ppm, equal to 28.9 mg/kg bw per day

[a] Dietary administration.
[b] Gavage administration.
[c] Atrazine molar equivalent dose.

(c) Deisopropyl-atrazine (DIA)

Species	Study	Effect	NOAEL	LOAEL
Rat	Thirteen-week study of toxicity[a]	Toxicity	50 ppm, equal to 3.2 mg/kg bw per day	500 ppm, equal to 34.9 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	5 mg/kg bw per day	25 mg/kg bw per day
		Embryo/fetotoxicity	5 mg/kg bw per day	25 mg/kg bw per day
	Special 30-day study[b]	Male pubertal delay	12.5 mg/kg bw per day[c]	25 mg/kg bw per day[c]
Dog	Thirteen-week study of toxicity[a]	Toxicity	100 ppm, equal to 3.8 mg/kg bw per day	500 ppm, equal to 18.0 mg/kg bw per day

[a] Dietary administration.
[b] Gavage administration.
[c] Atrazine molar equivalent dose.

(d) Diaminochlorotriazine (DACT)

Species	Study	Effect	NOAEL	LOAEL
Rat	Thirteen-week study of toxicity[a]	Endocrine disrup-tion (estrous cycle)	100 ppm, equal to 7.6 mg/kg bw per day	250 ppm, equal to 19.7 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	2.5 mg/kg bw per day	25 mg/kg bw per day
		Embryo/fetotoxicity	2.5 mg/kg bw per day	25 mg/kg bw per day
	Special study, 29/52 weeks[a]	Endocrine disruption (LH surge)	48 ppm, equal to 3.4 mg/kg bw per day	270 ppm, equal to 18.8 mg/kg bw per day
	Special 19-day study[b]	Female pubertal delay	25 mg/kg bw per day[c]	50 mg/kg bw per day[c]
	Special 30-day study[b]	Male pubertal delay	6.25 mg/kg bw per day[c]	12.5 mg/kg bw per day[c]
Dog	Thirteen-/52-week study of toxicity[a]	Toxicity	100 ppm, equal to 3.5 mg/kg bw per day	1500/750 ppm, equal to 23.8 mg/kg bw per day

LH, luteinizing hormone.
[a] Dietary administration.
[b] Gavage administration.
[c] Atrazine molar equivalent dose.

(e) Hydroxyatrazine

Species	Study	Effect	NOAEL	LOAEL
Rat	Thirteen-week study of toxicity[a]	Toxicity	100 ppm, equal to 6.3 mg/kg bw per day	300 ppm, equal to 18.9 mg/kg bw per day
	Two-year study of toxic-ity and carcinogenicity[a] (Sprague-Dawley rats)	Toxicity	25 ppm, equal to 1.0 mg/ kg bw per day	200 ppm, equal to 7.8 mg/kg bw per day
		Carcinogenicity	400 ppm, equal to 17.4 mg/kg bw per day[c]	—
	Developmental toxicity[b]	Maternal toxicity	25 mg/kg bw per day	125 mg/kg bw per day
		Embryo/fetotoxicity	25 mg/kg bw per day	125 mg/kg bw per day
	Special 19-day study[b]	Female pubertal delay	200 mg/kg bw per day[c,d]	—
Dog	Thirteen-/52-week study of toxicity[a]	Toxicity	150 ppm, equal to 5.8 mg/kg bw per day	1500 ppm, equal to 59.6 mg/kg bw per day

[a] Dietary administration.
[b] Gavage administration.
[c] Highest dose tested.
[d] Atrazine molar equivalent dose.

Estimate of acceptable daily intake for humans

Group ADI for atrazine, deethyl-atrazine (DEA), deisopropyl-atrazine (DIA) and diaminochlorotriazine (DACT)

0–0.02 mg/kg bw

Hydroxyatrazine

0–0.04 mg/kg bw

Estimate of acute reference dose

Group ARfD for atrazine, deethyl-atrazine(DEA), deisopropyl-atrazine (DIA) and diaminochlorotriazine (DACT)

0.1 mg/kg bw

Hydroxyatrazine

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposures

Critical end-points for setting guidance values for exposure to atrazine

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of oral absorption	Rapid, > 80% in rats
Distribution	Widely distributed
Rate and extent of excretion	> 50% in 24 h and > 93% within 7 days; approximately 73% via urine, approximately 20% via faeces (approximately 7% via bile)
Potential for accumulation	Low; binding to rat haemoglobin, not relevant to humans
Metabolism in mammals	Extensive (> 95%) to at least 25 metabolites; major pathway is *N*-dealkylation
Toxicologically significant compounds in animals, plants and the environment	Parent compound, chloro-*s*-triazine metabolites DEA, DIA, DACT (animals, environment), hydroxyatrazine (plants, environment)
Acute toxicity	
Rat, LD_{50}, oral	1870–3090 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, $LC_{50,}$ inhalation	> 5.8 mg/l
Rabbit, skin irritation	Not an irritant
Rabbit, eye irritation	Not an irritant
Guinea-pig, skin sensitization	Sensitizer (Magnusson & Kligman; Maurer optimization test)
Short-term studies of toxicity	
Target/critical effect	Reduced body-weight gain, ovaries (inhibition of ovulation), cardiotoxicity (in dogs only)
Lowest relevant oral NOAEL	3.3 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	100 mg/kg bw per day (25-day study in rabbits)
Lowest relevant inhalation NOAEC	No data
Genotoxicity	
	Unlikely to be genotoxic in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Ovaries (inhibition of ovulation) and related endocrine changes
Lowest relevant NOAEL	1.8 mg/kg bw per day (6-month –study of LH-surge in Sprague-Dawley rats)
Carcinogenicity	No relevant carcinogenicity
Reproductive toxicity	
Reproductive target/critical effect	Reduced body-weight gain in pups at parentally toxic doses
Lowest relevant reproductive NOAEL	3.6 mg/kg bw per day

Developmental target/critical effect	Increased resorptions and incomplete ossification at maternally toxic doses; delayed sexual development
Lowest relevant developmental NOAEL	6.25 mg/kg bw per day (rat; male pubertal development)
	5 mg/kg bw per day (rabbit)
Neurotoxicity	
	No evidence of neurotoxicity in standard tests for toxicity; however, neuroendocrine mode of action has been established for atrazine and its chloro-*s*-triazine metabolites
Other toxicological studies	
Studies on metabolites	DEA, DIA, DACT have the same neuroendocrine mode of action and similar potency to atrazine
	Hydoxyatrazine has a different mode of action and toxicity profile to atrazine
Mode of neuroendocrine action	Atrazine and its chlorometabolites modify hypothalamic catecholamine function and regulation, leading to alterations in pituitary LH and prolactin secretion
Mode of carcinogenic action	The postulated mode of carcinogenic action in female Sprague-Dawley rats involves acceleration of the reproductive ageing process (suppression of LH surge, subsequent estrous cycle disruption), which is not relevant to humans
Direct estrogenic activity	Atrazine has no intrinsic estrogenic activity
Aromatase expression	No effect on aromatase expression in rats
Effects on sexual development	Evidence of delayed sexual development in male and/or female rats by atrazine, DEA, DIA and DACT
Effects on neuronal development	Evidence of impaired postnatal CNS development (and subsequent alterations in prolactin regulation)
Immunotoxicity	Evidence for immune system modulation at doses greater than LOAELs for neuroendocrine disruption or reproductive and developmental effects
Medical data	
	No evidence of atrazine causing effects in manufacturing plant personnel.
	Epidemiology studies do not support a causal association between exposure to atrazine and cancer in humans.

Summary

Atrazine

	Value	**Study**	**Safety factor**
Group ADI[a]	0–0.02 mg/kg bw	Sprague-Dawley rats; 6-month study of LH surge/estrous cycle disruption	100
Group ARfD[a]	0.1 mg/kg bw	Rat; special 4-day study of prolactin release, supported by studies of developmental toxicity in rats and rabbits	100
Hydroxyatrazine			
ADI	0–0.04 mg/kg bw	Sprague-Dawley rats; 2-year study	25
ARfD	Unnecessary	—	—

[a] Group ADI or ARfD for atrazine, deethyl-atrazine (DEA), deisopropyl-atrazine (DIA) and diaminochlorotriazine (DACT).

CNS, central nervous sytem; GnRH, gonadotrophin-releasing hormone; LH, luteinizing hormone.

Appendix 1

Application of the IPCS Conceptual Framework for Cancer Risk Assessment (IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans): consideration of mammary gland tumours in female Sprague-Dawley rats exposed to atrazine

This framework, developed by an International Programme on Chemical safety (IPCS) working group, provides a generic approach to the principles commonly used in evaluating a postulated mode of action (MOA) for tumour induction by a chemical (Boobis et al., 2006). The framework was used by the 2007 JMPR to provide a structured approach to the assessment of the overall weight-of-evidence for the postulated MOA for the increased incidence of mammary tumours (adenomas, carcinomas, fibroadenomas) in rats that was observed after long-term administration of atrazine.

The first stage of the framework is to determine whether it is possible to establish an MOA. This process comprises a series of key events along the causal pathway to cancer, identified using a weight-of-evidence approach on the basis of the Bradford-Hill criteria. The key events are then compared first qualitatively and then quantitatively between the experimental animals and humans. Finally, a statement of confidence, analysis, and implications is provided.

Mammary gland tumours associated with atrazine exposure in female Sprague-Dawley rats

I. Is the weight of evidence sufficient to establish an MOA in animals?

1. Introduction

In seven studies of carcinogenicity in Sprague-Dawley rats fed diets containing atrazine at concentrations of up to 1000 ppm (equal to about 42 and 65 mg/kg bw per day in males and females, respectively), an increased incidence and/or an earlier appearance of spontaneously occurring mammary tumours (adenomas, carcinomas, fibroadenomas) was observed in four studies, while in two studies, there was an earlier appearance of mammary tumours, without any increase in their overall lifetime incidence (see monograph section 2.3).

2. Postulated MOA (theory of the case)

The postulated MOA for mammary tumours induced by atrazine in female Sprague-Dawley rats involves disruption of the hypothalamic–pituitary–ovary axis. Atrazine modifies catecholamine function and the regulation of gonadotropin-releasing hormone (GnRH) pulsatility in the rat hypothalamus, with the consequence that the pulse of luteinizing hormone (LH) released from the pituitary gland is of insufficient amplitude or duration to trigger the ovulation. The failure to ovulate results in persistent secretion of estrogen, which provides a feedback to the pituitary leading to increased secretion of prolactin. The persistent stimulation of the mammary gland by estrogen and prolactin translates into a proliferative response characterized by an earlier appearance and/or a higher incidence of adenocarcinomas (high estrogen, moderate prolactin levels) or fibroadenomas (high prolactin with a background of estrogen) (McConnell, 1989; O'Connor et al., 2000; Simpkins, 2000; Simpkins et al., 2000; Cooper et al., 2007).

3. Key events

The sequence of key events in the mode of carcinogenic action of atrazine in the mammary gland of female Sprague-Dawley rats includes:

- Effect on the hypothalamus, leading to a modification of catecholamine function and the regulation of GnRH pulsatility;
- In consequence, the pulse of LH released from the pituitary gland is of insufficient amplitude or duration to trigger the ovulation;

- The failure to ovulate results in persistent secretion of estrogen, which provides a feedback to the pituitary leading to increased secretion of prolactin;
- The prolonged exposure to estrogen and prolactin causes a hyperstimulation of the mammary gland and an earlier appearance and/or a higher incidence of mammary tumours.

The key events as described above include effects on the hypothalamic control of pituitary function, leading to disruption of the estrous cycle and a persistent stimulation of the mammary gland by endogenous estrogen and prolactin. These effects have been investigated and observed in female Sprague-Dawley rats in short-term and/or mechanistic studies, and at interim and terminal kills in a long-term study. The dose–response relationship and temporal analyses of the key events and tumour response are presented below.

4. Concordance of dose–response relationships

The no-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs) for the key effects in the MOA of atrazine in the mammary gland are provided in Table A1.

In ovariectomized rats, a biologically significant suppression of the serum LH surge was observed after 3 days or 3 weeks of treatment with atrazine at doses of 50 mg/kg bw per day and

Table A1. Summary of dose–response relationship in rats receiving atrazine

Effect	NOAEL/LOAEL	Reference
Hypothalamus		
Increase in GnRH and dopamine	< 25/25 mg/kg bw per day (4-day mechanistic study)	Cooper et al., (2007)
Pituitary		
Attenuation of LH surge	< 300/300 mg/kg bw per day (1-day mechanistic study)	Cooper et al. (2000)
	< 50/50 mg/kg bw per day (3-day mechanistic study)	Cooper et al. (2000)
	< 6.25/6.25 mg/kg bw per day (4-day mechanistic study)	Cooper et al. (2007)
	1.8/3.65 mg/kg bw per day (26-week mechanistic study)	Morseth (1996c)
Ovary		
Disruption of estrous cycle	< 300/300 mg/kg bw per day (1-day mechanistic study)	Cooper et al. (2000)
	< 75/75 mg/kg bw per day (21-day mechanistic study)	Cooper et al. (1996)
	1.8/3.65 mg/kg bw per day (26-week mechanistic study)	Morseth (1996c)
Mammary gland		
Increase in acinar-lobular development, secretory activity and galactocoele formation	3.5/20 mg/kg bw per day (2-year study, 1-year interim kill)	McConnell (1995)
Increase in incidence of palpable mammary masses	3.5/20 mg/kg bw per day (2-year study, 1-year interim kill)	Thakur (1991a, 1992a)
Increase in incidence of mammary tumours	0.5/3.5 mg/kg bw per day (2-year study)	Mayhew (1986)
	1.5/3.1 mg/kg bw per day (2-year study)	Morseth (1996d, 1998)

GnRH, gonadotrophin-releasing hormone; LH, luteinizing hormone.

greater, and in a 26-week study with atrazine at doses of 3.65 mg/kg bw per day and greater. Consistent with attenuation of the LH surge, disruption of the estrous cycle was seen in a 3-week study with atrazine at doses of 75 mg/kg bw per day and greater, and in a 26-week study at doses of 3.65 mg/kg bw per day and greater. Owing to the failure to ovulate and the subsequent persistent exposure to endogenous estrogen and prolactin, hyperstimulation of the mammary gland (increase in acinar-lobular development indicative of increased exposure to estrogen; increase in secretory activity and galactocoele formation indicative of increased exposure to prolactin) was observed with atrazine at doses of 20 mg/kg bw per day and greater, while increased incidences of mammary tumours were found at doses of 3.1 mg/kg bw per day and greater.

Generally, there was a good correlation between the doses causing attenuation of the LH surge and those causing an earlier onset and/or an increased incidence of mammary tumours.

5. Temporal association

The key events, such as attenuation of the LH surge and disruption of the estrous cycle were observed after a single high dose of atrazine at 300 mg/kg bw, after a 3-day or 3-week exposure at doses of 50 or 75 mg/kg bw per day and greater, respectively, and after a 26-week exposure at doses of 3.65 mg/kg bw per day and greater. In 2-year studies in rats, the onset-time for initial palpation of mammary masses was decreased when compared with controls (14 weeks at approximately 20 mg/kg bw per day vs 29 weeks in controls), while the incidence of palpable mammary masses was increased at interim kill (weeks 52–54) after exposure to atrazine at approximately 20 mg/kg bw per day. Thus, there is a logical temporal response with all key events preceding tumour formation.

6. Strength, consistency and specificity of association of tumour response with key events

The key events were observed consistently in a number of studies with differing experimental designs. On the basis of information from the studies described in the monograph, there is sufficient weight of evidence that the key events (attenuation of the LH surge, disruption of the estrous cycle) are linked to the morphological changes in the mammary gland indicative of stimulation of estrogen and prolactin (increase in acinar-lobular development, increase in secretory activity and galactocoele formation) which precede the occurrence of tumours. In addition, there is a substantial independent literature on the role of estrogen and prolactin in the pathogenesis of mammary tumours in rats. There are no significant contradictory data.

7. Biological plausibility and coherence

The relationship between sustained perturbation of the hypothalamic–pituitary axis (change of the regulation of GnRH pulsatility, attenuation of the LH surge), disruption of the estrous cycle, persistent secretion of estrogen and prolactin, prolonged stimulation of the mammary gland by endogenous estrogen and prolactin, and the development of mammary gland tumours is considered to be biologically plausible and has been shown in several studies in laboratory rats.

In long-term bioassays with natural and synthetic estrogens, it has been established that prolonged stimulation of the mammary gland with estrogen leads to development of adenocarcinomas. In contrast, high-level stimulation of the mammary gland with prolactin has been shown to be linked to the development of fibroadenoma.

The tumour response elicited by atrazine is typical of a rodent mammary-gland carcinogen in that mammary tumours are found in female Sprague-Dawley rats but not in male rats or mice. Rats tend to be more sensitive to mammary-gland carcinogenesis than mice, and female rats are frequently found to be more sensitive than male rats with respect to the proportion of chemicals that induce mammary tumours. Consistent with this, concentrations of estrogen and prolactin are typically higher in female rats than in males.

The relationship between increased concentrations of estradiol (E2) and prolactin and tumours of the mammary gland in female rats is further supported by the fact that tumours of the mammary gland tumours do not occur in female Sprague-Dawley rats ovariectomized at an early age and treated with atrazine, as concentrations of E2 and prolactin are both minimal in these females.

8. Other modes of action

Genotoxicity is always one possible MOA to consider, but in a large range of in studies of genotoxicity in vitro and in vivo with atrazine, mostly negative results were obtained in tests using standard methods. The weight of evidence suggests that it is unlikely that atrazine is genotoxic. This conclusion is also supported by the fact that atrazine did not induce a tumorigenic response in ovariectomized rats, while genotoxic carcinogens like dimethylbenz[a]anthracene and *N*-methyl-*N*-nitrosourea are capable of inducing mammary tumours in ovariectomized rats.

In addition, a possible intrinsic estrogenic activity of atrazine was considered. However, the majority of in-vitro studies reported that atrazine did not competitively bind to the rat cytosolic estrogen or progesterone receptors. Also, exposure to atrazine in vivo did not induce uterine growth nor increase the number of progesterone receptors in ovariectomized rats. Thus, the development of tumours of the mammary gland in female rats exposed to atrazine does not seem to be related to any intrinsic estrogenic activity of the compound.

Furthermore, studies in rats indicated no evidence for any atrazine-induced upregulation of aromatase expression in the brain, testes, or mammary gland.

9. Uncertainties, inconsistencies, and data gaps

No inconsistencies were identified in the database for atrazine with regard to the postulated MOA for mammary-gland tumours in female rats.

However, the precise mechanism by which atrazine disrupts the neuronal control of hypothalamic GnRH secretion in the rat remains to be determined.

10. Assessment of postulated MOA

There is sufficient experimental evidence that atrazine disrupts the neuroendocrine control of ovarian function in Sprague-Dawley rats, which leads to premature reproductive senescence (i.e. constant estrus) and a hormonal milieu conducive to the development of mammary gland tumours. The strength, consistency, and specificity of the available MOA information for female Sprague-Dawley rats is further confirmed by data showing that Fischer 344 rats, which have a different reproductive senescence, do not develop atrazine-related mammary tumours. Alternative hypotheses have been ruled out, such as genotoxicity, estrogenic activity or upregulation of aromatase expression, further showing the strength and specificity of the proposed MOA

II. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

The MOA for the formation of mammary tumours in female Sprague-Dawley rats after exposure to atrazine depends on the rat-specific nature of the reproductive cycle and reproductive senescence. Because of the fundamental differences between female Sprague-Dawley rats and humans with regard to both the normal regulation of the pre-ovulatory LH surge and the reproductive senescence, the mammary tumorigenic effects of atrazine in female Sprague-Dawley rats are not expected to occur in humans.

1. Comparison of the reproductive cycles in rodents and humans

In the rodent, the estrous cycle is short and the pre-ovulatory LH surge is brief, timed by the light cycle and dependent on the brain (Simpkins, 2000; Goldman et al., 2007). The brain plays a deterministic role in the LH surge in rodents. Every afternoon during a critical period, a brain signal for LH secretion occurs that is driven by the increased activity of noradrenergic neurons (Wise et al., 1997). As such, selective blockage of this increased activity in noradrenergic neurons during this brief period blocks the pre-ovulatory LH surge (Ordog et al., 1998).

The human menstrual cycle is long, exhibits protracted pre-ovulatory LH surge and ends with menses due to the death of the corpus luteum and the resulting decline in estrogens and progestins. The driving force for the pre-ovulatory LH surge in women is ovarian estrogen secretion (Ordog et al., 1998; Simpkins, 2000). The role of brain regulation of GnRH is that the pre-ovulatory LH surge is permissive in women and other primates. Indeed, the entire menstrual cycle can be recapitulated in Rhesus monkeys in which the source of GnRH has been destroyed, by exogenous administration of pulses of GnRH (Pohl & Knobil, 1982; Ordog et al., 1998). In contrast to the observations in rodents, inhibitors of NE neurotransmission do not affect the pre-ovulatory LH surge in women or other primates (Knobil, 1974; Weiss et al., 1977).

2. Comparison of reproductive ageing in rodents and humans

Reproductive ageing in rodents and women is distinctively different. In female Sprague-Dawley rats, reproductive senescence is a result of a breakdown of the brain regulation of the LH surge, while the ovaries are functional very late into life. The decline in reproductive function is primarily a result of the inability of brain NE neurons to transmit the estrogen signal to GnRH neurons (Wise et al., 1997). The inability to stimulate a pre-ovulatory LH surge results in the maintenance of ovarian follicles and the persistent secretion of estrogens. Sequentially, the increased secretion of estrogens causes a persistent state of hyperprolactinaemia (Welsch et al., 1970; Sarkar et al., 1982; O'Connor et al., 2000). Thus in the Sprague-Dawley rat, reproductive senescence is characterized by persistent hyperestrogenaemia and hyperprolactinaemia with low concentrations of LH and follicle-stimulating hormone (FSH) (Simpkins, 2000).

Advancing age in the female Sprague-Dawley rat is associated with increasing numbers of days spent in estrus with eventual entry into a constant estrous state associated with elevated estrogen and prolactin concentrations and a high incidence of mammary tumours. Other rat strains (such as Fischer 344) are more likely to show age-related increases in the number of days spent in diestrus followed by a constant diestrus or pseudopregnant-like condition. This reproductive state is associated with the development of a number of corpora lutea that are stimulated to secrete progesterone by prolactin. Rats in this reproductive state show a much lower incidence of mammary tumours.

In women, reproductive ageing is characterized by exhaustion of ovarian follicles and the resulting menopause (Taylor, 1998). During menopause, the ability to induce a pre-ovulatory LH surge is normal, but estrogens, the driving force for the cycle, are absent. The menopause is characterized by low concentrations of estrogen and high concentrations of LH and FSH, while prolactin concentrations are usually unchanged or slightly reduced secondary to decreased estrogen (Simpkins, 2000). The major differences between the parameters of reproductive senescence in female Sprague-Dawley rats, female Fischer 344 rats and women are summarized in Table A2.

3. Similarities with reproductive pathologies in women

Polycystic ovarian syndrome (PCOS) is an anovulatory state in women that is characterized by the presence of a cystic ovary associated with elevated concentrations of LH, increased production of estrogen, elevated concentrations of androgens and marked hirsutism. It is associated with obesity,

Table A2. Comparison of parameters of reproductive senescence in female Sprague-Dawley rats, female Fischer 344 rats and humans

Parameter	Sprague-Dawley rats	Fischer 344 rats	Humans
Start of senescence (% of normal lifespan)	30–40%	60–70%	60–70%
Principle cause of senescence	Hypothalamic failure to stimulate LH/FSH	Hypothalamic failure to control prolactin surges	Depletion of ovarian follicle content
LH-surge capability	Lost	Maintained	Maintained
Predominant cycle pattern	Persistent estrus	Pseudopregnancy episodes	Menopause
Estrogen/progesterone ratio	Elevated/prolonged	Reduced	Reduced
Prolactin secretion	Persistently elevated	Episodically elevated	Reduced
Spontaneous incidence of mammary tumours (lifetime)	30–40%	2–5%	8–10%
Principal known factors that increase risk of mammary tumour s	Prolactin, estrogen, chemical mutagens	Prolactin, estrogen, chemical mutagens	Family history, parity, diet, and body weight
Prolactin dependence	High	Median	None

From Simpkins et al. (2000)
LH, luteinizing hormone; FSH, follicle-stimulating hormone.

insulin resistance and altered metabolism of carbohydrate and lipid. An association between the occurrence of PCOS and endometrial hyperplasia and/or cancer has been reported and is biologically plausible on the basis of estrogenic stimulation of the endometrium unopposed by progesterone. Epidemiological evaluation of the association between the occurrence of PCOS and ovarian and breast cancer is not compelling (Eldridge & Delzell, 2000a).

In contrast to women with PCOS, female Sprague-Dawley rats treated with atrazine at high doses display decreased LH, decreased or unaltered concentrations of androgen, weight loss and no association with endometrial or ovarian cancer. The earlier appearance and/or elevated incidence of mammary tumours observed in female Sprague-Dawley rats is attributed to persistent exposure to endogenous estrogen and prolactin resulting from an earlier failure of the neuro-endocrinological control mechanisms that regulate the estrous cycle in this strain of rat.

Hypothalamic amenorrhoea (HA) is characterized by decreased activity in the hypothalamic–pituitary ovarian axis and low-level exposure to endogenous estrogen (Eldridge & Delzell, 2000b). Amenorrhoea may occur as a result of a diverse number of conditions, including stress, anorexia-induced weight loss, exercise-induced weight loss or failure to gain weight, and the occurrence of lactation, and results in failure to have a normal menstrual cycle. Amenorrhoea, from whatever cause, is generally associated with a decreased risk for developing endocrine-mediated tumours in the breast, ovary and uterus. Exposure to atrazine would not lead to a state of persistently increased concentrations of estrogen in women and would not cause an increased incidence of estrogen-mediated tumours in women.

In contrast to hypothalamic amenorrhoea in women, female Sprague-Dawley rats given atrazine at high doses experience prolonged periods of high-level stimulation of estrogen-sensitive tissues by endogenous estrogens (endocrine ageing), which leads to the earlier appearance of mammary tumours.

III. Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic of dynamic factors between experimental animals and humans?

It is not necessary to consider this section in this case because the postulated MOA for the carcinogenesis in the mammary gland of female Sprague-Dawley rats is unique to this strain and, thus, not relevant for risk assessment in humans.

IV. Statement on confidence, analysis, and implications

The postulated MOA is supported by data showing that Sprague-Dawley rats treated with atrazine maintain constant estrus as a result of reproductive senescence. The strength, consistency, and specificity of the available information on MOA for Sprague-Dawley females is further confirmed by data showing that Fischer 344 rats, which have a different reproductive senescence, do not form atrazine-related mammary tumours. A concordance analysis comparing key events in the animal MOA with related reproductive processes in the human female shows distinct differences. Alternative hypotheses have been ruled out, such as genotoxicity or estrogenic activity, further showing the strength and specificity of the proposed MOA. Because hypothalamic dysfunction leading to cessation of ovulation as the MOA for tumour formation appears to be specific to the female Sprague-Dawley rat and does not appear to have a counterpart in the human female, atrazine-related mammary tumours formed by this MOA in the Sprague-Dawley rat are qualitatively not relevant for risk assessment in humans (Meek et al., 2003).

In addition, epidemiological studies indicate that there is no known association between atrazine and any cancer. Three types of studies (cohort studies, case–control studies, and ecological or correlational studies) have assessed possible relationships between atrazine or other triazine herbicides and cancer in humans. Each study has shortcomings that limit conclusions. For example, limitations of the ecological studies include: (a) atrazine and other exposures were not measured at the level of the individual subject, but rather at the level of geographic region; (b) exposures were measured virtually concurrently with cancer occurrence; (c) factors such as duration of exposure and time since first exposure could not be analysed; and (d) controls for confounding was not adequate. Nonetheless, the weight of evidence from these studies indicated that atrazine is unlikely to cause cancer in humans.

References

Adler, I.D. (1980) A review of the coordinated research effort on the comparison of test systems for the detection of mutagenic effects, sponsored by the E.E.C. *Mutat. Res*., **74**, 77–93.

Alavanja, M.C.R., Samanic, C., Dosemeci, M., Lubin, J., Tarone, R., Lynch, C.F., Knott, C., Thomas, K., Hoppin, J.A., Barker, J., Coble, J., Sandler, D.P. & Blair, A. (2003) Use of agricultural pesticides and prostate cancer risk in the agricultural health study cohort. *Am. J. Epidemiol.,* **157**, 800–814.

Arni, P. (1978). Salmonella/mammalian-microsome mutagenicity test with G 30027. Unpublished report No. 782527 dated 18 July 1978, from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Arthur, A.T. (1984). A teratology of atrazine technical in New Zealand White rabbits. Unpublished report No. 832110 dated 18 September 1984 from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection AG.

Ashby, J., Tinwell, H., Stevens, J., Pastoor, T. & Breckenridge, C.B. (2002) The effects of atrazine on the sexual maturation of female rats. *Regul. Toxicol. Pharmacol.,* **35**, 468–473.

Bachmann, M. (1994) G 30027. 3-Month oral toxicity study in rats. Unpublished report No. 931063 dated 5 August 1994, from Ciba Geigy Ltd, Stein, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Ballantine, L., Murphy, T. G. & Simoneaux, B.J. (1985) Metabolism of ^{14}C atrazine in orally dosed rats. Unpublished report No. ABR-85104 dated 6 February 1985, from Ciba-Geigy Corp., Greensboro, North Carolina, USA.. Submitted to WHO by Syngenta Crop Protection AG.

Basler, A. & Rohrborn, G. (1978) SCE test in vivo: chromosome aberrations in bone marrow cells. Unpublished report No. 175-77-1 ENV D from University of Düsseldorf, Germany.

Blondell, J. & Dellarco, V. (2003) Review of atrazine cancer epidemiology. Unpublished report No. D295200 dated 28 October 2004 from United States Environmental Protection Agency, Health Effects Division (7509C).

Boobis, A.R., Cohen, S.M., Dellarco, V., McGregor, D., Meek, M.E., Vickers, C., Willcocks, D., Farland, W. (2006) IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Crit. Rev. Toxicol*., **36**, 78–192.

Brusick, D.J. (1994) An assessment of the genetic toxicity of atrazine: relevance to human health and environmental effects. *Mutat. Res*., **317**, 133–144.

Brusick, D. (2000) An assessment of the genetic toxicology database for atrazine 1994–2000. Unpublished report dated 21 June 2000, pp 1–15. Submitted to WHO by Syngenta Crop Protection AG.

Butler, M.A. & Hoagland, R.E. (1989) Genotoxicity assessment of atrazine and some major metabolites in the Ames test. *Bull. Environ. Contam. Toxicol*., **43**, 797–804.

Catenacci, G., Barbieri, F., Bersani, M., Feriolo, A., Cottica, D. & Maroni, M. (199). Biological monitoring of human exposure to atrazine. *Toxicol. Lett*., **69**, 217–222.

Ceresa, C. (1988a) G 34048 - micronucleus test, mouse. Unpublished report No. 871373 dated 31 August 1988, from Ciba-Geigy Ltd, Basle, Switzerland, Submitted to WHO by Syngenta Crop Protection AG.

Ceresa, C. (1988b) G 30027 - micronucleus test, mouse. Unpublished report No. 871546 dated 31 May 1988, from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Chau, R.Y., McCormick, G.C. & Arthur, A.T. (1990) Hydroxyatrazine - 13-week feeding study in dogs. Unpublished report No. 892076 dated 20 March 1990, from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA, Submitted to WHO by Syngenta Crop Protection AG.

Chollet, M.C., Degraeve, N., Gilot-Delhalle, J., Colizzi, A., Moutschen, J. & Moutschen-Dahmen, M. (1982). Mutagenic efficiency of atrazine with and without metabolic activation. *Mutation Res*. **97**, 237–238. Abstract presented at the 1st Meeting of the Belgian Environmental Mutagen Society, Brussels, 24 October 1981.

Chow, E. & Hart, E. (1995) Two-year dietary chronic toxicity/oncogenicity study with G-34048 technical in rats. Unpublished report No. F-00125 dated 27 January 1995, from Environmental Health Center, Farmington, Connecticut, USA.. Submitted to WHO by Ciba-Geigy Ltd.

Connor, K., Howell, J., Chen, I., Liu, H., Berhane, K., Sciarretta, C., Safe, S. & Zacharewski, T. (1996) Failure of chloro-s-triazine-derived compounds to induce estrogen receptor-mediated responses in vivo and in vitro. *Fundam. Appl. Toxicol.,* **30**, 93–101.

Connor, K., Howell, J., Safe, S., Chen, I., Liu, H., Berhane, K., Sciarretta, C., & Zacharewski, T. (1998) Failure of chloro-s-triazine-derived compounds to induce estrogenic responses in vivo and in vitro. In: Ballantine, L.G., McFarland, J.E. & Hackett, D.S., eds, *Triazine herbicides: risk assessment.* Washington, DC, Oxford University Press,.pp 424–431.

Cooper, R.L., Stoker, T.E., Goldman, J.M., Parrish, M.B. & Tyrey, L. (1996) Effect of atrazine on ovarian function in the rat. *Reprod. Toxicol*., **10**, 257–264.

Cooper, R.L., Laws, S.C., Das, P.C., Narotsky, M.G., Goldman, J.M., Tyrey, E.L. & Stoker, T.E. (2007) Atrazine and reproductive function: mode and mechanism of action studies. *Birth Defects Res. B Dev. Reprod. Toxicol*., **80**, 98–112.

Cooper, R.L., Stoker, T.E., Tyrey, L., Goldman, J.M. & McElroy, W.K. (2000) Atrazine disrupts the hypothalamic control of pituitary-ovarian function. *Toxicol. Sci.,* **53**, 297–307.

Cummings, A.M., Rhodes, B.E. & Cooper, R. L. 2000. Effect of atrazine on implantation and early pregnancy in four strains of rats. *Toxicol. Sci.,* **58**, 135–143.

Das, P.C., McElroy, W.K. & Cooper, R.L. (2000) Differential modulation of catecholamines by chlorotriazine herbicides in pheochromocytoma (PC12) cells in vitro. *Toxicol. Sci.,* **56**, 324–331.

Das, P.C., McElroy, W.K. & Cooper, R.L. (2001) Alteration of catecholamines in pheochromocytoma (PC12) cells in vitro by the metabolites of chlorotriazine herbicide. *Toxicol. Sci.,* **59**, 127–137.

Das, P.C., McElroy, W.K. & Cooper, R.L. (2003) Potential mechanisms responsible for chlorotriazine-induced alterations in catecholamines in pheochromocytoma (PC12) cells. *Life Sci.,* **73**, 3123–3138.

Davidson, I.W.F. (1988) Metabolism and kinetics of atrazine in man. Unpublished report No. 101947 dated 1989, from Bowman Gray School of Medicine, Department of Pysiology/ Pharmacology, North Carolina, USA. Submitted to WHO by Syngenta Crop Protection AG.

De Roos, A.J., Zahm, S.H., Cantor, K.P., Weisenburger, D.D., Holmes, F.F., Burmeister, L.F. & Blair, A. (2003) Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup. Environ. Med.,* **60**, E11.

De Veer, I., Moriske, H.J., & Ruden, H. (1994) Photochemical decomposition of organic compounds in water after UV-irradiation: investigation of positive mutagenic effects. *Toxicol. Lett.,* **72**, 113–119.

Della Croce, C., Morichetti, E., Intorre, L., Soldani, G., Bertini, S. & Bronzetti, G. (1996) Biochemical and genetic interactions of two commercial pesticides with the monooxygenase ystem and chlorophyllin. *J. Environ. Pathol. Toxicol. Oncol*., **15**, 21–28.

Deparade, E. (1986) G-30027 - Salmonella/mammalian microsome mutagenicity test. Unpublished report No. 861172 dated 5 December 1986, from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Deparade, E. (1987) G 28273 - Salmonella/mammalian-microsome mutagenicity test. Unpublished report No. 871372 dated 10 November 1987 from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Deparade, E. (1988) G 34048 - Salmonella/mammalian-microsome mutagenicity test. Unpublished report No. 871376 dated 15 February 1988 from Ciba-Geigy Ltd, Basle, Switzerland.. Submitted to WHO by Syngenta Crop Protection AG.

Deparade, E. (1989) G 30033 - Salmonella and Escherichia/liver-microsome test. Unpublished report No. 891236 dated 18 December 1989 (amended 21 December 1993) from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Deparade, E. (1990) G 28279 - Salmonella and *Escherichia*/liver-microsome test. Unpublished report No. 891243 dated 18 January 1990 (amended 21 December 1993) from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Ciba-Geigy Ltd.

Devos, S., De Bosscher, K., Staels, B., Bauer, E., Roels, F., Berghe, W., Haegeman, G., Hooghe, R. & Hooghe-Peters, E.L. (2003) Inhibition of cytokine production by the herbicide atrazine. Search for nuclear receptor targets. *Biochem. Pharmacol.,* **65**, 303–308.

Devos, S., Van Den Heuvel, R., Hooghe, R. & Hooghe-Peters, E.L. (2004) Limited effect of selected organic pollutants on cytokine production by peripheral blood leukocytes. *Eur.Cytokine Netw.*, **15**, 145–151.

Dooley, G.P., Prenni, J.E., Prentiss, P.L., Cranmer, B.K., Andersen, M.E. & Tessari, J.D. (2006) Identification of a novel hemoglobin adduct in Sprague Dawley rats exposed to atrazine. *Chem. Res. Toxicol.,* **19**, 692–700.

Duchosal, F., Vogel, O., Chevalier, H.J., Luetkemeier, H., & Biedermann, K. (1990a) 28-Day oral toxicity (feeding) study with G 28279 tech. in the rat. Unpublished report No. RCC 252090 dated 21 September 1990 from RCC AG, Itingen, Switzerland.

Duchosal, F., Vogel, O., Wilson, J.T., Luetkemeier, H., & Biedermann, K. (1990b) 28-Day oral toxicity (feeding) study with G 30033 tech in the rat. Unpublished report No. RCC 252088 / CG 891235 dated 21 September 1990 from RCC AG, Itingen, Switzerland.

Dunkelberg, H., Fuchs, J., Hengstler, J.G., Klein, E., Oesch, F. & Struder, K. (1994) Genotoxic effects of the herbicides alachlor, atrazine, pendimethaline, and simazine in mammalian cells. *Bull. Environ. Contam. Toxicol.,* **52**, 498–504.

Ehling, U.H. 1979. Micronucleus test in mouse bone marrow. Progress report (March. 1979–December. 1979). Unpublished report No. 136-77-1 ENV D GSF from Gesellschaft für Strahlenschutz und Umweltforschung (GSF), Neuherberg, Germany.

Eldridge, J.C. & Delzell, E. (2000a) An evaluation of the association between polycystic ovarian syndrome in women and the occurrence of ovarian, breast, and endometrial cancer and an assessment of the relevance of finding in female Sprague-Dawley rats administered high doses of atrazine to PCOS in women. Unpublished report from Department of Physiology and Pharmacology, Wake Forest University School of Medicine, and Department of Epidemiology, University of Alabama School of Public Health. Submitted to WHO by Syngenta Crop Protection AG.

Eldridge, J.C. & Delzell, E. (2000b) Endocrinological characterization of hypothalic amenorrhea (HA) in women and an evaluation of the relevance of the endocrine effects observed in female Sprague-Dawley rats treated with high doses of atrazine to HA in women. Unpublished report from Department of Physiology and Pharmacology, Wake Forest University School of Medicine, and Department of Epidemiology, University of Alabama School of Public Health. Submitted to WHO by Syngenta Crop Protection AG.

Eldridge, J.C., Tennant, M.K., Wetzel, L.T., Breckenridge, C.B. & Stevens, J.T. (1994) Factors affecting mammary tumor incidence in chlorotriazine-treated female rats: hormonal properties, dosage, and animal strain. *Environ. Health Perspect*., **102**, 29–36.

Eldridge, J.C., McConnell, R.F., Wetzel, L.T. & Tisdel, M.O. (1998) Appearance of mammary tumors in atrazine-treated female rats: probable mode of action involving strain-related control of ovulation and estrous cycling. In: Ballantine, L.G., McFarland, J.E. &. Hackett, D.S., eds, *Triazine herbicides: risk assessment,* Washington, DC: Oxford University Press, pp. 414–423.

Eldridge, J.C., Wetzel, L.T., Stevens, J.T. & Simpkins, J.W. (1999a) The mammary tumor response in triazine-treated female rats: a threshold-mediated interaction with strain and species-specific reproductive senescence. *Steroids*, **64**, 672–678.

Eldridge, J.C., Wetzel, L.T., Tisdel, M.O. & Luempert, L.G. (1993a) Revised supplement to final report: Determination of hormone levels in Sprague-Dawley rats treated with atrazine technical. Unpublished report dated 8 April 1993 from Bowman Gray School of Medicine, Winston-Salem, North Carolina; HWA study No. 483-278. Submitted to WHO by Syngenta Crop Protection AG.

Eldridge, J.C., Wetzel, L.T., Tisdel, M.O. & Luempert, L.G. (1993b) Revised supplement to final report: Determination of hormone levels in Fischer-344 rats treated with atrazine technical. Unpublished report dated 8 April 1993 from Bowman Gray School of Medicine, Winston-Salem, North Carolina; HWA study No. 483-279. Submitted to WHO by Syngenta Crop Protection AG.

Eldridge, J.C., Wetzel, L.T. & Tyrey, L. (1999b) Estrous cycle patterns of Sprague-Dawley rats during acute and chronic atrazine administration. *Reprod. Toxicol.*, **13**, 491–499.

Eldridge, J.C., Minnema, D., Breckenridge, C.B., McFarland, J. & Stevens, J.T. (2001) The effect of 6 months of feeding of atrazine or hydroxyatrazine on the leutinizing hormone surge in female Sprague-Dawley and Fischer 344 rats. *Soc. Toxicol. 28* March, Abstract No. 1525.

Engel, L.S., Hill, D.A., Hoppin, J.A., Lubin, J.H., Lynch, C.F., Pierce, J., Samanic, C., Sandler, D.P., Blair, A. & Alavanja, M.C. (2005) Pesticide use and breast cancer risk among farmers' wives in the agricultural health study. *Am. J. Epidemiol*., **161**, 121–135.

Enoch, R.R., Stanko, J.P., Greiner, S.N., Youngblood, G.L., Rayner, J.L. & Fenton, S.E. (2007) Mammary gland development as a sensitive end point after acute prenatal exposure to an atrazine metabolite mixture in female Long-Evans rats. *Environ. Health Perspect*., **115**, 541–547.

Fan, W., Yanase, T., Morinaga, H., Gondo, S., Okabe, T., Nomura, M., Komatsu, T., Morohashi, K.-I., Hayes, T.B., Takayanagi, R. & Nawata, H. (2007) Atrazine-induced aromatase expression is SF-1 dependent:

implications for endocrine disruption in wildlife and reproductive cancers in humans. *Environ. Health Perspect.,* **115**, 720–727.

Filipov, N.M., Pinchuk, L.M., Boyd, B.L. & Crittenden, P.L. (2005) Immunotoxic effects of short-term atrazine exposure in young male C57BL/6 mice. Toxicol. Sci., 86, 324–332.

Fournier, M., Friborg, J., Girard, D., Mansour, S. & Krzystyniak, K. (1992) Limited immunotoxic potential of technical formulation of the herbicide atrazine (AAtrex) in mice. *Toxicol. Lett*., **60**, 263–274.

Gebel, T., Kevekordes, S., Pav, K., Edenharder, R. & Dunkelberg, H. (1997) In vivo genotoxicity of selected herbicides in the mouse bone-marrow micronucleus test. *Arch. Toxicol.,* **71**, 193–197.

Geleick, D. (1991a) G 30033 – autoradiographic DNA repair test on rat hepatocytes in vitro. Unpublished report No. 901310 dated 26 April 1991 (amended 21 December 1993) from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Geleick, D. (1991b) G 28279 - autoradiographic DNA repair test on rat hepatocytes in vitro. Unpublished report No. 901308 dated 12 April 1991 (amended 21 December 1993) from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Ciba-Geigy Ltd.

Gerspach, R. (1991) G 30033 - 3-month oral toxicity study in rats (administration in food). Unpublished report No. 901264 dated 22 October 1991 from Ciba-Geigy Ltd, Stein, Switzerland, Submitted to WHO by Syngenta Crop Protection AG.

Giknis, M.L.A. (1989) Atrazine technical - a teratology (segment II) study in rats. Unpublished report No. 882049 dated 12 February 1989 from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection AG.

Giurgea, R. & Koszta, M. (1979) Action of herbicide “Atrazine” on thymus and bursa in chickens. *Arch. Exp. Veterinarmed.,* **33**, 703–707.

Giurgea, R., Borsa, M. & Bucur, N. (1981) Immunological reactions of Wistar rats following administration of atrazine and prometryne. *Arch. Exp. Veterinarmed.,* **35**, 811–815.

Goldman, J.M., Murr, A.S. & Cooper, R.L. (2007) The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies. *Birth Defects Res. B Dev. Reprod. Toxicol*., **80**, 84–97.

Guigas, C., Pool-Zobel, B.L. & Diehl, J.F. (1993) The combination effects of quercetin with the herbicides atrazine, cyanazine and gesamprim in mutagenicity tests. *Z Ernahrungswiss.,* **32**, 131–138.

Hamboeck, H., Fischer, R.W., Di Iorio, E.E. & Winterhalter, K.H. (1981) The binding of s-triazine metabolites to rodent haemoglobins appears irrelevant to other species. *Mol. Pharmacol.,* **20**, 579–584.

Hartmann, H.R. (1989) G 30027 - acute inhalation toxicity in the rat. Unpublished report No.891162 dated 24 August 1989 from Ciba-Geigy Ltd, Stein, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Hartmann, H.R. (1993) G 30027 - acute dermal toxicity study of G 30027 in rats. Unpublished report No. 931184 dated 2 December 1993 from Ciba-Geigy Ltd, Stein, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Hazelette, J.R. & Green, J. (1987) G 30027 - oncogenicity study in mice. Unpublished report No. 842120 dated 30 October 1987 from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection AG.

Hazelette, J.R. & Arthur, A.T. (1989a) Hydroxy-atrazine - pilot 4-week oral feeding toxicity study in rats. Unpublished report No. 872282 dated 22 March 1989 from Ciba-Geigy Corp., Research Department, Pharmaceutical Division, Summit, New Jersey, USA.

Hazelette, J.R. & Arthur, A.T. (1989b) Hydroxy-atrazine - pilot 4-week oral feeding toxicity study in dogs. Unpublished report No. 872156 dated 22 March 1989 from Ciba-Geigy Corp., Research Department, Pharmaceutical Division, Summit, New Jersey, USA.

Heneweer, M., van den Berg, M. & Sanderson, J.T. (2004) A comparison of human H295R and rat R2C cell lines as in vitro screening tools for effects on aromatase. *Toxicol. Lett*., **146**, 183–194.

Hertner, Th. (1988a) G 34048 - autoradiographic DNA repair test on rat hepatocytes. Unpublished report No. 871374 dated 22 January 1988 from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Hertner, Th. (1988b) G 28273 - autoradiographic DNA repair test on rat hepatocytes. Unpublished report No. 871370 dated 10 March 1988 from Ciba-Geigy Ltd, Basle, Switzerland.. Submitted to WHO by Syngenta Crop Protection AG.

Hertner, Th. (1992) G 30027 - autoradiographic DNA repair test on rat hepatocytes. Unpublished report No. 911246 dated 14 April 1992 from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Hertner, Th. (1993) G 30027 - dominant lethal test, mouse, 8-weeks. Unpublished report No. 911247 dated 7 January 1993 from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Hessel, P.A., Kalmes, R., Smith, T.J., Lau, E., Mink, P.J. & Mandel, J. (2004) A nested case–control study of prostate cancer and atrazine exposure. *J. Occup. Environ. Med.*, **46**, 379–385.

Holbert, M. S. (1991) G 30027 - Acute inhalation toxicity study in rats. Unpublished report No. 8079-91 dated 18 July 1991 from Stillmeadow, Inc., Houston, Texas, USA. Submitted to WHO by Syngenta Crop Protection AG.

Hooghe, R.J., Devos, S. & Hooghe-Peters, E.L. (2000) Effects of selected herbicides on cytokine production in vitro. *Life Sci.,* **66**, 2519–2525.

Hool, G. (1981a) G 30027 - nucleus anomaly test in somatic interphase nuclei of Chinese hamster. Unpublished report No. 783027 dated 20 January 1981 (supplemented 6 October 1986) from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Hool, G. (1981b) G 30027 - chromosome studies in male germinal epithelium in mouse (test for mutagenic effects on spermatogonia). Unpublished report No. 800209 dated 25 January 1981 (supplemented 6 October 1986) from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Hool, G. (1981c) G 30027 - chromosome studies in male germinal epithelium in mouse (test for mutagenic effects on spermatocytes). Unpublished report No. 800210 dated 28 January 1981 (supplemented 6 October 1986) from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Hool, G., (1981d) G 30027 – dominant lethal test in mouse. Unpublished report No. 801380 dated 8 August 1981 from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Huber, K.R., Batastini, G., & Arthur, A.T. (1989) G 30027 - 21-day dermal toxicity study in the rabbit. Unpublished report No. 882035 dated 1 December 1989 from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection AG.

Hui, X., Wester, R., Maibach, H.I., Gilman, S.D., Gee, S.J., Hammock, B.D., Simoneaux, B., Breckenridge, C. & Kahr, R. (1996a) Disposition of atrazine in Rhesus monkey following intravenous administration. Unpublished report of Study Nos UCSF 95SU04 (UCSF), BDH-081-1 (UC Davis), ABR 96066, 96073 dated 3 September 1996 from Ciba Crop Protection. Submitted to WHO by Syngenta Crop Protection AG.

Hui, X., Wester, R., Maibach, H.I., Simoneaux, B. & Breckenridge, C. (1996b) Disposition of atrazine in Rhesus monkey following oral administration. Unpublished report of study Nos UCSF 96SU01 (UCSF) ABR-96094; Ciba Study Number 306-96, dated 30 October 1996, from Ciba Crop Protection. Submitted to WHO by Syngenta Crop Protection AG.

Hummel, H., Youreneff, M., Giknis, M. & Yau, E.T. (1989) G 28273 - a teratology (segment II) study in rats. Unpublished report No. 872177 dated 15 August 1989 from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection AG.

IARC (1999) Atrazine. In: *Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances*. Vol. 73, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. IARCPress, Lyon.

Infurna, R.N. (1984) Atrazine technical, a teratology study (segment II) in rats. Unpublished report No. 832109 dated 18 September 1984 from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection AG.

Innes, J.R.M., Ulland, B.M., Valerio, M.G., Pertrucelli, L., Fishbein, L., Hart, E.R., Pallota, A.J., Bates, R.R., Falk, H.L., Gart, J.J., Klein, M., Mitchell, I. & Peters, J. (1969) Bioassay of pesticides and industrial chemicals for tumorogenicity in mice: a preliminary note. *J. Natl. Cancer Inst*., **42**, 1101–1114.

Kahrs, R. & Thede, B. (1987) Study of ^{14}C-atrazine dose/response relationship in the rat. Unpublished report No. ABR-87087 dated 23 October 1987 form Ciba-Geigy Corp., Greensboro, North Carolina, USA. Submitted to WHO by Syngenta Crop Protection AG.

Karrow, N.A., McCay, J.A., Brown, R.D., Musgrove, D.L., Guo, T.L., Germolec, D.R. & White, K.L., Jr. (2005) Oral exposure to atrazine modulates cell-mediated immune function and decreases host resistance to the B16F10 tumor model in female B6C3F1 mice. *Toxicol.,* **209**, 15–28.

Kligerman, A.D., Doerr, C.L., Tennant, A.H. & Peng, B. (2000a) Cytogenetic studies of three triazine herbicides. In vivo micronucleus studies in mouse bone marrow. *Mutat. Res.,* **471**, 107–112.

Kligerman, A.D., Doerr, C.L., Tennant, A.H. & Zucker, R.M. (2000b) Cytogenetic studies of three triazine herbicides. In vitro studies. *Mutat. Res.,* **465**, 53–59.

Knobil, E. (1974) On the control of gonadotropin secretion in the Rhesus monkey. *Recent Prog. Hormone Res.,* **30**, 1–36.

Kuhn, J.O. (1988) G 30027 - acute oral toxicity study (mouse). Unpublished report No. 5421-88 dated 27 July 1988 from Stillmeadow Inc., Houston, Texas, USA. Submitted to WHO by Syngenta Crop Protection AG.

Kuhn, J.O. (1991a) G 30027 - acute oral toxicity study in rats. Unpublished report No. 7800-91 dated 18 March 1991 from Stillmeadow Inc., Sugar Land, Texas, USA. Submitted to WHO by Syngenta Crop Protection AG.

Kuhn, J.O. (1991b) DACT (G 28273) - technical ground FL-871776 - acute oral toxicitity study in rats. Unpublished report No. 7801-91 dated 21 March 1991 from Stillmeadow Inc., Sugar Land, Texas, USA. Submitted to WHO by Syngenta Crop Protection AG.

Kuhn, J.O. (1991c) G 30033 - acute oral toxicity study in rats. Unpublished report No. 7802-91 dated 22 March 1991 from Stillmeadow Inc., Sugar Land, Texas, USA. Submitted to WHO by Syngenta Crop Protection AG.

Kuhn, J.O. (1991d) Deisopropyl-atrazine (G 28279) - acute oral toxicity study in rats. Unpublished report No. 7803-91 dated 25 March 1991 from Stillmeadow Inc., Sugar Land, Texas, USA. Submitted to WHO by Syngenta Crop Protection AG.

Kuhn, J.O. (1991e) Hydroxyatrazine (G 34048) - technical FL-870869 - acute oral toxicity study in rats. Unpublished report No. 7938-91 dated 3 April 1991 from Stillmeadow Inc., Sugar Land, Texas, USA. Submitted to WHO by Syngenta Crop Protection AG.

Laws, S.C., Ferrell, J.M., Stoker, T.E., Schmid, J. & Cooper, R.L. (2000) The effects of atrazine on female Wistar rats: an evaluation of the protocol for assessing pubertal development and thyroid function. *Toxicol. Sci.*, **58**, 366–376.

Laws, S.C., Ferrell, J.M., Stoker, T.E. & Cooper, R.L. (2003) Pubertal development in female Wistar rats following exposure to propazine and atrazine biotransformation by-products, diamino-*S*-chlorotriazine and hydroxyatrazine. *Toxicol. Sci.*, **76**, 190–200.

Laws, S.C., Ferrell, J., Best, D., Buckalew, A., Murr, A. & Cooper, R.L. (2006) Atrazine stimulates the release of serum ACTH and adrenal steroids in male Wistar rats. Unpublished report (abstract) from Reprod. Tox. Division, NHEERL, ORD, U.S. EPA, Research Triangle Park, NC, USA.

Lindsay, L.A., Wimbert, K.V., Giknis, M.L.A. & Yau, E.T. (1989) Hydroxyatrazine (G 34048) - a teratology (segment II) study in rats. Unpublished report No. 872202 dated 14 February 1989 (supplemented 2 November 1990 and 14 May 1993) from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection AG.

Lioi, M.B., Scarfi, M.R., Santoro, A., Barbieri, R., Zeni, O., Salvemini, F., Di Berardino, D. & Ursini, M.V. (1998) Cytogenetic damage and induction of pro-oxidant state in human lymphocytes exposed in vitro to glyphosate, vinclozolin, atrazine, and DPX-E9636. *Environ. Mol. Mutagen*., **32**, 39–46.

Liu, C.Y. & Thakur, A.K. (1999) Statistical report for survival and mammary tumor analyses from the Fischer 344/Lati rat study (Pinter, 1990). Unpublished report No. 6117-998 dated 2 September 1999 from Covance Laboratories Inc., Vienna, Virginia, USA. Submitted to WHO by Syngenta Crop Protection AG.

Luebke, R.W., Chen, D.H., Dietert, R., Yang, Y., King, M. & Luster, M.I. (2006) The comparative immunotoxicity of five selected compounds following developmental or adult exposure. *J. Toxicol. Environ. Health B Crit. Rev.*, **9**, 1–26.

Lynch, S.M., Rusiecki, J.A., Blair, A., Dosemeci, M., Lubin, J., Sandler, D., Hoppin, J.A., Lynch, C.F. & Alavanja, M.C.R. (2006) Cancer incidence among pesticide applicators exposed to cyanazine in the Agricultural Health Study. *Environ. Health Perspect*., **114**, 1248–1252.

MacLennan, P.A., Delzell, E., Sathiakumar, N., Myers, S.L., Cheng, H., Grizzle, W., Chen, V.W. & Wu, X.C. (2002) Cancer incidence among triazine herbicide manufacturing workers. *J. Occup. Environ. Med.,* **44**, 1048–1058.

MacLennan, P.A., Delzell, E., Sathiakumar, N. & Myers, S.L. (2003) Mortality among triazine herbicide manufacturing workers. *J. Toxicol. Environ. Health A.,* **66**, 501–517.

Mainiero, J., Youreneff, M. & Giknis, M. (1987) Two-generation reproduction study in rats: atrazine technical. Final report. Unpublished report No. MIN 852063 dated 2 September 1987 from Safety Evaluation Facility. Summit, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection AG.

Marty, J.H. (1992a) G 28279 - developmental toxicity (teratogenicity) study in rats (oral administration). Unpublished report No. 901262 dated 1 June 1992 from Ciba-Geigy Ltd, Stein, Switzerland. Submitted to WHO by Ciba-Geigy Ltd.

Marty, J.H. (1992b) Developmental toxicity (teratogenicity) study in rats with G 30033 technical (oral administration). Unpublished report No. 901265 dated 1 June 1992 from Ciba-Geigy Ltd, Stein, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Maurer, Th. (1983) G 30027 - skin sensitization effects in guinea pigs (optimization test). Unpublished report No. 830644 dated 29 November 1983 from Ciba-Geigy Ltd, Basle, Switzerland.. Submitted to WHO by Syngenta Crop Protection AG.

Mayhew, D.A. (1986) Two-year chronic feeding/oncogenicity study in rats administered atrazine. Unpublished report No. 410-1102 dated 29 April 1986 from American Biogenics Corporation, Decatur, IL, USA. Submitted to WHO by Syngenta Crop Protection AG.

McConnell, R.F. (1989) Comparative aspects of contraceptive steroids: effects observed in rats. *Toxicol. Pathol.,* **17**, 385–388.

McConnell, R.F. (1995) A histomorphologic reevaluation of the ovaries, uterus, vagina, mammary gland, and pituitary gland from Sprague-Dawley and Fischer 344 female rats treated with atrazine. Unpublished report Nos 483-278 & 483-279 dated 10 March 1995 from Hazleton Laboratories, Vienna, Virginia, USA. Submitted to WHO by Syngenta Crop Protection AG.

McElroy, J.A., Gangnon, R.E., Newcomb, P.A., Kanarek, M.S., Anderson, H.A., Vanden Brook, J., Trentham-Dietz, A.M. & Remington, P.L. (2007) Risk of breast cancer for women living in rural areas from adult exposure to atrazine from well water in Wisconsin. *J. Expo. Sci. Environ. Epidemiol*., **17**, 207–214.

McMullin, T.S., Andersen, M.E., Nagahara, A., Lund, T.D., Pak, T., Handa, R.J. & Hanneman, W.H. (2004) Evidence that atrazine and diaminochlorotriazine inhibit the estrogen/progesterone induced surge of luteinizing hormone in female Sprague-Dawley rats without changing estrogen receptor action. *Toxicol. Sci.,* **79**, 278–286.

Meek, M.E., Bucher, J.R., Cohen, S.M., Dellarco, V., Hill, R.N., Lehman-McKeeman, L.D., Longfellow, D.G., Pastoor, T., Seed, J. & Patton, D.E. (2003) A framework for human relevance analysis of information on carcinogenic modes of action. *Crit. Rev. Toxicol.,* **33**, 591–653.

Meisner, L.F., Belluck, D.A. & Roloff, B.D. (1992) Cytogenetic effects of alachlor and/or atrazine in vivo and in vitro. *Environ. Mol. Mutagen.,* **19**, 77–82.

Mencoboni, M., Lerza, R., Bogliolo, G., Flego, G. & Pannacciulli, I. (1992) Effect of atrazine on hemopoietic system. *In Vivo.,* **6**, 41–44.

Mehta, C.S. (1980a) 2,4-Diamino-6-chloro-s-triazine (DACT, G 28273) FL-781538 ARS 1857 - rat acute oral toxicity. Unpublished report No. 1290A-79 dated 6 August 1980 from Stillmeadow Inc., Houston, Texas, USA. Submitted to WHO by Syngenta Crop Protection AG.

Mehta, C. S. (1980b) 2,4-Diamino-6-chloro-s-triazine (DACT, G 28273) FL-781538 ARS 1857 - rat acute oral toxicity. Unpublished report No. 1290B-79 dated 7 August 1980 from Stillmeadow Inc., Houston, Texas, USA. Submitted to WHO by Syngenta Crop Protection AG. Submitted by: Syngenta Crop Protection AG.

Meyer, A. (1987) G 28273 - autoradiographic DNA repair test on human fibroblasts. Unpublished report No. 871371 dated 20 November 1987 from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Meyer, A. (1988) G 34048 - autoradiographic DNA repair test on human fibroblasts. Unpublished report No. 871375 dated 11 January 1988 from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Mills, P.K. (1998). Correlation analysis of pesticide use data and cancer incidence rates in California counties. *Arch. Environ. Health.,* **53**, 410–413.

Mills, P.K. & Yang, R. (2003) Prostate cancer risk in California farm workers. *J. Occup. Environ. Med.,* **45**, 249–258.

Mills, P.K. & Yang, R. (2006) Regression analysis of pesticide use and breast cancer incidence in California Latinas. *J. Environ. Health*., **68**, 15–22.

Minnema, D. (2001) Comparison of the LH surge in female rats administered atrazine, simazine or DACT via oral gavage for one month. Final report. Unpublished report No. 1198-98 dated 21 March 2001 from Covance Laboratories Inc., Vienna, Virginia, USA. Submitted to WHO by Syngenta Crop Protection AG.

Minnema, D. (2002) 52-Week toxicity study of simazine, atrazine, and DACT administered in the diet to female rats. Unpublished report No. 2214-01 dated 21 February 2002 from Covance Laboratories Inc., Vienna, Virginia, USA. Submitted to WHO by Syngenta Crop Protection AG.

Modic, W., Ferrell, J., Wood, C., Laskey, J., Cooper, R., & Laws, S. (2004) Atrazine alters steroidogenesis in male Wistar rats. *The Toxicologist*. Abstract. 568. Society of Toxicologists Annual Meeting 2004.

Modic, W.M. (2004) The role of testicular aromatase in the atrazine mediated changes of estrone and estradiol the male Wistar rat. Thesis submitted to Department of Molecular & Structural Biochemistry, North Carolina State University, Raleigh, NC, USA. pp. 1–61.

Moon, H.J, Han, S.Y., Shin, J.H., Kang, I.H., Kim, T.S., Hong, J.H., Kim, S.H .& Fenton, S.E. (2007) Gestational exposure to nonylphenol causes precocious mammary gland development in female rat offspring. *J. Reprod. Dev*., **53**, 333–344.

Morseth, S.L. (1996a) Evaluation of the luteinizing hormone (LH) in female Sprague-Dawley rats - pilot study: final report. Unpublished report No. 2386-109:6791 dated 18 January 1996 from Corning Hazleton, Inc., Vienna, Virginia, USA. Submitted to WHO by Syngenta Crop Protection AG.

Morseth, S.L. (1996b) Evaluation of the luteinizing hormone (LH) surge in female Sprague-Dawley rats-method validation: final report. Unpublished report No. 2386-110 dated 18 January 1996 from Corning Hazleton, Inc., Vienna, Virginia, USA. Submitted to WHO by Syngenta Crop Protection AG.

Morseth, S.L. (1996c) Evaluation of the luteinizing hormone (LH) surge in atrazine-exposed female Sprague-Dawley rats: 6-month report. Unpublished report No. 2386-111 dated 25 October 1996 from Corning Hazleton, Inc., Vienna, Virginia, USA. Submitted to WHO by Syngenta Crop Protection AG.

Morseth, S.L. (1996d) Chronic (12–24 months) study in rats with atrazine technical: final 12-month report. Unpublished report No. 2386-108 dated 24 October 1996 from Corning Hazleton, Inc., Vienna, Virginia, USA. Submitted to WHO by Syngenta Crop Protection AG.

Morseth, S.L. (1998) Chronic (12–24 months) study in rats with atrazine technical. Unpublished report No. 2386-108 dated 15 April 1998 from Corning Hazleton, Inc., Vienna, Virginia., USA. Submitted to WHO by Syngenta Crop Protection AG.

Narotsky, M.G., Best, D.S., Guidici, D.L. & Cooper, R.L. (2001) Strain comparisons of atrazine-induced pregnancy loss in the rat. *Reprod. Toxicol.,* **15**, 61–69.

Neuberger, J.S. (1996) Atrazine and/or triazine herbicides exposure and cancer: an epidemiologic review. *J. Agromedicine,* **3**, 9–30.

O'Connor, D.J., McCormick, G.C. & Green, J.D. (1987) G 30027 – 52-week oral feeding study in dogs. Unpublished report No. MIN 852008 dated 27 October 1987 from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection AG.

O'Connor, J.C., Plowchalk, D.R., Van Pelt, C.S., Davis, L.G. & Cook, J.C. (2000) Role of prolactin in chloro-*S*-triazine rat mammary tumorigenesis. *Drug Chem. Toxicol.,* **23**, 575–601.

Ogorek, B. (1991a) G 28279 - micronucleus test, mouse. Unpublished report No. 901307 dated 23 February 1991 (amended 21 December 1993) from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Ciba-Geigy Ltd.

Ogorek, B. (1991b) G 30033 - Micronucleus test, mouse. Unpublished report No. 901309 dated 25 March 1991 (amended 21 December 1993) from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Oh, S.M., Shim, S.H., & Chung, K.H. (2003) Antiestrogenic action of atrazine and its major metabolites in vivo. *J. Health Sci*., **49**, 65–71.

Ohno, K., Araki, N., Yanase, T., Nawata, H. & Iida, H. (2004) A novel nonradioactive method for measuring aromatase activity using a human ovarian granulosa-like tumor cell line and an estrone ELISA. *Toxicol. Sci*., **82**, 443–450.

Ordog T., Goldsmith J.R., Chen M.D., Connaughton M.A., Hotchkiss J. & Knobil E. (1998) On the mechanism of the positive feedback action of estradiol on luteinizing hormone secretion in the Rhesus monkey. *J. Clin. Endocrinol. Metab.,* **83**, 4047–4053

Orr, G.R., Simoneaux, B. & Davidson, I.W.F. (1987) Disposition of atrazine in the rat. Unpublished report No. ABR-87048 dated 23 October 1987 from Ciba-Geigy Corp., Greensboro, North Carolina, USA. Submitted to WHO by Syngenta Crop Protection AG.

Osterburg, I., & Breckenridge, C. (2004) Oral (gavage) study on the effect of atrazine on pituitary hormone secretion of ovariectomized, estrogen-replaced female Rhesus monkeys. Unpublished report No. 1893-002 dated 14 July 2004 from Covance Laboratories, Munich, Germany. Submitted to WHO by Syngenta Crop Protection AG.

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. *Mutat. Res.*, **116**, 407–415.

Paul, H.J., Dunsire, J.P., & Hedley, D. (1993) The absorption, distribution, degradation and excretion of U-^{14}C triazine G 30027 in the rat. Unpublished report No. IRI 153138 dated 8 July 1993 from Inveresk Research International Ltd, Scotland. Submitted to WHO by Syngenta Crop Protection AG.

Petterson, J.A. & Turnier, J. (1995) 1-Year chronic toxicity study with atrazine technical in rats: final report. Unpublished report No. F-00171 dated 8 December 1995. Submitted to WHO by Syngenta Crop Protection AG.

Pettersen, J.C., Richter, A.D. & Gilles, P.A. (1991) G 28273 - 90-day oral toxicity study in rats. Unpublished report No. F-00006 dated 5 November 1991 from Ciba-Geigy Corp., Environmental Health Center, Farmington, Connecticut, USA. Submitted to WHO by Syngenta Crop Protection AG.

Pino, A., Maura, A. & Grillo, P. (1988) DNA damage in stomach, kidney, liver and lung of rats treated with atrazine. *Mutat. Res.*, **209**,145–147.

Pintér, A., Török, G., Börzsönyl, M., Surján, A., Csík, M., Kelecsényi, Z., & Kocsis, Z. (1990) Long-term carcinogenicity bioassay of the herbicide atrazine in F 344 rats. *Neoplasma,* **37**, 533–544.

Pistl, J., Kovalkovicova, N., Holovska, V., Legath, J. & Mikula, I. (2003) Determination of the immunotoxic potential of pesticides on functional activity of sheep leukocytes in vitro. *Toxicology.* **188**, 73–81.

Pohl, C.R. & Knobil, E. (1982) The role of the central nervous system in the control of ovarian function in higher primates. *Annu. Rev. Physiol*., **44**, 583–593.

Porter, W.P., Jaeger, J.W. & Carlson, I.H. (1999) Endocrine, immune, and behavioral effects of aldicarb (carbamate), atrazine (triazine) and nitrate (fertilizer) mixtures at groundwater concentrations. *Toxicol. Ind. Health.,* **15**, 133–150.

Pruett, S.B., Fan, R., Zheng, Q., Myers, L.P. & Hebert P. (2003) Modeling and predicting immunological effects of chemical stressors: characterization of a quantitative biomarker for immunological changes caused by atrazine and ethanol. *Toxicol. Sci.,* **75**, 343–354.

Puri, E. (1984a) G 30027 - autoradiographic DNA repair test on rat hepatocytes. Unpublished report No. 831171 dated 16 May 1984 (supplemented 14 October 1986) from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Puri, E. (1984b) G 30027 - autoradiographic DNA repair test on human fibroblasts. Unpublished report No. 831172 dated 16 May 1984 from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Rayner, J.L., Wood, C. & Fenton, S.E. (2004) Exposure parameters necessary for delayed puberty and mammary gland development in Long-Evans rats exposed in utero to atrazine. *Toxicol. Appl. Pharmacol.,* **195**, 23–34.

Rayner, J.L., Enoch, R.R. & Fenton, S.E. (2005) Adverse effects of prenatal exposure to atrazine during a critical period of mammary gland growth. *Toxicol. Sci.,* **87**, 255–266.

Ribas, G., Frenzilli, G., Barale, R. & Marcos, R. (1995) Herbicide-induced DNA damage in human lymphocytes evaluated by the single-cell gel electrophoresis (SCGE) assay. *Mutat. Res*., **344**, 41–54.

Ribas, G., Surrallés, J., Carbonell, E., Creus, A., Xamena, N. & Marcos, R. (1998) Lack of genotoxicity of the herbicide atrazine in cultured human lymphocytes. *Mutat. Res.,* **416**, 93–99.

Roberge, M., Hakk, H. & Larsen, G. (2004) Atrazine is a competitive inhibitor of phosphodiesterase but does not affect the estrogen receptor. *Toxicol. Lett,* **154**, 61–68.

Roberge, M., Hakk, H., & Larsen, G. (2006) Cytosolic and localized inhibition of phosphodiesterase by atrazine in swine tissue homogenates. *Food Chem. Toxicol*., **44**, 885–890.

Rodriguez, V.M., Thiruchelvam, M. & Cory-Slechta, D.A. (2005) Sustained exposure to the widely Used herbicide atrazine: altered function and loss of neurons in brain monoamine systems. *Environ. Health Perspect.,* **113**, 708–715.

Rooney, A.A., Matulka, R.A., Luebke, R.W. (2003) Developmental atrazine exposure suppresses immune function in male, but not female Sprague-Dawley rats. *Toxicol. Sci.,* **76**, 366–375.

Rowe, A.M., Brundage, K.M., Schafer, R. & Barnett, J.B. (2006) Immunodulatory effects of maternal atrazine exposure on male Balb/c mice. *Toxicol. Appl. Pharmacol*., **214**, 69–77.

Rudzki, M.W., McCormick, G.C. & Arthur, A.T. (1989) Hydroxy-atrazine (G 34038) - 90-day oral toxicity study in rats. Unpublished report No. 882146 dated 25 October 1989 from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection AG.

Rudzki, M.W., McCormick, G.C. & Arthur, A.T. (1991) Atrazine technical: chronic toxicity study in rats. Unpublished report No. 852214 dated 28 January 1991 from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection AG.

Rudzki, M.W., Batastina, G., & Arthur, A.T. (1992) G-30033: 13-week feeding study in dogs. Unpublished report No. 902187 dated 16 April 1992 from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection AG.

Ruiz, M.J. & Marzin, D. (1997) Genotoxicity of six pesticides by Salmonella mutagenicity test and SOS chromotest. *Mutat. Res.,* **390**, 245–255.

Rusiecki, J.A., De Roos, A., Lee, W.J., Dosemeci, M., Lubin, J.H., Hoppin, J.A., Blair, A. & Alavanja, M.C.R. (2004) Cancer incidence among pesticide applicators exposed to atrazine in the Agricultural Health Study. *J. Natl. Cancer Inst*. **96**, 1375–1382.

Sachsse, K. & Bathe, R. (1975a) Acute intraperitoneal LD_{50} of technical atrazine in the rat. Unpublished report No. Siss 4569 dated 10 March 1975 from Ciba-Geigy Ltd, Stein, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Sachsse, K. & Bathe, R. (1975b) Acute oral LD_{50} of atrazine in the rat. Unpublished report No. Siss 4569 dated 10 March 1975 from Ciba-Geigy Ltd, Stein, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Sachsse, K. & Bathe, R. (1975c) Acute oral LD_{50} of technical atrazine in the mouse. Unpublished report No. Siss 4569 dated 7 April 1975 from Ciba-Geigy Ltd, Stein, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Sachsse, K. & Bathe, R. (1976) Acute dermal LD_{50} in the rat. Unpublished report No. Siss 5663 dated 6 December 1976 from Ciba-Geigy Ltd, Stein, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Sanderson, J.T., Seinen, W., Giesy, J.P. & van den Berg, M. (2000) 2-Chloro-s-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: a novel mechanism for estrogenicity*? Toxicol. Sci*., **54**, 121–127.

Sanderson, J.T., Letcher, R.J., Heneweer, M., Giesy, J.P. & van den Berg, M. (2001) Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes. *Environ. Health Perspect.,* **109**, 1027–1031.

Sanderson, J.T., Boerma, J., Lansbergen, G.W. & van den Berg, M. (2002) Induction and inhibition of aromatase (CYP19) activity by various classes of pesticides in H295R human adrenocortical carcinoma cells. *Toxicol. Appl. Pharmacol.,* **182**, 44–54.

Sarkar, D.K., Gottschall, P.E. & Meites, J. (1982) Damage to hypothalamic dopaminergic neurons is associated with development of prolactin-secreting pituitary tumors. *Science,* **218**, 684–686.

Sathiakumar, N. & Delzell, E. (1997) A review of epidemiologic studies of triazine herbicides and cancer. *Crit. Rev. Toxicol.,* **27**, 599–612.

Schneider, M. (1992) G 28279 - 3-month oral toxicity study in rats (administration in food). Unpublished report No. 901261 dated 8 May 1992 from Ciba-Geigy Ltd, Stein, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Schoch, M. (1985) G 30027 - skin sensitization test in the guinea pig (maximization test). Unpublished report No. 841072 dated 4 June 1985 from Ciba-Geigy Ltd, Stein, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Schwab, C.L., Fan, R., Zheng, Q., Myers, L.P., Herbert, P. & Pruett, S.B. (2005) Modeling and predicting stress-induced immunosuppression in mice using blood parameters. *Toxicol. Sci.,* **83**, 101–113.

Shafer, T.J., Ward, T.R., Meacham, C.A. & Cooper, R.L. (1999) Effects of the chlorotriazine herbicide, cyanazine on $GABA_A$ receptors in cortical tissue from rat brain. *Toxicology,* **142**, 57–68.

Shelanski, M.V. & Gittes, H.R. (1965) Atrazine 80 W - repeated insult patch test (in humans). Unpublished report No. IBL 2430 dated 21 January 1965 from Industrial Biology Laboratories Inc. Submitted to WHO by Syngenta Crop Protection AG.

Sielken, R.L. & Holden, L. (2002) Comparison of the LH surge in female rats administered atrazine, simazine, or DACT for six months. Statistical analysis of the LH surge: supplemental analysis. Unpublished report

No. 2214-01 dated 4 March 2002 from Sielken & Associates Consulting, Inc., Bryan, Texas, USA. Submitted to WHO by Syngenta Crop Protection AG.

Simoneaux, B.J. (2001). Comparative metabolism of two s-triazine herbicides: atrazine and ametryn in the rat. Unpublished report No. ABR-97077 dated 11 October 2001 from Norvartis Crop Protection, Inc., Greensboro, North Carolina, USA. Submitted to WHO by Syngenta Crop Protection AG.

Simpkins, J.W. (2000) Relevance of the female Sprague-Dawley (SD) rat for human risk assessment of chloro-s-triazines. A report to Novartis Crop Protection. Unpublished report dated 20 June 2000 frm Center for the Neurobiology of Aging, University of Florida, Gainesville, Florida, USA. Submitted to WHO by Syngenta Crop Protection AG.

Simpkins, J. & Eldridge, J.C. (2004) Evaluation of the potential effects of atrazine on pituitary hormones in estrogen replaced ovariectomized female Rhesus monkey. Unpublished report No. T020244-04 dated 15 September 2004 from Syngenta Crop Protection, Inc, Greensboro, North Carolina, USA. Submitted to WHO by Syngenta Crop Protection AG.

Simpkins, J.W., Andersen, M.E., Brusick, D., Eldridge, J.C., Delzell, E., Lamb, J.C., McConnell, R.F., Safe, S., Tyrey, L. & Wilkinson, C. (2000) Evaluation of hormonal mechanism for mammary tumorigenesis of the chloro-s-triazine herbicides: Fourth Consensus Panel report. Unpublished report dated 13 January 2000. Submitted to WHO by Syngenta Crop Protection AG.

Simpson, E.R., Clyne, C., Rubin, G., Boon, W.C., Robertson, K., Britt, K., Speed, C. & Jones, M. (2002) Aromatase – a brief overview. *Annu. Rev. Physiol*., **64**, 93–127

Spindler, M. & Sumner, D.D. (1981) Two-year chronic toxicity study with technical atrazine in albino rats. Unpublished report No. 622-06769 dated 15 January 1981 from Industrial Bio-Test Laboratories, Northbrook, IL, USA. Submitted to WHO by Syngenta Crop Protection AG.

Stoker, T.E., Robinette, C.L. & Cooper, R.L. (1999) Maternal exposure to atrazine during lactation suppresses suckling-induced prolactin release and results in prostatitis in the adult offspring. *Toxicol. Sci.,* **52**, 68–79.

Stoker, T.E., Laws, S.C., Guidici, D.L. & Cooper, R.L. (2000) The effect of atrazine on puberty in male Wistar rats: an evaluation in the protocol for the assessment of pubertal development and thyroid function. *Toxicol. Sci.,* **58**, 50–59.

Stoker, T.E., Guidici, D.L., Laws, S.C. & Cooper, R.L. (2002) The effects of atrazine metabolites on puberty and thyroid function in the male Wistar rat. *Toxicol. Sci.,* **67**, 198–206.

Strasser, F. (1988) G 28273 - micronucleus test, mouse. Unpublished report No. 871369 30 March 1988 from Ciba-Geigy Ltd, Basle, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Sumner, D.D. (1981) Carcinogenicity study with atrazine technical in albino mice. Unpublished report No. IBT 8580-8906 dated 20 June 1981 from Industrial Bio-Test Laboratories, Inc., Wedge's Creek Research Facility, Neillsville, Wisconsin, USA. Submitted to WHO by Syngenta Crop Protection AG.

Surrallés, J., Xamena, N., Creus, A. & Marcos, R. (1995) The suitability of the micronucleus assay in human lymphocytes as a new biomarker of excision repair. *Mutat. Res*., **342**, 43–59.

Sutou, S., Kimura, Y., Yamamoto, K. & Ichihara, A. (1979) In-vitro microbial assays for mutagenicity testing of atrazine.Unpublished report No. NRI 79-2884 dated August 1979 from Nomura Research Institute. Submitted to WHO by Syngenta Crop Protection AG.

Taylor, A.E. (1998) Polycystic ovary syndrome. *Endocrinol. Metab. Clin. North Am*., **27**, 877–902

Tennant, M.K., Hill, D.S., Eldridge, J.C., Wetzel, L.T., Breckenridge, C.B. & Stevens, J.T. (1994a) Possible antiestrogenic properties of chloro-s-triazines in rat uterus. *J. Toxicol. Environ. Health.,* **43**, 183–196.

Tennant, M.K., Hill, D.S., Eldridge, J.C., Wetzel, L.T., Breckenridge, C.B. & Stevens, J.T. (1994b) Chloro-s-triazines antagonism of estrogen action: interaction with estrogen receptor binding. *J. Toxicol. Environ. Health.,* **43**, 197–211.

Tennant, A.H., Peng, B. & Kligerman, A.D. (2001) Genotoxicity studies of three triazine herbicides: in vivo studies using the alkaline single cell gel (SCG) assay. *Mutat. Res.*, **493**, 1–10.

Terranova, P. (1991) F-00006: 90-day subchronic dietary toxicity study with G-28273 in rats. Report addendum: effects of G-28273 technical administration on estrous cycle parameters in female Sprague-Dawley rats. Unpublished report (addendum to report No. F-00006) dated 27 March 1991 from Department of Physiology, University of Kansas School of Medicine, Kansas City, Kansas, USA. Submitted to WHO by Syngenta Crop Protection AG.

Thakur, A.K. (1991a) Determination of hormone levels in Sprague-Dawley rats treated with atrazine technical. Unpublished report No. HWA 483-278 dated 17 October 1991 from Hazleton Laboratories, Vienna, Virginia, USA. Submitted to WHO by Syngenta Crop Protection AG.

Thakur, A.K. (1991b) Determination of hormone levels in Fisher-344 rats treated with atrazine technical. Unpublished report No. HWA 483-279 dated 8 November 1991 from Hazleton Laboratories, Vienna, Virginia, USA. Submitted to WHO by Syngenta Crop Protection AG.

Thakur, A.K. (1992a) Oncogenicity study in Sprague-Dawley rats with atrazine technical. Unpublished report No. HWA 483-275 dated 27 January 1992 from Hazleton Laboratories, Vienna, Virginia, USA. Submitted to WHO by Syngenta Crop Protection AG.

Thakur, A.K. (1992b) Oncogenicity study in Fisher-344 rats with atrazine technical. Unpublished reort No. 483-277 dated 18 February 1992 from Hazleton Laboratories, Vienna, Virginia, USA. Submitted to WHO by Syngenta Crop Protection AG.

Thede, B. (1988) Comparative metabolism of atrazine by mammalian hepatocytes: progress report. Unpublished report No. ABR-88139 dated 13 November 1988 from Ciba-Geigy Corp., Greensboro, North Carolina, USA. Submitted to WHO by Syngenta Crop Protection AG.

Thompson, S.S., Batastini, G.G. & Arthur A.T. (1990) G 28273 - 13/52-week oral toxicity study in dogs. Unpublished report No. 872151 dated 17 January 1990 from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Submitted to WHO by Syngenta Crop Protection AG.

Thompson, S.S., Batastini, G. & Arthur A.T. (1992) G 28279 - 13-week feeding study in dogs. Unpublished report No. 912021 dated 22 April 1992 from Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Submitted to WHO by Ciba-Geigy Ltd.

Timchalk, C., Dryzga, M.D., Langvardt, P.W., Kastl, P.E. & Osborne, D.W. (1990) Determination of the effect of tridiphane on the pharmacokinetics of [^{14}C]-atrazine following oral administration to male Fischer 344 rats. *Toxicology*. **61**, 27–40.

Trentacoste, S.V., Friedmann, A.S., Youker, R.T., Breckenridge, C.B. & Zirkin, B.R. (2001) Atrazine effects on testosterone levels and androgen-dependent reproductive organs in peripubertal male rats. *J. Androl.,* **22**, 142–148.

Ullmann, L. (1976a) Eye irritation in the rabbit after single application of G 30027. Unpublished report No. SISS-5663 dated 24 November 1976 from Ciba-Geigy Ltd, Stein, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Ullmann, L. (1976b) Skin irritation in the rabbit after single application of G 30027. Unpublished report No. SISS-5663 dated 24 November 1976 from Ciba-Geigy Ltd, Stein, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.

Welsch, C.W., Jenkins, T.W. & Meites, J. (1970) Increased incidence of mammary tumors in the female rat grafted with multiple pituitaries. *Cancer Res.,* **30**, 1024–1029.

Whalen, M.M., Loganathan, B.G., Yamashita, N. & Saito, T. (2003) Immunomodulation of human natural killer cell cytotoxic function by triazine and carbamate pesticides. *Chem. Biol. Interact.*, **145**, 311–319.

Weiss, G., Schmidt, C., Kleinberg, D.L. & Ganguly, M. (1977) Positive feedback effects of estrogen on LH secretion in women in neuroleptic drugs. *Clin. Endocrinol*. **7**, 423–427.

Wise, P.M., Kashon, M.L., Krajnak, K.M., Rosewell, K.L., Cai, A., Scarbrough, K., Harney, J.P., McShane, T., Lloyd, J.M. & Weiland, N.G. (1997) Aging of the female reproductive system: a window into brain aging. *Recent Prog. Horm. Res.* **52,** 279–303.

Young, H.A., Mills, P.K., Riordan, D.G. & Cress, R.D. (2005) Triazine herbicides and epithelial ovarian cancer risk in central California. *J. Occup. Environ. Med.,* **47**, 1148–1156.