.

5.2 ATRAZINE

TOXICOLOGY

Atrazine, 6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine (International Union of Pure and Applied Chemistry, IUPAC) (CAS No. 1912-24-9), is a selective systemic herbicide of the

chlorotriazine class, 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 ground water as a result of the use of atrazine as a pre-emergent or early post-emergent herbicide. Hydroxyatrazine is more commonly detected in ground than in surface water. The relative order of concentrations in rural wells in the USA was generally as follows: atrazine ~DEA ~DACT > DIA > hydroxyatrazine. However, concentrations of DEA that are several-fold higher than 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 based on a 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 had not been previously evaluated by JMPR, and no 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 GLP-compliant guideline studies with atrazine and its four key metabolites, 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 its 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.

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 deisopropyl-atrazine (DIA) or deethyl-atrazine (DEA) to diaminochlorotriazine (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, based on 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 Fischer-344 (F344) rats fed diets containing atrazine 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 luteinizing hormone (LH) surge and subsequent disruption of the oestrous cycle (characterized by an increase in days in oestrous) were observed at ≥ 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 found in the studies of carcinogenicity. The effects on the LH surge and disruption of the oestrous 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 gonadotropin-releasing hormone (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 oestrogen, 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 oestrogen and prolactin. In contrast, women respond to reduced levels of LH by reductions in levels of oestrogen. Thus, the Meeting considered that the mode of carcinogenic action in certain susceptible rat strains is not relevant for human risk assessment.

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 post-implantation losses, including full-litter resorptions. Effects were seen at doses of ≥ 50 mg/kg bw per day 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 prolactin release in lactating rats was seen with atrazine at doses of ≥ 25 mg/kg bw per day, 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 prior to 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 ≥ 30 mg/kg bw per day, with a NOAEL of 10 mg/kg bw per day, and in male rats after exposure on postnatal days 23–53 at doses ≥ 12.5 mg/kg bw per day, 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 oestrous 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 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 oestrous 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 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 foetuses, 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 (DEA, DIA) and ≥ 12.5 mg/kg bw per day (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 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 oestrous 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 foetuses 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 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 based on 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 oestrous 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 is 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 based on 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., post-implantation 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 based on 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 safety factor of 25. A modified safety factor based on 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.

A toxicological monograph was prepared.

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]	—

Species	Study	Effect	NOAEL	LOAEL
		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 (luteinizing hormone 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]	Post-implantation 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 fetotoxicity	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 disruption (oestrous 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

[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 toxicity 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, deisopropyl-atrazine and diaminochlorotriazine
0–0.02 mg/kg bw
Hydroxyatrazine
0–0.04 mg/kg bw

Estimate of acute reference dose

Group ARfD for atrazine, deethyl-atrazine, deisopropyl-atrazine and diaminochlorotriazine

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 luteinizing-hormone surge study 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 toxicity tests; 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
	Hydroxyatrazine has a different mode of action and toxicity profile to atrazine
Mode of neuroendocrine action	Atrazine and its chlorometabolites modify hypothalamic catecholamine function and gonadotrophin-releasing hormone (GnRH) regulation, leading to alterations in pituitary luteinizing hormone (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 oestrous 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 post-natal CNS development (and subsequent alterations in prolactin regulation)
Immunotoxicity	Evidence for immune system modulation at doses above 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-Dawleys rat; 6-month study of LH surge/oestrous 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)