AMITROLE

This substance was considered by previous working groups, in 1974 (IARC, 1974), 1986 (IARC, 1986a) and 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

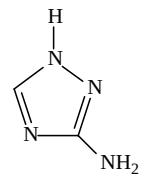
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 61-82-5
Deleted CAS Reg. Nos: 155-25-9; 6051-75-8; 11121-00-9; 16681-74-6; 29212-82-6; 30922-30-6
Chem. Abstr. Name: 1*H*-1,2,4-Triazol-3-amine
IUPAC Systematic Name: 3-Amino-*s*-triazole; 1*H*-1,2,4-triazol-3-ylamine
Synonyms: Aminotriazole; 2-amino-1,3,4-triazole; 3-aminotriazole; 3-amino-1,2,4-triazole; 3-amino-1*H*-1,2,4-triazole; 5-amino-1,2,4-triazole; 5-amino-1*H*-1,2,4-triazole; AT; 3,A-T; ATA; ENT 25 445

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_4N_4$ Relative molecular mass: 84.08

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: White to yellowish crystalline powder (FAO/WHO, 1999)
(*b*) *Melting-point*: 159 °C (Lide & Milne, 1996)
(*c*) *Spectroscopy data*: Infrared [prism (8667), grating (21258)], nuclear magnetic resonance [proton (9499), C-13 (6254)] and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996).
(*d*) *Solubility*: Soluble in water (280 g/L at 25 °C), chloroform, ethanol, and methanol; sparingly soluble in ethyl acetate; insoluble in acetone and diethyl ether (WHO, 1994; Lide & Milne, 1996; Budavari, 2000)
(*e*) *Volatility*: Vapour pressure, < 1 mPa at 20 °C (FAO/WHO, 1999; Tomlin, 1999)
(*f*) *Ionization constant*: $pK_a = 4.0$ (FAO/WHO, 1999)
(*g*) *Octanol/water partition coefficient (P)*: log P, –0.77 at pH 7.1 (FAO/WHO, 1999)
(*h*) *Conversion factor*[1]: $mg/m^3 = 3.44 \times ppm$

1.1.4 *Technical products and impurities*

As of 1990, two types of product were commercially available: soluble concentrates containing 200–500 g/L amitrole and water-soluble powders containing 50–90% amitrole. A water-soluble granule containing 86% amitrole is in the process of registration in many countries worldwide (FAO/WHO, 1999)

Impurities that have been identified in commercial formulations of amitrole include 3-(*N*-formylamino)-1,2,4-triazole, 4*H*-1,2,4-triazole-3,4-diamine and 4*H*-1,2,4-triazole-3,5-diamine (WHO, 1994; FAO/WHO, 1999).

Trade names for amitrole include Amizol, Amitrol, Amitrol 90, ATA (amine), Azaplant, Cytrol, Cytrole, Herbidal total and Weedazol.

1.1.5 *Analysis*

Selected methods for the analysis of amitrole in air, water, soil, plant materials and foods are presented in Table 1.

1.2 Production

The synthesis of amitrole was first reported by J. Thiele and W. Manchot in 1898, involving the reaction of aminoguanidine with formic acid (Carter, 1976). The industrial process, described by Allen and Bell (1946) and patented in 1954 by Allen, involves the

[1] Calculated from: $mg/m^3 = (\text{molecular weight}/24.45) \times ppm$, assuming standard temperature (25 °C) and pressure (760 mm Hg [101.3 kPa])

Table 1. Methods for the analysis of amitrole

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Draw air through impinger containing water	HPLC/UV	0.004 mg/m^3	Occupational Safety and Health Administration (1998)
Fruit, crops, wine, soil	Extract with ethanol:water (2:1) or rotary evaporate; acetylate with acetic anhydride; partition into dichloromethane; clean-up	GLC/NPD	0.01 mg/kg	H.J. Jarczyk & E. Möllhoff (1991), cited in FAO/WHO (1999)
Blackberries	Extract with ethanol:water; treat with H_2O_2; clean-up with ion exchange; convert to a complex with fluorescamine	HPLC/FD	0.02 mg/kg	FAO/WHO (1999)
Grapes[a]	Extract with acetone:water; partition into dichloromethane; acidify; clean-up; evaporate to dryness; resuspend in pyridine; derivatize	GC/MS	Method validated over the range 0.005–0.5 mg/kg	C.H. McGuire (1997), cited in FAO/WHO (1999)
Water	Apply sample to cation exchange column; elute with ammonia; clean-up with column chromatography	HPLC/ECD	0.1 µg/L	E. Weber (1988), cited in FAO/WHO (1999)
Crops, soil, milk, eggs, muscle	Extract with acetone:water (1:3); partition into dichloromethane; clean-up	HPLC/ECD	0.005 mg/kg	E. Weber (1997), cited in FAO/WHO (1999)

HPLC/UV, high-performance liquid chromatography/ultraviolet detection; HPLC/FD, high performance liquid chromatography/fluorescence detection; GC/MS, gas chromatography/mass spectrometry; GLC/NPD, gas–liquid chromatography/nitrogen–phosphorus detection; HPLC/ECD, high-performance liquid chromatography/electrochemical detection

[a] This method is now being validated for must and wine, barley, wheat, peas and canola seeds.

same reaction, in which an aminoguanidine salt is heated to 100–120 °C with formic acid in an inert solvent (Carter, 1976; Sittig, 1980).

Information available in 2000 indicated that 3-aminotriazole was manufactured by two companies each in China, France and Japan and one company each in Armenia, Belgium, Canada, India, Switzerland and the USA (CIS Information Services, 2000).

1.3 Use

Amitrole was introduced in the USA in the mid-1950s as a herbicide and plant-growth regulator (Carter, 1976). Registrations for use in food crop production were cancelled in 1971, but it remains an important specialty herbicide (Carter, 1976; Environmental Protection Agency, 1984).

Amitrole is a fast acting herbicide which is taken up predominantly through the leaves of plants. It is used on industrial land, roadsides, railways and ditches and is also used worldwide as a herbicide in vineyards and orchards against all kinds of weeds (grasses and dicotyledons, annual, biannual and perennial) and as a total weed killer after harvest and before the next annual sowing (FAO/WHO, 1999).

1.4 Occurrence

1.4.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 2000), about 700 workers in the USA were potentially exposed to amitrole, including assemblers and technicians in the manufacture of transport equipment. Farmers were not included in the survey. According to the Finnish Register of Employees Exposed to Carcinogens, about 100 chemical process workers and laboratory workers were exposed in Finland in 1997 (Savela *et al.*, 1999).

Amitrole was reported to be released during dry crushing, and to a lesser extent from bagging, in a plant where the chemical was manufactured, but the concentrations of amitrole in the workroom air were not reported (Alary *et al.*, 1984). No data on levels of exposure to amitrole during its application were available.

1.4.2 *Environmental occurrence*

Amitrole undergoes rapid degradation in the environment, and no systematic measurements of environmental concentrations have been reported. A review (WHO, 1994) presented some anecdotal data on environmental concentrations of this chemical. Air concentrations as high as 100 μg/m^3 were reported in the vicinity of a facility where amitrole was produced (Alary *et al.*, 1984). The concentration in pond-water immediately after application for aquatic weed control was reported to be 1340 μg/L initially, decreasing to 80 μg/L after 27 weeks. Similar spraying of a larger watershed in Oregon, USA, showed an initial concentration of 155 μg/L within 30 min after spraying, which decreased to below detectable (2 μg/L) within 6 days (WHO, 1994). Measurements in an aeration pond treated with amitrole showed concentrations up to 200 mg/L, but downstream, rapid degradation and dilution reduced the concentration to 0.5 mg/L (Alary *et al.*, 1984). A series of studies carried out in Japan in 1984 and in France in 1991 indicated

no detectable (< 4 µg/L and < 0.1 µg/L, respectively) amounts in typical ponds. Amitrole is readily degraded in soil or attaches irreversibly to soil particles (WHO, 1994).

1.5 Regulations and guidelines

Occupational exposure limits and guidelines for amitrole have been established in several countries (see Table 2).

Amitrole was first considered by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1974 and given a conditional acceptable daily intake (ADI) of 0–0.00003 mg/kg bw. In 1993, the Codex Committee on Pesticide Residues set a temporary ADI of 0–0.0005 mg/kg bw. A full ADI (0–0.002 mg/kg bw) was allocated in 1997. The 1998 JMPR recommended a maximum residue limit (MRL) of 0.05 mg/kg in grapes and 0.05 mg/kg (limit of detection) in pome fruits and stone fruits (FAO/WHO, 1999; WHO, 1999).

Table 2. Occupational exposure limits and guidelines for amitrole

Country	Year	Concentration (mg/m^3)	Interpretation
Australia	1993	0.2	TWA
Austria	1993	0.2	TWA
Belgium	1993	0.2	TWA
Denmark	1993	0.2 (Ca)	TWA
Finland	1993	(Ca)	TWA
Germany	2000	0.2 (IF) (3B)	TWA
Ireland	1997	0.2	TWA
Netherlands	1999	0.2	TWA
Switzerland	1993	0.2	TWA
USA			
ACGIH (TLV)	2000	0.2 (A3)	TWA
NIOSH (REL)	1999	0.2 (Ca)	TWA

From American Conference of Governmental Industrial Hygienists (ACGIH) (2000); Deutsche Forschungsgemeinschaft (2000)

TWA, time-weighted average; TLV, threshold limit value; NIOSH, National Institute for Occupational Safety and Health; REL, recommended exposure limit; Ca, carcinogen; IF, inhalable fraction of the aerosol; A3, confirmed animal carcinogen of unknown relevance to humans; 3B, substances for which in-vitro or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories; further studies are required before a final decision can be made. A maximum acceptable concentration (MAC) value can be established provided no genotoxic effects have been detected.

2. Studies of Cancer in Humans

2.1 Cohort study

In a study of mortality in Sweden, a cohort of 348 male railroad workers who had been exposed for 45 days or more to amitrole and/or chlorophenoxy herbicides (see IARC, 1986b) was followed up from 1957 to 1972 and again in 1978 (Axelson & Sundell, 1974; Axelson *et al*., 1980). There was a deficit of deaths from all causes (45 observed, 49 expected) but an excess of deaths from malignant neoplasms (17 observed, 11.9 expected). In a subcohort exposed to amitrole but not chlorophenoxy herbicides, there were five deaths from cancer (two lung cancers, one pancreatic cancer, one reticulum-cell sarcoma and one maxillary sinus cancer), with 3.3 expected (not significant); three of the deaths (with 2.0 expected) occurred in workers first exposed 10 years or more before death. In a subcohort of men exposed to both amitrole and chlorophenoxy herbicides, there were six deaths from cancer with 2.9 expected. All six (with 1.8 expected; $p < 0.005$) occurred in workers first exposed 10 years or more before death. The men were also exposed to other organic (e.g., monuron and diuron) and inorganic chemicals (e.g., potassium chlorate). The results obtained during the extended follow-up period (1972–78), in which the exposure assessment would not have been influenced by knowledge of the disease, are similar to the results for the whole period 1957–78 reported above, i.e. a statistically significant excess of deaths from cancers at all sites in the subcohort exposed to both amitrole and chlorophenoxy herbicides but not in the subcohort exposed to amitrole alone. No thyroid tumours were reported.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Mouse: In a preliminary report of a screening study, groups of 18 male and 18 female (C57BL/6×C3H/Anf)F_1 and (C57BL/6×AKR)F_1 mice, 7 days of age, were given 0 (control) or 1000 mg/kg bw (maximum tolerated dose) amitrole [purity unspecified] in distilled water daily by stomach tube until 4 weeks of age. Subsequently, the animals were fed diets containing 0 (control) or 2192 mg/kg amitrole (maximum tolerated dose) until the end of the observation period (53–60 weeks). Thyroid follicular-cell tumours (carcinomas) were reported in 64/72 treated males and females of both strains combined. ‘Hepatomas’ were observed in 34/36 pooled treated male and female (C57BL/6×C3H/Anf)F_1 mice and in 33/36 pooled treated male and female (C57BL/6×AKR)F_1 mice. In pooled control groups, 8/166 (C57BL/6×CeH/Anf)F_1 mice and 6/172 (C57BL/6×AKR)F_1 mice had ‘hepatomas’ (National Cancer Institute,

1968; Innes *et al*., 1969). [The Working Group noted the lack of a published final report that would have provided more details on the study.]

Groups of C3H female mice were treated at weaning with neutron irradiation or fed a diet containing amitrole [purity unspecified], or both. Amitrole was mixed into the diet at a concentration of 1% (10 000 mg/kg) during the first 4 weeks of a 5-week cycle, and this cycle was repeated continuously for life. The groups that received amitrole alone or after neutron irradiation had a 100% incidence of liver tumours (29/29 and 33/33, respectively), whereas in the group treated with neutrons but not fed amitrole in the diet, 2/37 (5%) mice had liver tumours (Feinstein *et al*., 1978a). [The Working Group noted the lack of an untreated control group and that the liver tumours were not specified as adenomas or carcinomas.]

Groups of 75 male and 75 female NMRI mice, 6 weeks of age, were fed diets containing 0 (control), 1, 10 or 100 mg/kg amitrole (technical grade, 97% pure) for life. There was no difference in body weights or survival rate (average survival time, 637–734 days) between amitrole-treated and control mice. No indication of a carcinogenic effect was seen (Steinhoff *et al*., 1983). [The Working Group noted the low range of doses administered.]

Groups of male and female $B6C3F_1$ mice were fed diets containing 0 (control) or 500 mg/kg amitrole [purity unspecified] continuously from weaning until 90 weeks. In the 55 males, nine hepatocellular adenomas and 11 hepatocellular carcinomas were observed; in the 49 females, there were five hepatocellular adenomas and four hepatocellular carcinomas. In control mice, held for 90 weeks only, one hepatocellular adenoma was observed among 98 males and 96 females (Vesselinovitch, 1983). [The Working Group noted that the increased tumour incidences were statistically significant, as calculated by a previous working group (IARC, 1986a).]

Groups of 6-week-old stocks and strains of female DS, ICR (Crj:CD-1®) and NOD (derived from ICR) mice were given amitrole [purity not specified] in the drinking-water at 1% for up to 6 months in two experiments. The mice were treated for 3 months in the first experiment and for 6 months in the second experiment, killed and evaluated for the presence of hyperplastic nodules and neoplastic lesions in the liver. The incidences of hyperplastic nodules were 15/19 in NOD, 3/5 in DS and 0/5 in ICR mice in the first experiment and 19/19 in NOD, 18/18 in DS and 17/19 in ICR mice in the second experiment. One hepatocellular carcinoma was also observed in a NOD mouse treated for 6 months. The hyperplastic nodules were larger in NOD mice than in either DS or ICR mice (Mori *et al*., 1985). [The Working Group noted the lack of a matched untreated control group for each strain and experiment.]

Rat: Groups of rats [initial numbers, sex, strain and age unspecified] were fed diets containing 0 (control), 10, 50 or 100 mg/kg amitrole [purity unspecified] for 104 weeks. Thyroid follicular-cell adenomas were observed in 1/10, 2/15 (one ‘adenocarcinomatous’) and 17/26 (four ‘adenocarcinomatous’) rats at the three concentrations, respectively. No thyroid tumour was found in the five controls examined (Jukes & Shaffer,

1960). [The Working Group noted the small number of control rats and the lack of detail in this report.]

Groups of 27–32 male and female random-bred white rats (weighing 100–120 g) were either given drinking-water containing amitrole to provide a dose of 20–25 mg/rat per day, or were fed diets providing a dose of 250 or 500 mg/rat per day for life (5–23 months). Of the group receiving amitrole in the drinking-water, eight were alive at the time of appearance of the first thyroid follicular-cell tumour, and three thyroid and six hepatocellular tumours were observed. Of the groups receiving amitrole in the diet, 10 and 11 rats were alive at the time of appearance of the first tumour in the groups given 250 and 500 mg/rat, respectively; two thyroid and eight liver tumours were observed in the group fed 250 mg amitrole; five thyroid and 10 liver tumours were seen in the group receiving 500 mg amitrole (Napalkov, 1962). [The Working Group noted the lack of matching control groups.]

Groups of rats [initial numbers, sex, strain and age unspecified] were fed diets containing 0, 10, 50 or 100 mg/kg amitrole [purity unspecified] for 104 weeks. Thyroid follicular-cell adenomas were observed in 15/27 rats at the highest concentration and in 1–3/27 in the other two treated groups (Hodge *et al*., 1966). [The Working Group noted that the experiment was inadequately reported and that control data were not included.]

Six groups of female Wistar rats, weighing approximately 200 g, received the following treatments: group 1 (40 rats) received drinking-water containing 2500 mg/L amitrole [purity unspecified] for 70 weeks; group 2 (30 rats) received a partial thyroidectomy and then, 2 weeks later, amitrole in the drinking-water; group 3 (30 rats) received a partial thyroidectomy followed by re-implantation of the autochthonous thyroid tissue and, 2 weeks later, amitrole in the drinking-water; group 4 (10 rats) served as untreated controls; group 5 (10 rats) received a partial thyroidectomy with no further treatment; and group 6 (10 rats) received a partial thyroidectomy followed by re-implantation of the autochthonous thyroid tissue with no further treatment. Rats that lived longer than 30 weeks comprised the effective animals. Premature deaths were largely a result of infection; the survival rate at 30 weeks was 70–80% for rats not receiving amitrole and 47–65% for amitrole-treated rats. Papillary follicular-cell adenomas were observed in the thyroid in 3/26, 1/14 and 1/10 rats in groups 1, 2 and 3, respectively, and invasive follicular-cell tumours of the thyroid were found in 19/26, 14/14 and 10/10 rats in groups 1, 2 and 3, respectively. These results were significantly different ($p < 0.001$) from those in the matching control groups (0/7, 0/7 and 0/8 in groups 4, 5 and 6, respectively) (Tsuda *et al*., 1976).

Groups of female Wistar rats weighing approximately 200 g received one of the following treatments: 20 rats received drinking-water containing 2500 mg/L amitrole [purity unspecified] and a standard diet (containing 5 mg/kg iodine); 20 rats were fed a low-iodine (0.25 mg/kg) diet; and 20 rats were fed a standard diet (containing 5 mg/kg iodine) and served as untreated controls. The experiment was terminated at 60 weeks; rats that lived 30 weeks or more were considered to be the effective animals. Follicular-cell carcinomas of the thyroid were observed in 9/13 rats treated

with amitrole alone, in 4/9 rats fed the low-iodine diet and in 0/16 untreated controls (Tsuda *et al*., 1978).

Groups of 75 male and 75 female Wistar rats, 6 weeks of age, were fed diets containing 0 (control), 1, 10 or 100 mg/kg amitrole (technical grade, 97% pure) for life. No difference was observed in body-weight gain, and the average survival exceeded 900 days in all groups. Increased incidences of thyroid follicular-cell tumours were observed in the group at the high dietary concentration. The incidences of benign thyroid tumours in the four groups were 5/36, 9/41, 4/44 and 45/53 for males and 7/59, 12/67, 8/60 and 44/71 for females, and the incidences of malignant thyroid tumours were 3/36, 0/41, 3/44 and 18/53 for males and 0/59, 1/67, 4/60 and 28/71 for females. The incidences of benign pituitary tumours (adenomas) were marginally increased in females, being 4/36, 9/41, 10/44 and 10/53 for males and 14/59, 20/67, 15/60 and 36/41 for females (Steinhoff *et al*., 1983).

Hamster: Groups of 76 male and 76 female golden hamsters, 6 weeks of age, were fed diets containing 0 (control), 1, 10 or 100 mg/kg amitrole (technical grade, 97% pure) for life. No difference was observed in the body-weight gains or survival of controls and animals at the two lower dietary concentrations. Reduced body-weight gain and a significant reduction in survival were observed at the high concentration. There was no indication of a carcinogenic effect (Steinhoff *et al*., 1983).

3.2 Dermal application

Mouse: Groups of 50 male and 50 female C3H/Anf mice, 2–4 months old, received weekly applications to the skin of 0.1 or 10 mg analytical-grade amitrole in 0.2 mL acetone:methanol (65:35) for life. The median length of survival ranged from 44 to 57 weeks. No skin tumour was observed (Hodge *et al*., 1966).

3.3 Subcutaneous administration

Rat: A group of 19 male and female random-bred white rats, weighing 100–120 g, received twice-weekly subcutaneous injections of 125 mg amitrole [purity unspecified] in water for 11 months and were observed up to 23 months. Of the seven rats alive at the appearance of the first tumour, five had liver tumours and five had thyroid follicular-cell tumours (Napalkov, 1962). [The Working Group noted the lack of a matching control group.]

3.4 Perinatal exposure

Mouse: Pregnant C57BL/6 mice (mated with C3H males) were fed a diet containing 500 mg/kg amitrole [purity unspecified] from day 12 of gestation until delivery, and the $B6C3F_1$ offspring were maintained on a standard diet without amitrole for 90 weeks. Four hepatocellular adenomas and two hepatocellular carcinomas were observed in 74

male B6C3F$_1$ offspring, but there were no liver tumours in the 83 females. In unexposed B6C3F$_1$ mice held for 90 weeks, only one hepatocellular adenoma was observed in 98 males, and there were no liver tumours in 96 females (Vesselinovitch, 1983).

Groups of B6C3F$_1$ mice were exposed to amitrole perinatally, being nursed by dams fed diets containing 500 mg/kg amitrole [purity unspecified] from birth until weaning. The offspring were then maintained on a standard diet until the end of the study at 90 weeks. In 45 males, six hepatocellular adenomas and four hepatocellular carcinomas were observed; no liver tumours occurred in 55 females. In untreated controls observed for 90 weeks, one hepatocellular adenoma was observed in a group of 98 males; none was observed among 96 females (Vesselinovitch, 1983). [The Working Group noted that the increased tumour incidences were statistically significant as calculated by a previous working group (IARC, 1986).]

3.5 Administration with known carcinogens or modifying factors

Rat: Two groups of 30 male albino rats, 2–3 months of age, were fed a diet containing 0.06% 4-dimethylaminoazobenzene (DAB), and one of the groups also received an intraperitoneal injection of amitrole [purity unspecified] every 2 days as a 10% solution in water until the end of the study to provide a dose of 1000 mg/kg bw. The surviving 16 DAB-treated and 19 DAB plus amitrole-treated rats were killed at 21 weeks. The incidences of liver tumours were 12/16 in the group receiving DAB alone and 4/19 in the group that received DAB plus amitrole ($p < 0.01$). The liver tumours produced by DAB alone were mostly hepatocellular carcinomas, whereas those in the group treated with DAB plus amitrole were hepatocellular carcinomas and cholangiocarcinomas (Hoshino, 1960).

Groups of 12 male Wistar rats, 6 weeks of age, received the following treatments: group 1 received four weekly subcutaneous injections to provide a dose of 700 mg/kg bw *N*-nitrosobis(2-hydroxypropyl)amine (NBHPA), followed by a diet containing 2000 mg/kg amitrole [purity unspecified] for a total of 12 weeks; group 2 received four weekly injections of NBHPA only; group 3 was fed a diet containing 2000 mg/kg amitrole beginning at week 4 for 12 weeks; group 4 received eight weekly injections of NBHPA followed by a diet containing 2000 mg/kg amitrole for 12 weeks; group 5 received eight weekly injections of NBHPA only; group 6 was fed a diet containing 2000 mg/kg diet amitrole beginning at week 8; and group 7 was fed a standard diet and served as untreated controls. All animals were killed after 20 weeks. No thyroid tumours were found in group 2, 3, 6 or 7, but thyroid tumours were observed in 7/12 rats in group 5. A significantly increased incidence ($p < 0.05$) of thyroid follicular-cell tumours was observed in rats in group 1 (9/11) when compared with groups 7 and 2. A significant increase in tumour incidence ($p < 0.05$) was also observed in rats in group 4 (12/12) when compared with groups 7 and 5. The tumours found in groups 1, 4 and 5 were mainly thyroid follicular-cell tumours. Thus, amitrole promoted NBHPA-induced thyroid neoplasia (Hiasa *et al*., 1982).

A group of 75 male Wistar-Furth rats, castrated at 40 days of age, were divided into six groups: group 1 (five rats) received no further treatment and served as untreated controls; group 2 (10 rats) was given drinking-water containing 1500 mg/L amitrole [purity unspecified] starting 7 days after castration; group 3 (10 rats) received a subcutaneous implant of a pellet on the back containing 5 mg diethylstilbestrol and 45 mg cholesterol, which was replaced every 2 months; group 4 (11 rats) received the pellet plus amitrole in the drinking-water; group 5 (20 rats) received the pellet followed by administration of drinking-water providing a dose of 5 mg/day of *N*-butyl-*N*-nitrosourea (BNU) for 30 days starting at 50–55 days of age; and group 6 (19 rats) received the pellet followed by administration of BNU and, 7 days after BNU treatment, amitrole in the drinking-water. Groups 3–6 received the implants at the same time as they were castrated. Rats that lived beyond 230 days of age were considered to be effective animals; all survivors were killed at 14 months of age. Neoplastic nodules and hepatocellular carcinomas developed in 4/9 rats in group 3 and 15/17 in group 5, and pituitary tumours developed in 7/9 rats in group 3 and 12/17 in group 5. Addition of amitrole to these regimens (groups 4 and 6, respectively) had no effect on the incidence of pituitary tumours (8/11 and 10/14) but slightly (group 4; 2/11) and significantly (group 6; 3/14) reduced the incidences of neoplastic nodules and hepatocellular carcinomas. There were no pituitary or liver tumours in the untreated controls or in rats receiving amitrole alone (Sumi *et al.*, 1985).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Urinary excretion of unchanged amitrole at a concentration of 1 g/L was reported in a woman who ingested approximately 20 mg/kg bw of the herbicide (Geldmacher-von Mallinckrodt & Schmidt, 1970).

4.1.2 *Experimental systems*

Amitrole is rapidly and almost completely absorbed from the gut and lungs (Fang *et al.*, 1964; Burton *et al.*, 1974; Tjälve, 1975; Brown & Schanker, 1983). After intravenous or intragastric administration of radiolabelled amitrole to mice, the label accumulated in the bone marrow, spleen, thymus, liver and gut mucosa (Tjälve, 1975). After administration of an oral dose of radiolabelled compound to rats, the label disappeared from the heart, lung, spleen, testis and brain with a half-time of approximately 2.5 h. It was excreted mainly in the urine, with a small, variable amount found in the faeces

(Fang *et al.*, 1964). Six per cent of a 50-mg/kg bw oral dose was excreted in the urine of rats as 3-amino-5-mercapto-1,2,4-triazole and 3-amino-1,2,4-triazoyl-(5)-mercapturic acid (Grunow *et al.*, 1975).

When mice were given an intravenous dose of 3.4 mg/kg bw [^{14}C]amitrole, approximately 10% [estimated from data presented by the authors] of the radiolabel appeared to be irreversibly bound to liver tissue. The bound radiolabel was apparently located mainly centrilobularly, and the amount decreased very little over 24 h (Fujii *et al.*, 1984).

4.1.3 *Comparison of animals and humans*

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

Intentional ingestion of a commercial mixture of amitrole and diuron at a dose equivalent to 20 mg/kg bw of amitrole was reported to have caused no symptoms of poisoning in a woman (Geldmacher-von Mallinckrodt & Schmidt, 1970).

English *et al.* (1986) reported on a 41-year-old weed control operator with a 6-month history of dermatitis involving his face, hands, back, thighs and feet. Patch testing with 1% amitrole revealed a strong positive vesicular reaction at 2 and 4 days, suggesting allergic contact dermatitis. Balkisson *et al.* (1992) reported on a 74-year-old, previously healthy man who sprayed an amitrole formulation (containing 19% amitrole, 17% ammonium thiocyanate) in a strong head-wind without any protective clothing for 2 h using 500 mL of the formulated material in 10 L of water. He developed a dry non-productive cough after 6–8 h. Diffuse, asymmetrical, severe alveolar damage in the lungs was detected, which was reversed by intravenous administration of high doses of corticosteroids.

The results of a study conducted on five men involved in spraying amitrole over 10 working days on utility right-of-ways in West Virginia (USA) were reported by WHO (1994; D.G. Baugher *et al.*, 1982). The amitrole was applied at a concentration of approximately 500 g of active ingredient per 100 L of water from hand-held hydraulic spray guns. Medical monitoring, particularly of the thyroid, was carried out both before and after spraying and included palpation of the thyroid gland and neck measurements 19 days before and 14 days after exposure, and thyroid function tests 19, 11 and 4 days before exposure and 0, 7 and 14 days after the last exposure. The thyroid function of all men was within the normal range, and no differences were found in any comparisons of thyroid function. It was estimated that the dermal exposure of each man over the 10-day period was approximately 340 mg/day. In a study reported by Miksche (1983), thyroid function was evaluated in five employees who had been engaged in the production and packaging of amitrole for between 3 and 16 years. Thyroid scintigrams

and measurements of triiodothyronine (T3) and thyroxine (T4) revealed no evidence of thyroid dysfunction.

Legras *et al.* (1996) described a fatal case of poisoning with Radoxone TL (in which amitrole is the main active ingredient at 240 g/L, with ammonium thiocyanate at 215 g/L, which enhances the activity of amitrole). A 54-year-old man was hospitalized with unexplained coma, myoclonic contractions and vascular collapse. The concentrations of thiocyanate and amitrole in his blood were 750 mg/L and 138 mg/L, respectively, more than 12 h after ingestion of the preparation. Experimental studies and previously reported fatal cases suggest that thiocyanate is the predominant toxic substance in the herbicide mixture.

4.2.2 *Experimental systems*

Amitrole generally has little acute toxicity to experimental animals. The acute oral LD_{50} has been reported to be 14.7 g/kg bw in mice and 25 g/kg bw in rats (Kröller, 1966). An oral dose of 2 g/kg bw administered to sheep was fatal [details not given] (Hapke *et al*., 1965). No pronounced toxicity was seen in mice, cats or dogs after intravenous injection of 1.6, 1.7 or 1.2 g/kg bw, respectively; mice tolerated an intraperitoneal injection of 4 g/kg bw amitrole (C.B. Shaffer, 1956; cited in Kröller, 1996). Acatalasaemic substrains of highly inbred C3H and C57BL mice were more resistant to the effects of amitrole, a known catalase inhibitor, on weight than mice with normal catalase activity (Feinstein *et al.*, 1978b). No sign of acute toxicity was seen in specific pathogen-free adult male or female rats given amitrole (99% pure) in aqueous solution by stomach tube at 4.0 g/kg bw or by dermal application at 2.5 g/kg bw (Gaines *et al*., 1973). Intraperitoneal administration of 4.0 g/kg bw amitrole every 4 h for 24 h to male rats did not produce toxic effects (Kato, 1967).

Adult specific pathogen-free rats given diets containing 500 or 1000 mg/kg amitrole (99% pure) for 107–110 days gained 14–26% less body weight than did controls, but no reduction in weight gain was observed in rats fed diets containing 25 or 100 mg/kg amitrole for 240–247 days (Gaines *et al*., 1973). The weight gain of specific pathogen-free rats, mice and golden hamsters was not affected by life-long administration of diets containing 10 mg/kg technical-grade amitrole (97% pure); however, a diet with 100 mg/kg amitrole resulted in a slight reduction in body weight in golden hamsters (Steinhoff *et al*., 1983).

Amitrole markedly inhibited thyroid iodine uptake and the organic binding of iodine in rats (Alexander, 1959), and Strum and Karnovsky (1970) showed that amitrole reversibly inhibits thyroid peroxidase in this species. [Thyroperoxidase is the coupling enzyme that enhances the combination of thyroid hormone precursor molecules (e.g. mono- with diiodotyrosine, or diiodotyrosine with diiodotyrosine) into the biologically active forms of thyroid hormones (T3 and T4, respectively); see Figure 1 in General Remarks.] Male rats given drinking-water containing 0.04% (400 mg/L) amitrole developed goitre (i.e. thyroid gland enlargement due to follicular cell hypertrophy and hyper-

plasia) by 7 days (Strum & Karnovsky, 1971). In female rats given drinking-water containing 2.5 mg/mL amitrole, a small increase in the size of the thyroid was visible after 3 days, and the size of the gland had doubled by 10 days (Tsuda *et al*., 1973). Continuous feeding of a diet containing 100 mg/kg amitrole resulted in the development of goitre in rats of each sex within 3 months, 25 mg/kg caused goitre in 4/10 females killed at 240 days, while rats receiving 10 mg/kg showed no goitrogenic effect within 24 months (Gaines *et al*., 1973; Steinhoff *et al*., 1983). Continuous feeding (up to 18 months) of a diet containing 100 mg/kg amitrole produced goitre in mice but not in golden hamsters; a dietary concentration of 10 mg/kg had no effect in either species (Steinhoff *et al*., 1983).

In male rats (Blue Spruce Farms strain) fed diets containing 0, 0.25, 0.5, 2, 10 or 50 mg/kg amitrole for 11–13 weeks, several measures of thyroid function were affected: dose-related decreases in serum protein-bound iodine concentrations and thyroid ^{131}I uptake were observed in groups receiving > 2 mg/kg of diet 24 h after injection of the isotope; and morphological changes in the thyroid, consisting of follicular-cell hypertrophy and reduced luminal colloid, were observed at the two higher concentrations (Fregly, 1968).

Amitrole caused rapid inactivation of lactoperoxidase only in the presence of hydrogen peroxide. The kinetics is consistent with a suicide mechanism (Doerge & Niemczura, 1989). In view of the similarities between lactoperoxidase and thyroid peroxidase, the authors suggested a similar mechanism of inhibition of thyroid hormone synthesis by amitrole. In addition to its direct inhibitory effect on thyroperoxidase in thyroid follicular cells, amitrole has been reported to affect the peripheral metabolism and deiodination of T4, resulting in increased formation of reverse T3 (Cartier *et al.*, 1985). Amitrole did not inhibit 5′-deiodinase activity as did other goitrogens (e.g. propylthiouracil; see monograph in this volume) but rather stimulated the T4 5-deiodination pathway in peripheral tissues.

Studies on the time-course of the response of the thyroid to amitrole treatment in male Wistar rats given drinking-water containing 0.1% for 7 or 12 months showed a rapid rise in the concentration of thyroid-stimulating hormone (TSH) after a short lag phase of a few days (Wynford-Thomas *et al.*, 1983) that was paralleled by thyroid hypertrophy and hyperplasia. These effects peaked and plateaued after 3–4 months and thereafter remained relatively stable despite further exposure. Wynford-Thomas *et al*. (1982) reported striking decreases in T4 and T3 and marked increases in serum TSH concentrations resulting in increased thyroid weight due to increased follicular-cell numbers in male Wistar rats (aged 10–11 weeks at the start of the experiment) given amitrole at 0.1% in drinking-water (estimated equivalent dose, 500 mg/kg bw) daily for 3, 7, 14, 24, 46, 83, 116 or 153 days. Control groups (age-matched) were killed after 0, 25, 83 and 154 days. Mattioli *et al*. (1994) gave lower concentrations of amitrole in the drinking-water (1 g/L; estimated daily intake, 200 mg/kg bw) to male Sprague-Dawley albino rats (90–100 g) for 5, 8 or 12 days and found similar marked reductions in plasma T4 and T3 concentrations associated with a concurrent increase in both the

mitotic index and frequency of S-phase cells, indicative of thyroid follicular-cell hyperplasia. A number of studies have shown that the goitrogenic action of amitrole is reversible on cessation of exposure (Jukes & Shaffer, 1960).

Enzymatically dispersed rat thyrocytes from the early plateau phase and involuting goitres were analysed for their capacity to form thyroid follicular units after transplantation into syngeneic recipients. The clonogenic fractions of goitres induced by either amitrole or $KClO_4$/Remington low-iodine diet were significantly smaller than in cells from control glands, and the clonogenic fraction of cells from the $KClO_4$-induced goitres was smaller than that of cells from amitrole-induced goitres, despite similar circulating TSH concentrations in the donor rats. The authors concluded that the capacity to proliferate clonally into follicular units is a specific trait that characterizes a unique subset of follicular cells and suggested that the hormonally responsive tumours that often develop in continuously stimulated rat thyroid glands arise from cells within this subset (Groch & Clifton, 1992).

In a study of the histopathological changes induced by amitrole in the liver, groups of male albino mice were given amitrole in the drinking-water at a concentration of 0.5, 1 or 2% for 30 days. Light microscopy revealed dose-related hypertrophy of hepatocytes, increased pyknotic nucleoli, increased vacuoles and lipid droplets in the cytoplasm. Electron microscopy revealed a dose-related proliferation of smooth endoplasmic reticulum (Reitze & Seitz, 1985).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

When amitrole was last evaluated (IARC, 1986a), limited studies were reviewed in which Sherman rats were exposed to a dietary concentration of amitrole of 25, 100, 500 or 1000 mg/kg (equivalent to 2.5, 9.6, 43 or 87 mg/kg bw per day) for up to two generations. Pup weights were reduced at the two higher concentrations, atrophy of the thymus and spleen was observed, and the majority of pups died within 1 week of weaning. Reproduction was not affected at the two lower concentrations, but thyroid hyperplasia was observed at ≥ 100 mg/kg of diet (Gaines *et al*., 1973).

4.4 Effects on enzyme induction or inhibition and gene expression

Amitrole inhibited catalase activity in the liver and iris–ciliary body in rats (Williams *et al*., 1985) and in human cultured fibroblasts (Middelkoop *et al*., 1991), and inhibited thyroperoxidase activity in rat thyroid follicular cells (Strum & Karnovsky, 1971).

4.5 Genetic and related effects

4.5.1 *Humans*

No data were available to the Working Group.

4.5.2 *Experimental systems* (see Table 3 for references)

Amitrole did not induce DNA damage or mutations in bacteria or mutations or chromosomal damage in cultured mammalian cells. It induced transformation in Syrian hamster embryo cells and chromosomal aberrations in plant root tips. Sporadic aneuploidy and recombinational effects were produced in yeast and fungi and in mammalian cells *in vitro*. No recessive lethal mutation, recombination or aneuploidy was seen in *Drosophila melanogaster*. Amitrole did not induce micronuclei in bone-marrow cells or unscheduled DNA synthesis in hepatocytes of mice treated *in vivo*.

Ki-*ras* mutation was detected in only 1/10 (10%) rat thyroid tumours induced by amitrole, while it was found in 8/15 (53%) radiation-induced tumours (Lemoine *et al.*, 1988).

4.6 Mechanistic considerations

An overall evaluation of the available data supports a mechanism of thyroid hormone imbalance in the development of follicular-cell neoplasia caused by amitrole in rats and mice because:

- Amitrole is considered not to be a genotoxic agent because of lack of activity in appropriate tests with bacteria, cultured mammalian cells and with rats and mice treated *in vivo*.
- Amitrole alters thyroid hormone homeostasis, decreases T4 and T3 and increases TSH concentrations in rats treated with doses that are within the range of those that produced tumours in the studies of carcinogenicity.
- The mechanism resulting in disturbed thyroid hormone synthesis is based on interference with the functioning of thyroid peroxidase.

On the basis of this information, which meets the criteria laid out in the IARC consensus report (Capen *et al.*, 1999), amitrole would be expected not to be carcinogenic to humans exposed to concentrations that do not lead to alterations in thyroid hormone homeostasis. Amitrole produces thyroid gland enlargement (goitre) in rats and mice as a result of follicular-cell hypertrophy and hyperplasia. The hyperplasia induced by amitrole in the thyroid gland is diffuse, in analogy with the morphological changes induced by TSH stimulation, rather than only multifocal, as would be induced by a genotoxic thyroid carcinogen (Hard, 1998). On the basis of the lack of genotoxicity, the liver tumours in mice and the benign pituitary tumours in rats were considered not to be produced by a genotoxic mechanism.

Table 3. Genetic and related effects of amitrole

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Prophage induction, SOS repair, DNA strand breaks, cross-links	NT	–	1000	Mamber *et al.* (1984)
Prophage induction, SOS repair, DNA strand breaks, cross-links	–	–	NR	Quillardet *et al.* (1985)
Prophage induction, SOS repair, DNA strand breaks, cross-links	–	–	1670	Nakamura *et al.* (1987)
Escherichia coli pol A, differential toxicity	–	–	250	Rosenkranz & Poirier (1979)
Escherichia coli rec, differential toxicity	–	NT	5000	Bamford *et al.* (1976)
Escherichia coli rec, differential toxicity	NT	–	4000	Mamber *et al.* (1983)
Bacteriophage, forward mutation	–	NT	25	Andersen *et al.* (1972)
Bacteriophage, reverse mutation	–	NT	200	Andersen *et al.* (1972)
Salmonella typhimurium TM677, forward mutation	NT	–	100	Skopek *et al.* (1981)
Salmonella typhimurium TA100, reverse mutation	NT	–	500 µg/plate	Hubbard *et al.* (1981)
Salmonella typhimurium TA100, reverse mutation	–	–	5000 µg/plate	MacDonald (1981)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	NT	–	5000 µg/plate	McCann *et al.* (1975)
Salmonella typhimurium TA100, TA1535, TA1536, TA1537, TA1538, TA98, reverse mutation	NT	–	250 µg/plate	Simmon (1979a)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	333.3 µg/plate	Dunkel *et al.* (1984)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	NT	–	4000 µg/plate	Mamber *et al.* (1984)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1000 µg/plate	Falck *et al.* (1985)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	10 000 µg/plate	Richold & Jones (1981)
Salmonella typhimurium TA100, TA1535, TA98, TA97, reverse mutation	–	–	3333 µg/plate	Zeiger *et al.* (1988)
Salmonella typhimurium TA1535, TA1537, TA1538, TA1536, reverse mutation	–	–	2000 µg/plate	Carere *et al.* (1978)
Salmonella typhimurium TA1535, TA1537, TA98, reverse mutation	–	–	500 µg/plate	Gatehouse (1981)
Salmonella typhimurium TA1535, TA1538, reverse mutation	–	–	250 µg/plate	Rosenkranz & Poirier (1979)
Salmonella typhimurium TA1537, TA98, reverse mutation	–	–	2000 µg/plate	MacDonald (1981)
Salmonella typhimurium TA98, reverse mutation	NT	–	10 000 µg/plate	Croker *et al.* (1992)
Salmonella typhimurium LT2 *trp*, reverse mutation	–	NT	4000 µg/plate	Bamford *et al.* (1976)
Escherichia coli, WP2 *uvr*A, reverse mutation	–	–	333.3 µg/plate	Dunkel *et al.* (1984)
Escherichia coli, WP2 *uvr*A, reverse mutation	–	–	1000 µg/plate	Falck *et al.* (1985)
Escherichia coli, WP2 *uvr*A, reverse mutation	–	–	500	Gatehouse (1981)
Saccharomyces wild type strain, differential toxicity	+	–	100	Sharp & Parry (1981a)
Saccharomyces cerevisiae, gene conversion	+	NT	300	Sharp & Parry (1981b)
Saccharomyces cerevisiae, gene conversion	NT	–	12 500	Zimmermann & Scheel (1981)
Saccharomyces cerevisiae, homozygosis	–	–	50 000	Simmon (1979b)
Saccharomyces cerevisiae, homozygosis	–	–	1000	Kassinova *et al.* (1981)
Saccharomyces cerevisiae, reverse mutation	–	–	1000	Mehta & von Borstel (1981)
Saccharomyces cerevisiae, aneuploidy	+	+	50	Parry & Sharp (1981)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Saccharomyces coelicolor, forward mutation	(+)	NT	1000	Carere *et al.* (1978)
Aspergillus nidulans, forward mutation	–	NT	2000	Bignami *et al.* (1977)
Aspergillus nidulans, forward mutation	–	NT	10 000	Crebelli *et al.* (1986)
Aspergillus nidulans, crossing-over	(+)	NT	2000	Bignami *et al.* (1977)
Aspergillus nidulans, crossing-over	–	NT	10 000	Crebelli *et al.* (1977)
Aspergillus nidulans, aneuploidy	(+)	NT	400	Bignami *et al.* (1977)
Aspergillus nidulans, aneuploidy	–	NT	10 000	Crebelli *et al.* (1986)
Chromosomal aberrations, *Hordeum* spp.	+	NT	100	Wuu & Grant (1966)
Chromosomal aberrations, *Vicia faba*	+	NT	50	Wuu & Grant (1967)
Chromosomal aberrations, *Neatby's virescens* (wheat)	+	NT	1.25	Rédei & Sandhu (1988)
Drosophila melanogaster, mitotic recombination	–		1680	Vogel & Nivard (1993)
Drosophila melanogaster, wing spot test	?		840	Tripathy *et al.* (1990)
Drosophila melanogaster, white-ivory assay	?		1680	Consuegra *et al.* (1996)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		10	Laamanen *et al.* (1976)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		2000	Vogel *et al.* (1981)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		20 000 feed[c]	Woodruff *et al.* (1985)
Drosophila melanogaster, aneuploidy and sex-linked recessive lethal mutations	–		10	Laamanen *et al.* (1976)
DNA fragmentation, rat hepatocytes *in vitro*	–	NT	1510	Mattioli *et al.* (1994)
Binding to RNA or protein *in vitro*	–	+	4	Krauss & Eling (1987)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	5000	McGregor *et al.* (1987)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	6000	Mitchell *et al.* (1988)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	5000	Myhr & Caspary (1988)
Gene mutation, Syrian hamster embryo cells, *Hprt* and Na^+/K^+ ATPase *in vitro*	+	NT	0.3	Tsutsui *et al.* (1984)
Gene mutation, Syrian hamster embryo BP6T cells *in vitro*	–	NT	8400	Lesko *et al.* (1985)
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	–	+	1	Perry & Thomson (1981)
Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	–	NT	1680	Ochi & Ohsawa (1985)
Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	–	NT	2000	Sofuni & Ishidate (1988)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	1	Pienta *et al.* (1977)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	50	Inoue *et al.* (1981)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	0.3	Tsutsui *et al.* (1984)
Cell transformation, Syrian hamster embryo cells, clonal assay	(+)	NT	100	Mikalsen *et al.* (1990)
DNA fragmentation, human hepatocytes, human thyroid cells *in vitro*	–	NT	1510	Mattioli *et al.* (1994)
Gene mutation, diploid human fibroblasts HFW, *HPRT* gene mutation	–	NT	6720	Hwua & Yang (1998)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	10 000	Meretoja *et al.* (1976)
Cell transformation, diploid human fibroblasts HFW, anchorage independence	–	NT	6720	Hwua & Yang (1998)
Host-mediated assay, mice and *Salmonella typhimurium* TA1950	–		245 po × 1	Braun *et al.* (1977)
Host-mediated assay, mice and *Salmonella typhimurium* TA1530	+		12 im × 1	Simmon *et al.* (1979)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA fragmentation, thyroid and liver, rats *in vivo*	–		200 drinking-water, 12 days	Mattioli *et al.* (1994)
Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		1000 po × 1	Kornbrust *et al*. (1984)
Micronucleus formation, mice *in vivo*	–		135	Salamone *et al.* (1981)
Micronucleus formation, mice *in vivo*	–		500 ip × 2	Tsuchimoto & Matter (1981)

[a] +, positive; (+), weak positive; –, negative; ?, inconclusive; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day; NR, not reported; po, oral; im, intramuscular; ip, intraperitoneal
[c] Negative when given at 10 000 µg/mL to adults by injection

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Amitrole is a herbicide, which has been used since the 1950s to control a wide range of weeds and grasses along roadsides, in vineyards and in orchards and in other applications, although contact with food crops is avoided. Amitrole is rapidly degraded in the environment, but occupational exposure may occur during its production and application.

5.2 Human carcinogenicity data

In a small cohort study of mortality among Swedish railroad workers who had sprayed herbicides, there was a statistically significant excess of cancers at all sites combined among men exposed to both amitrole and chlorophenoxy herbicides but not among those exposed mainly to amitrole.

5.3 Animal carcinogenicity data

Amitrole was tested in mice by oral administration, skin application and transplacental and perinatal exposure, in rats by oral and subcutaneous administration and in hamsters by oral administration. In mice, thyroid follicular-cell and hepatocellular tumours were produced after oral administration of amitrole. In rats, amitrole administered orally induced thyroid follicular-cell adenomas and carcinomas in males and females and a marginal increase in the incidence of pituitary adenomas in female rats at the highest dose. No carcinogenic effect was observed in hamsters.

In one experiment in rats, amitrole promoted thyroid follicular-cell tumours induced by *N*-nitrosobis(2-hydroxypropyl)amine.

5.4 Other relevant data

Amitrole is rapidly absorbed from the gastrointestinal tract and lung.

Amitrole caused thyroid gland enlargement (goitre) in rats and mice as a result of diffuse hypertrophy and hyperplasia of thyroid follicular cells. Administration of amitrole to rats under bioassay conditions that caused predominantly benign follicular-cell tumours resulted in alteration of thyroid hormone homeostasis, including increased secretion of thyroid-stimulating hormone. The underlying mechanism for the changes induced by amitrole is interference with the functioning of thyroid peroxidase.

No data were available on the genetic and related effects of amitrole in humans. Amitrole was not genotoxic in appropriate tests in bacteria and cultured mammalian cells or in rats and mice exposed *in vivo*. Amitrole induced chromosomal aberrations in

plants, aneuploidy in some experiments in fungi and transformation of Syrian hamster embryo cells *in vitro*.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of amitrole.

There is *sufficient evidence* in experimental animals for the carcinogenicity of amitrole.

Overall evaluation

Amitrole is *not classifiable as to its carcinogenicity to humans (Group 3)*.

In making its evaluation, the Working Group concluded that amitrole produces thyroid tumours in mice and rats by a non-genotoxic mechanism, which involves interference with the functioning of thyroid peroxidase, resulting in a reduction in circulating thyroid hormone concentrations and increased secretion of thyroid-stimulating hormone. Consequently, amitrole would not be expected to produce thyroid cancer in humans exposed to concentrations that do not alter thyroid hormone homeostasis.

An additional consideration of the Working Group, based on the lack of genotoxicity of amitrole, was that the liver tumours in mice and benign pituitary tumours in rats were also produced by a non-genotoxic mechanism.

Evidence from epidemiological studies and from toxicological studies in experimental animals provide compelling evidence that rodents are substantially more sensitive than humans to the development of thyroid tumours in response to thyroid hormone imbalance.

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