

AMITRAZ

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Explanation

Amitraz [*N*-methylbis(2,4-xylyliminomethyl)amine] was evaluated by the Joint Meeting in 1980, 1984, and 1990 (Annex 1, references 34, 42, and 59). A toxicological monograph was prepared in 1980 (Annex I, reference 35) and a monograph addendum was prepared in 1984 (Annex I, reference 43). A temporary ADI of 0–0.0005 mg/kg bw was allocated in 1980, and an ADI of 0–0.003 mg/kg bw was established in 1984. The 1990 Meeting reviewed the compound at the request of a WHO Member State which asked for reconsideration of the ADI in view of the acute nature of the reported toxicological effects and potential dietary exposure. Since that Meeting, studies have become available on absorption, distribution, excretion, biotransformation, effects on liver enzymes and the oestrus cycle, long-term toxicity, dermal and ocular irritation, and dermal sensitization.

The compound was reviewed by the present Meeting within the CCPR periodic review programme. This monograph summarizes the new data and relevant data from the previous monograph and monograph addendum on this pesticide (Annex 1, references 35 and 43).

Evaluation for Acceptable Daily Intake

1. Biochemical aspects

(a) Absorption, distribution, and excretion

Male and female B6C3F₁ mice, either untreated or pre-dosed for three weeks with 400 ppm amitraz in the diet, were given a single oral intubation of 0 or 10 mg/kg bw ¹⁴C-amitraz (specific activity, 9 mCi/g). Urine and faeces were collected for 96 h after dosing, after which the mice were killed for tissue analysis. During the first 24 h, 86% of the radiolabelled dose was excreted and 62% was present in the urine. It was completely excreted by 96 h, with 73% in the urine. The route and rate of excretion were similar in each sex and in untreated and pre-dosed mice. The highest concentrations of radiolabel were found in the liver, adrenals, and eyes and the least in bone and muscle (Campbell & Needham, 1983).

In rats given oral doses of ¹⁴C-labelled amitraz (specific activity unspecified), 53–85% was recovered in urine within three days, with 17–47% in faeces and < 0.1% in expired air. Peak plasma concentrations were found about 1 h after dosing. The highest concentrations of residue were found in the liver, kidney, and muscle within 2 h, diminishing thereafter (Lewis, 1971).

During repeated oral dosing of groups of one male and one female rat with 4 mg/kg bw per day ¹⁴C-labelled amitraz (specific activity unspecified) for 28 days, the highest concentrations of residue were found in the thyroid and adrenal glands, liver, kidney, skin, spleen, and eyes. After dosing had ceased, a considerable decrease in concentration was observed. The radiolabel in blood was bound mainly to cells. Seven days after the final dose, small but significant concentrations of residue were detected in liver, spleen, skin, and adrenals (Somerville, 1973).

Three male and three female Sprague-Dawley rats were dosed orally with 10 mg/kg bw ¹⁴C-amitraz (specific activity, 7.8 mCi/g) dissolved in corn oil, and urine and faeces were collected over 96 h, after which the rats were killed and dissected for tissue analysis. Over the first 24 h, 82% of the dose was excreted, mainly in the urine; over 96 h, 94% of the dose was recovered, with 82% in urine and 12% in faeces. The concentrations of residue were highest (0.4–0.5%) in liver (Campbell & Needham, 1981).

Ten male rats were treated dermally with 1 mg ¹⁴C-amitraz (specific activity, 253 mCi/g), formulated as Mitac™ wettable powder (technical product), diluted with water to a concentration approximately 20 times the maximum recommended spray dilution. After 10 h, the treated skin was washed with soap and water. Half of the rats were killed 24 h after treatment, and the remainder were maintained in metabolism cages for five days. Urine and faeces were collected at 24-h intervals after the beginning of treatment and were radioassayed with gastrointestinal tracts, carcasses, treatment sites, dressings, and washings. Washing of the skin 10 h after treatment removed 92% of the applied amitraz, while approximately 3% remained on the skin by 24 h; this percent fell to 1.4% after five days. The small amount of amitraz absorbed over five days (approximately 3–8% of that applied) was excreted in the urine and faeces. Excretion by this route was 90% complete 96 h after treatment. Very low concentrations of residue were detected in the carcass (0.06%) and gastrointestinal tract (0.01%) after five days (Challis, 1990).

Male CrI:CD(SD)BR rats ($n = 112$) were treated dermally with 0.01, 0.1, 1, or 10 mg per animal ¹⁴C-amitraz (specific activity, 281 mCi/g), formulated as diluted Mitac™ wettable powder (technical product) for 0.5, 1, 2, 4, or 10 h, and radiolabel was measured in urine, faeces, residual carcass, and the application site. The results for animals at 0.01 mg were not reported because of poor, inconsistent recovery of radiolabel. The mean total recovery of radiolabel was 104% at 0.1 mg, 100% at 1 mg, and 107% at 10 mg. The distribution of radiolabel was similar at the three doses. Most of the applied dose was recovered in application site

washings (61–99%) and dressing washings (10–31%). The concentration of radiolabel retained at the application site peaked 4 h after application. When the site was washed, 10 h after application, the concentration of radiolabel retained at the application site fell to < 3% by 24 h and to 0.06% by 120 h in all three groups. The extent absorbed (percent applied dose) fell with increasing dose, implying that absorption was nearing saturation at 0.1 mg/animal. Maximum absorption (12% at 0.1 mg/animal) was achieved at 120 h after a 10-h exposure. The concentrations of radiolabelled amitraz and its metabolites in blood were low at all sacrifices and at all doses. The absorbed radiolabel was eliminated primarily in urine, with minor quantities in faeces. The concentrations of radiolabel in residual carcass were low (maximum, 2% at 24 h in animals receiving 10 mg) at all three doses and all sacrifices. By 120 h after application, the concentration in residual carcass was below the limit of detection in all animals (Stewart, 1993).

In separate studies, groups of two male and two female beagle dogs were dosed orally (4 mg/kg bw by capsule) or dermally (20–21 mg on an area of 400–500 cm²) with ¹⁴C-amitraz (specific activity, 8.6 mCi/g). Peak blood concentrations of radiolabel were observed during the first 8 h after oral administration. About 80% of the oral dose was excreted within the first 24 h and 100% within 72 h, preferentially in the urine. After dermal treatment, peak blood concentrations occurred within 24–72 h, and only 25–40% was recovered in urine and faeces over a 10-day collection period, demonstrating the poor dermal absorption of amitraz (Hornish & Nappier, 1983).

Two male and two female pigs received a single topical dose of 18 mg ¹⁴C-amitraz (chemical purity, 98.6%; specific activity, 9 mCi/g) on a shaven dorsal area. The treated area was subjected to a mild washing procedure 12 h after application, which removed 60–80% of the applied radiolabel. Over 60 h after dosing, 7% of the applied radiolabel was detected in excreta. Less than 0.05 ppm residues were found in most tissues (Campbell & Needham, 1984a).

One male and one female baboon received a single oral dose of 10 mg/kg bw ¹⁴C-amitraz (specific activity, 2 mCi/g), and urine and faeces were collected for 72 h, after which time the animals were killed. Within the first 24 h, 75–83% of the dose was excreted, with 58–76% in the urine. The concentrations of residues in tissue residues were similar in animals of each sex: highest in liver and eyes and lowest in muscle (Campbell & Needham, 1984b).

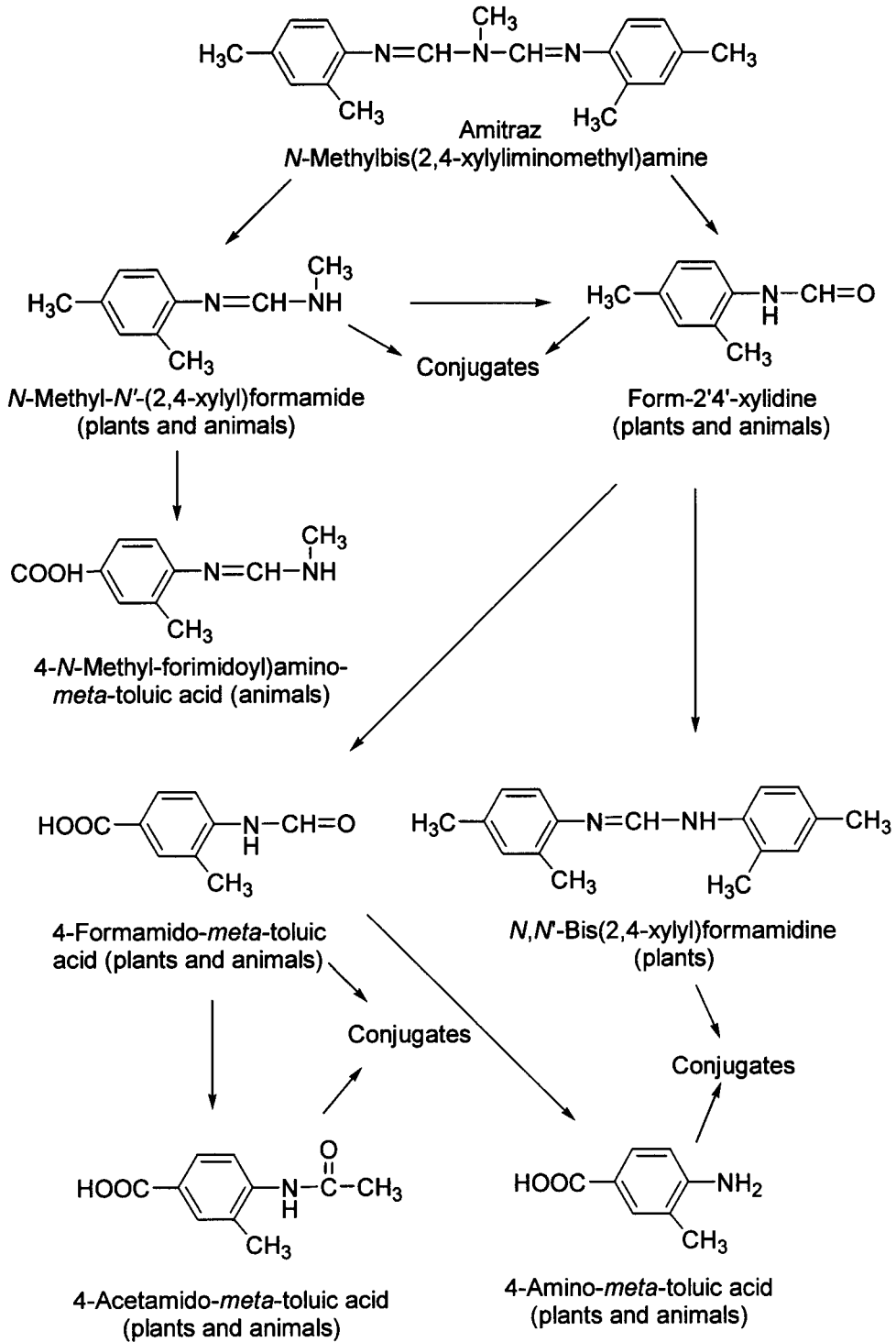
Two male volunteers received a single oral dose of 0.25 mg/kg bw ¹⁴C-amitraz; urine was collected for 72 h after dosing, and the radiolabel was determined. Urinary excretion was 58–68% of the dose within the first 24 h and 77–87% within 72 h. Within the first 4 h, dry mouth, drowsiness, disorientation, slurred speech, light-headedness, and decrease in pulse rate and blood pressure were recorded. One person fell asleep 2 h after treatment and subsequently complained of nausea and vivid dreams. The urinary metabolites were the same as those found in the other species examined (Campbell & Needham, 1984c).

(b) *Biotransformation*

In rats dosed orally with ¹⁴C-labelled amitraz (specific activity unspecified), at least four metabolites were found in urine and six in faeces, the major component of which was *N*-methyl-*N'*-(2,4-xylyl)formamidine (Lewis, 1971).

After administration of a single oral dose of ¹⁴C-amitraz (specific activity, 9.0 mCi/g; radiochemical purity, > 95%) to groups of two male and two female Sprague-Dawley CD rats at 1, 10, 50, or 100 mg/kg bw, the compound was rapidly excreted, effectively metabolized, and completely degraded, with no apparent sex difference. At all doses, 4-formamido-*meta*-toluic acid and 4-acetamido-*meta*-toluic acid were the major metabolites (see Figure 1), which together accounted for up to 32% of the metabolites excreted in the urine. Excretion of the hydrolysis product, *N*-methyl-*N'*-(2,4-xylyl)formamidine, was dose-dependent: about 4% of

Figure 1. Proposed metabolic routes for amitraz in crops and animals



Concentration of each metabolite, given as percent of total urinary excretion: *N*-Methyl-*N'*-(2,4-xylyl)formamide, 4–38% (dose-dependent); 4-acetamido-*meta*-toluic acid plus 4-formamido-*meta*-toluic acid, 32%; form-2',4'-xylylidine, < 2%; 4-amino-*meta*-toluic acid, < 2%

the dose at 1 mg/kg bw and 23–38% at 100 mg/kg bw. Minor metabolites identified accounted for 2% of the total excretion and included form-2',4'-xylylide and 4-amino-*meta*-toluic acid (Campbell & Needham, 1984d).

In the study of Challis (1990), described above, the metabolic profile of rats treated dermally was similar to that of rats treated orally.

In the study of Hornish & Nappier (1983), described above, the metabolism of amitraz was essentially the same after oral and dermal administration. 4-Formamido-*meta*-toluic acid was the predominant residue in both blood and urine. The parent compound and the first hydrolysis products, *N*-methyl-*N'*-(2,4-xylyl)formamidine and form-2',4'-xylylide, were not observed at measurable concentrations in either blood or urine.

One cow received 0.5 mg/kg bw per day ¹⁴C-amitraz (specific activity, 4.2 mCi/g; radiochemical purity, > 98.8%) by capsule twice daily for four days. All milk was collected, and samples of urine were taken. The cow was killed 17 h after the final dose. The urinary metabolites corresponded to 4-acetamido-*meta*-toluic acid and 4-formamido-*meta*-toluic acid, which were converted to 4-amino-*meta*-toluic acid by acid hydrolysis. The total residue in milk accounted for only 0.2% of the dose. The greatest concentration of residue (0.07 ppm) was found after the final dose. The residue was extracted with methanol (recovery, 90%), and the metabolites present identified by co-chromatography as 4-acetamido-*meta*-toluic acid, 4-formamido-*meta*-toluic acid, and 4-amino-*meta*-toluic acid (23%); form-2',4'-xylylide (9%); polar material that was converted to 4-amino-*meta*-toluic acid by acid hydrolysis (34%); and low-polarity material (24%). The latter broke down readily to form-2',4'-xylylide under neutral or basic conditions; it was not present in acetonitrile extracts of whole milk and was considered to be an artefact produced by the methanol Soxhlet extraction of the milk. 2,4-Dimethylaniline was not observed. The edible tissue with the highest concentration of residue was the liver (3.7 ppm).

Methanol Soxhlet extraction released about 60% of the residue, and the remainder was solubilized after enzymic digestion and acid hydrolysis. The residues were identified by co-chromatography as 4-acetamido-*meta*-toluic acid and 4-formamido-*meta*-toluic acid (14%), 4-amino-*meta*-toluic acid (15%), form-2',4'-xylylide (10%), and polar material (10%) which on acid hydrolysis was converted to 4-amino-*meta*-toluic acid and additional low-polarity material. Other polar material (29% of the residue) was not amenable to chromatography. 2,4-Dimethylaniline was not observed. The low-polarity material (14%) broke down on neutral or basic thin-layer chromatography to a range of compounds, suggesting that it was a product of condensation between 4-amino-*meta*-toluic acid and compounds released from acid-labile conjugates. When the non-methanol-extractable residue was fed to a dog, analysis of urine gave similar results to those after enzymic digestion (Phillips et al., 1987).

Six laying hens (*Gallus gallus domesticus*) were each dosed orally with 24.5 mg ¹⁴C-amitraz (specific activity, 0.14 mCi/g; radiochemical purity, > 99%) per day for four days, providing a dose 2200 times greater than the expected daily exposure of birds fed cotton-meal derived from amitraz-treated plants. The hens were killed 4 or 12 h after the final dose, and the concentrations and nature of radioactive residues in tissues were determined. The radiolabel was rapidly excreted, with 68% in 0–24-h excreta. The main route of metabolism was via 4-amino-*meta*-toluic acid, since 75% of the metabolites extracted from the excreta could be accounted for as either this metabolite or its acid-labile conjugates. The highest concentrations of tissue residues were found in the liver and kidney (17 and 25 mg/kg respectively) 4 h after the fourth dose, but these concentrations had fallen to 12 and 10 mg/kg, respectively, by 12 h after dosing. The major metabolite detected in the liver was 4-amino-*meta*-toluic acid, present as both free acid and labile conjugates, which represented 55% of the total residue. *N*-Methyl-*N'*-(2,4-xylyl)formamidine (4%), form-2',4'-xylylide (4%) and 2,4-dimethylaniline (2%) were also present. A mixture of at least seven highly polar compounds, believed to be mono- and di-acids similar to those previously seen in rats, was a further substantial residue (27%).

Unextractable fibre-bound material represented 2% of the residue. The concentrations in fat, muscle, and skin represented 0.6–2.7 mg/kg 4 and 12 h after the final dose. In fat, the residues consisted of form-2',4'-xylylide (42%), *N*-methyl-*N'*-(2,4-xylyl)formamidine (24%), unchanged amitraz (21%), 2,4-dimethylaniline (3%), and 4-amino-*meta*-toluic acid (3%). In muscle, 81% of the residues were identified as 4-amino-*meta*-toluic acid or its acid-labile conjugates; form-2',4'-xylylide was present as 7% of the residue.

The concentrations of amitraz residues in eggs, collected daily, were 0.28–0.46 mg/kg throughout the study and did not increase over the four days of dosing. The residue concentrations in the yolks rose from 0.1 to 1.4 mg/kg during the study; *N*-methyl-*N'*-(2,4-xylyl)formamidine (54%) and 4-amino-*meta*-toluic acid (34%) were the major metabolites. 4-Amino-*meta*-toluic acid accounted for 91% of the residue in the egg white as both free acid and labile conjugates, and form-2',4'-xylylide accounted for 4% of the residue (Needham & Hemmings, 1988).

Separately reported data on the metabolism of amitraz in various species were compared. After a single oral dose of ¹⁴C-amitraz (specific activity, 9 mCi/g; radiochemical purity, > 95%) to groups of four male and four female mice, three male and three female rats, one male and one female baboon, and two men, the administered radiolabel was rapidly excreted. In all species examined, urine was the major route of excretion, accounting for 65–84% of the dose (55–76% within the first 24 h). Analysis of urine obtained from volunteers dosed with ¹⁴C-amitraz indicated that the metabolism of amitraz was qualitatively similar to that in the other species. The major metabolites were 4-formamido-*meta*-toluic acid, 4-acetamido-*meta*-toluic acid and *N*-methyl-*N'*-(2,4-xylyl)formamidine. In addition, 40–60% of the metabolites excreted in urine was accounted for by a polar fraction containing conjugates of 4-formamido-*meta*-toluic acid and 4-acetamido-*meta*-toluic acid. The minor metabolites included 4-amino-*meta*-toluic acid and form-2',4'-xylylide. Excretion of *N*-methyl-*N'*-(2,4-xylyl)formamidine was dose-dependent. The metabolites identified in the volunteers were 4-formamido-*meta*-toluic acid plus 4-acetamido-*meta*-toluic acid (27% of the total radiolabel in urine), *N*-methyl-*N'*-(2,4-xylyl)formamidine (6%), 4-amino-*meta*-toluic acid (4%), form-2',4'-xylylide (4%), the product of acid hydrolysis of *N*-methyl-*N'*-(2,4-xylyl)formamidine and form-2',4'-xylylide (1%), and polar material (57%). In both rats and mice, increasing the dose of amitraz from 1 to 100 mg/kg bw increased the excretion of *N*-methyl-*N'*-(2,4-xylyl)formamidine from approximately 5 to 30% of the total excretion. Prior dosing of five male and five female mice with amitraz in the diet at 100 ppm for three weeks, followed by 400 ppm for a further three weeks, had no effect on the metabolism of a single oral dose of ¹⁴C-amitraz (Campbell & Needham, 1984e).

(c) *Effects on enzymes and other biochemical parameters*

Groups of 18 male and 18 female B6C3F₁ mice were dosed orally with corn oil or amitraz at 100 mg/kg bw per day for two days and, because of toxic symptoms, at 50 mg/kg bw per day for the following two days. The animals were then killed, and the livers were assayed for the activity of microsomal oxidases. A significant increase in liver weight and in the activity of cytochrome *b*₅ were recorded. The effect did not appear to be related to increased activity of the hepatic mixed-function oxidase system, since no significant increase was seen in the activities of cytochrome P450, aniline hydroxylase, or *para*-nitroanisole demethylase or in the concentration of microsomal protein (Needham, 1984).

2. Toxicological studies

(a) *Acute toxicity*

The acute toxicity of amitraz and its metabolites (purity unspecified) has been investigated in several species (Table 1). The toxic signs after oral administration of amitraz to mice and

Table 1. Acute toxicity of amitraz

Species	Route	LD ₅₀ or LC ₅₀ (mg/kg bw or mg/L air)	Reference
Mouse	Oral	> 1600	Patton & Sutton (1971)
Rat	Oral	600	Patton & Sutton (1971); Shaw (1973a)
Rat	Dermal	>1600	Patton & Sutton (1971)
Rat	Intraperitoneal	800	Shaw (1971, 1973a)
Rat	Inhalation (6 h)	65	Berczy et al. (1972)
Guinea-pig	Oral	400–800	Patton & Sutton (1971)
Rabbit	Oral	> 100	Patton & Sutton (1971)
Rabbit	Dermal	> 200	Sutton & Williams (1972)
Dog	Oral	100	Patton & Sutton (1971)
Baboon	Oral	100–250	Patton (1973)

rats were hyperexcitability, ataxia, tremor, and ptosis. Rats had intestinal irritation and bladder distension. The lowest effective doses were 400 mg/kg bw in mice and 200 mg/kg bw in rats. Dermal application of 1600 mg/kg bw to rats had no local or systemic effect. Guinea-pigs were hyperexcitable after receiving 400 mg/kg bw or more. Rabbits had central nervous system depression, decreased rectal temperature, pulse rate, and respiration, nasal discharge, and rales after receiving 100 mg/kg bw, with complete recovery by 48 h. The reactions of dogs to administration of 100 mg/kg bw and, to a lesser extent, 20 mg/kg bw, were central nervous system depression, ataxia, muscular weakness, muscular spasm, uncontrolled vocal spasm and micturition, and decreased rectal temperature and pulse rate. Haemoconcentration and increased blood sugar, urea nitrogen, and potassium concentrations were also seen. No specific pathological lesions were induced. The lowest dose, 4 mg/kg bw, affected temperature and pulse rate only slightly. The profile of toxic reactions was consistent with depression of hypothalamic function. All of the effects were reversible.

The dermal irritation potential of amitraz (purity, 99%) was studied in six New Zealand white rabbits weighing 1.9–2.5 kg. Approximately 24 h after hair had been removed from the dorso-lumbar region, 0.5 g of technical-grade amitraz was applied under a 2.5-cm gauze pad moistened with 0.5 ml distilled water, and each treatment site was occluded with an elastic adhesive dressing for 4 h. At the end of the exposure period, the dressing was removed and the treatment site was washed with water. The treated skin was examined on days 1, 2, 3, and 4 after treatment. There was no response to treatment (Liggett & Smith, 1987a).

The ocular irritation potential of amitraz (purity unspecified) was studied in six New Zealand white rabbits weighing 2.1–2.8 kg. The eyes of each animal were examined before instillation of 45 mg (0.1 ml crystalline powder) of technical-grade amitraz (purity, 98.4%) into one eye. Both eyes were examined 1 h before and 1, 2, 3, 4, and 7 days after instillation. Only minimal or slight conjunctival irritation was observed (Liggett & Smith, 1987b).

Groups of 12 guinea-pigs weighing 300–350 g were treated daily with 0.1 ml of acetone or 10% amitraz (purity unspecified) or with 1% dinitrochlorobenzene on the outer surface of each ear for three days. One week after the start of treatment, the backs and flanks were clipped and treated with challenge doses of 0.2 ml acetone, amitraz (10%), or dinitrochlorobenzene (0.25%). The degree of erythema was assessed after 24 h. Sensitizing activity was observed after treatment with the positive control but not with amitraz (Sutton, 1971).

The dermal sensitizing effect of amitraz was studied in the Buehler test. During induction, five male and five female guinea-pigs received 500 mg technical-grade amitraz (purity, 99%)

on the anterior right flank for 6 h under a dry compress on days 1, 8, and 15. A dry compress alone was applied on the anterior left flank. After a two-week rest period, each animal received a challenge of 500 mg technical-grade amitraz to the right flank and a dry compress alone to the left flank. The cutaneous reactions were evaluated 24 and 48 h after challenge. The animals were then sacrificed, and cutaneous samples were taken from the challenge site in all animals. No clinical signs or deaths related to treatment were observed, and no cutaneous reactions were found 24 and 48 h after the challenge with amitraz (Clouzeau, 1992).

Delayed contact hypersensitivity in response to amitraz (purity, 98.4%) was tested in the maximization test described by Magnusson and Kligman. After random allocation of 20 female guinea-pigs to a control group and 20 to a test group, the animals were induced by intradermal injection of 5% w/w amitraz in Alembicol D (a coconut oil) into a 4 x 6-cm clipped area of the dorsal skin on the scapular region. One week after the injections, the same area was clipped again and given a topical application of 15 or 30% w/w amitraz in Alembicol D on a 2 x 4-cm patch saturated with the test solution, which was placed on the skin, covered with impermeable plastic adhesive tape, secured by an elastic adhesive bandage, and left in place for 48 h. The test and control animals were challenged topically as described above, two weeks after the induction, with 15 or 30% w/w technical-grade amitraz in Alembicol D. The challenge sites were evaluated 24, 48, and 72 h after removal of the patches, and an arbitrary scale of 0–4 was used to score the reactions. The dermal reactions observed in the test animals were more marked and persistent than those seen in the controls (Kynoch & Parcell, 1988).

(b) *Short-term toxicity*

Mice

Groups of 20 male and 20 female B6C3F₁ mice were fed diets containing 0, 100, 200, 400, 600, or 800 ppm amitraz (purity, 98.3%), equal to 0, 13, 26, 53, 96, and 110 mg/kg bw per day for males and 0, 17, 35, 68, 110, and 150 mg/kg bw per day for females, for 13 weeks. Clinical signs, body weight, and food consumption were recorded throughout the study. All mice were killed at the end of treatment and examined grossly but not histopathologically. The only overt sign of reaction to treatment was an increase in aggressive behaviour, as evidenced by fighting and resultant cutaneous lesions, among males treated at doses \geq 400 ppm. The overall body-weight gain was statistically significant lower in the males (40%) treated at \geq 400 ppm and in females (34%) at \geq 200 ppm. Food consumption did not differ statistically significantly between the control and treated groups. The NOAEL was 100 ppm, equal to 17 mg/kg bw per day, on the basis of the overall reduced body-weight gain of females (Colley et al., 1981).

Rats

Groups of 21 male and 21 female Ash-Wistar rats were given amitraz (purity unspecified) suspended in 0.4% Cellosize solution by oral intubation at a dose of 0, 3, or 12 mg/kg bw per day for 90 days. They were then killed either immediately or after a three-week recovery. Treatment of rats with 50 mg/kg bw per day was discontinued after seven days because of depressed growth and behavioural disturbances, and treatment with 200 mg/kg bw per day was discontinued after seven days because of irritability and debilitation. Body weights were recorded three times per week. Haematological examinations were made on some control rats and on some rats receiving 12 mg/kg bw per day at 4, 8, and 12 weeks. At autopsy, blood was withdrawn from the heart to estimate plasma alkaline phosphatase activity, bilirubin, aspartate and alanine aminotransferase activity, and sodium and potassium concentrations. Organs were weighed and prepared for microscopic examination. Significant reductions were seen in the overall body-weight gain (8%) and absolute (8%) and relative weights (6%) of the liver of male rats receiving 12 mg/kg bw per day. The NOAEL was 3 mg/kg bw per day on the basis of these last effects (Sutton & Williams, 1971).

Groups of 12 rats (strain not given) were exposed daily for 6 h to dusts of amitraz (purity unspecified) at a concentration of 0, 0.01, 0.1, or 1 mg/L air, on 14 days over three weeks. During exposure to 0.1 mg/L, signs of mild dyspnoea, slight eye irritation, and hyposensitivity to noise were recorded. After termination of exposure, the rats were hypersensitive to touch and became aggressive. Exposure to 1 mg/L elicited signs similar to but more severe than those seen in the previous group. In addition, ataxia, increased nasal secretion, polyuria, body tremors, and coma were observed in the exposed rats. Consumption of food and water was reduced, and there was body-weight loss. Reductions in packed cell volume, haemoglobin, red cells, and plasma protein concentrations in blood may have been related to treatment. The body tremors, aggressive behaviour, and coma indicate that the central nervous system was affected (Berczy et al., 1973).

Rabbits

Groups of four to eight male and female New Zealand white rabbits weighing 2.5–4.5 kg (the health status of which must be regarded as suspect due to general infection) were treated on the intact skin of the back (without occlusion) with amitraz (purity unspecified) in acetone at a dose of 0, 50, or 200 mg/kg bw per day for a total of 15 doses over 21 days. Body weight, food consumption, heart rate, and rectal temperature were determined. Haematological and blood biochemical parameters and organ weights were measured, and histopathological examinations were carried out. A transient sedative effect and local skin reactions were seen in animals of each sex at 50 and 200 mg/kg bw per day. Body weights and food consumption were adversely affected, and some deaths occurred that were possibly related to treatment. Variable tubular degeneration was seen in the testes, which were underweight. At 50 mg/kg bw per day, body weights and food consumption were less adversely affected than at the high dose, and the death of only one male was considered related to treatment (Sutton, 1973a).

Dogs

Groups of two male and two female beagles received amitraz (purity unspecified) in capsules at a dose of 0, 0.25, 1, or 4 mg/kg bw per day for 90 days. The animals were examined clinically before dosing began and during weeks 2, 4, 8, and 13 of dosing. Food consumption was recorded daily and body weights weekly. Haematological, blood biochemical, and urinary parameters were measured before and at intervals during treatment. During week 13 of dosing, control animals and those at 4 mg/kg bw per day were placed in metabolism cages, and their water intake and urine excretion over 24 h were recorded. The faeces were tested daily for occult blood during the first week of dosing. At the end of treatment, the animals were sacrificed, and several organs were weighed and prepared for histological examination.

On the three first days of dosing, all four animals at 4 mg/kg bw per day showed central nervous system depression, vomiting, ataxia, and reduced rectal temperature and pulse rate, which recurred consistently within 6 h. Hyperglycaemia and occasional glycosuria were also induced. The livers were slightly enlarged and showed low-grade microscopic changes. The dose of 1 mg/kg bw per day had similar but less severe effects. At 0.25 mg/kg bw per day, only minor changes were seen, although central nervous system depression was seen in one dog on one occasion 3 h after dosing during week 8 of treatment. The NOAEL was 0.25 mg/kg bw per day on the basis of central nervous system depression and reduced rectal temperature and pulse rate (Patton & Williams, 1971).

Groups of four male and four female beagles were given 0, 0.1, 0.25, or 1 mg/kg bw per day amitraz (purity unspecified) in gelatine capsules for two years. They were examined clinically before and immediately after dosing on the first two days and again during weeks 4, 13, 26, 39, 52, 78, and 102. Heart rate and rectal temperature were monitored serially for up to 24 h after dosing on day 1 and during weeks 39, 52, 79, and 103. Signs of toxicity and faecal appearance were recorded daily and body weights weekly. Haematological, blood biochemical, and urinary parameters were measured before dosing and during weeks 4, 13, 26, 52, 78, and 102. In addition, serial blood samples were obtained during weeks 40 and 53 for estimating

blood sugar and during weeks 91 to 96 for estimating methaemoglobin. The animals were sacrificed and examined macroscopically on the day after the final dose, and their organs were weighed and prepared for histopathological examination.

All eight animals given 1 mg/kg bw per day showed signs of slight central nervous system depression 3 h after dosing on days 1 and 2, but all appeared normal by the following morning. On both days, one dog had a slightly subnormal temperature (38.3 °C) at 3 h, which had returned to normal (38.7 °C) by 24 h. Subsequently, all of the animals in this group appeared clinically normal, apart from one bitch that was slightly hypothermic 3 h after dosing during weeks 52 and 79. The NOAEL was 0.25 mg/kg bw per day on the basis of central nervous system depression (Morgan et al., 1973).

(c) *Long-term studies of toxicity and carcinogenicity*

Mice

Groups of 50 male and 50 female CFLP mice were fed diets containing 0, 25, 100, or 400 ppm amitraz (purity unspecified), equivalent to 3, 8, 15, and 60 mg/kg bw per day (wrongly given as 25, 100, and 400 mg/kg bw per day in reference 34, Annex 1), for 80 weeks. Animals at 100 or 400 ppm gained less weight than the controls over the first 40 weeks, whereas those at 25 ppm gained more weight than the controls from week 10 onwards and showed a 20% greater body-weight gain at the end of the experiment. Calculated over 80 weeks, food consumption was increased for male mice at 100 and 400 ppm and female mice at 400 ppm, and decreased for males at 25 and 100 ppm. The survival rate was similar in all groups. An increased incidence of lymphoreticular tumours was observed in female mice at 400 ppm (49% compared with 23% in controls). A slight, not statistically significant increase in the incidence of all types of liver-cell tumour was found in animals of each sex fed 400 ppm. No other pathological finding related to treatment was reported. The NOAEL for carcinogenicity was 100 ppm, equivalent to 15 mg/kg bw per day, on the basis of an increased incidence of lymphoreticular tumours in females at 400 ppm (Burnett et al, 1976; Kakuk, 1979).

Groups of 75 male and 75 female B6C3F₁ hybrid mice, 33 days old, were given diets containing 25, 100, or 400 ppm amitraz (purity, 97.9%), equal to 2.3, 9.6, and 45 mg/kg bw per day for males and 2.6, 11, and 50 mg/kg bw per day for females, for 104 weeks. The untreated controls consisted of 100 male and 100 female mice. Clinical signs, food consumption, and body weight were recorded throughout the study. Survivors were killed at the end of the dosing period and all animals were subjected to a complete gross autopsy. All tissues were collected from all animals and preserved for future examination. Histopathological examination was performed on all tissues from the high-dose group and controls, and on the liver, pancreas, spleen, lung, stomach, pituitary, thyroid, adrenals, ovaries, uterus, sternum, and all abnormal tissues found grossly from animals at the low and intermediate doses.

Males at 400 ppm were hyperactive and behaved aggressively. The incidence of cutaneous ulceration and inflammation of the perigenital and perianal areas was greater in mice at 100 and 400 ppm than in those at 25 ppm or in controls, and the incidence of urogenital swelling was greater in all treated mice than in controls. The incidences of gross adverse effects in treated females were comparable to the control incidences. There was clearly increased mortality of males at 400 ppm (20/75) and of males and females combined (37/150). Mean body-weight gain was reduced in mice at 400 ppm (by 30–50%) throughout the study, with a significant decrease in females at 100 ppm over the last 74 weeks of the study. Food consumption was marginally reduced initially in animals at 100 and 400 ppm but was comparable to that of controls from week 25 to termination. Increased liver and lymph node involvement was seen in males and females at 400 ppm, and the incidence of preputial gland enlargement in males at 400 ppm was greater than that in controls (20/75 vs 12/100). The prominence of the limiting ridge of the stomach was greater than in controls for females at all doses and for males at 100 and 400 ppm. Decreased production of myeloid elements accompanied by an increase in erythroid elements resulted in a significant decrease in the myeloid:erythroid ratio for males at 400 ppm and females at 100 or 400 ppm. The incidences of spleen haematopoiesis in males

and of stomach focal hyperkeratosis in males and females at all three doses were greater than that in controls. There was apparent liver involvement in females, with an increased incidence of hepatocellular carcinoma at 400 ppm (15/75 vs 2/100 hepatocarcinoma; 16/75 vs 4/100 hepatocellular adenoma). This was accompanied by a dose-related increase in the incidence of hyperplastic nodules and telangiectatic and basophilic foci of the liver in animals at 100 and 400 ppm. Males at 400 ppm also had an apparent increase in the incidence of hyperplastic nodules and telangiectatic and basophilic foci of the liver. The NOAEL for carcinogenicity was 100 ppm, equal to 11 mg/kg bw per day, on the basis of hepatocellular carcinoma in females at 400 ppm. This dose was considered to be greater than the conventional maximum tolerated dose. The NOAEL for long-term toxicity was 25 ppm, equal to 2.3 mg/kg bw per day, on the basis of general toxicity (Colley et al., 1983).

Rats

Groups of 40 male and 40 female Ash-Wistar rats received amitraz (purity, 97.8%) at a dietary concentration of 0, 15, 50, or 200 ppm (equal to 0, 0.77, 2.5, or 10 mg/kg bw per day in males and 0, 0.97, 3.1, or 13 mg/kg bw per day in females) for two years. Body weights were measured twice per week during the period of maximal growth and later weekly. Food consumption was determined weekly during this growth period and later monthly. Haematological, biochemical, and urinary analysis were performed during and at the end of the study, and organs were weighed and examined grossly and histologically at the end of study.

Rats at 200 ppm were occasionally nervous, excitable, and aggressive, and their food consumption was temporarily reduced. The overall body-weight gain of males was significantly reduced (10%). Rats at 50 and 15 ppm showed no adverse reactions to treatment. The incidence, type, and time to appearance of tumours were not significantly different in treated and control groups. The NOAEL for toxicity was 50 ppm, equal to 2.5 mg/kg bw per day, on the basis of effects on the central nervous system and reduced overall body-weight gain in males (Sutton & Offer, 1973).

(d) Genotoxicity

The results of tests for the genotoxicity of amitraz are summarized in Table 2. The results of the tests *in vitro* and *in vivo* were negative.

(e) Reproductive toxicity

(i) Multigeneration reproductive toxicity

In a three-generation study of reproductive toxicity, groups of 10 male and 20 female newly weaned Boots-Wistar rats were fed amitraz (purity, 99.8%) at a dietary concentration of 0, 15, 50, or 200 ppm, equal to 0, 1.3, 4.4, and 16 mg/kg bw per day in males and 0, 1.5, 5.1, and 20 mg/kg bw per day in females. After the F₁ generation had been weaned, 12 males and 24 females from each group were retained for breeding and maintained on the diet. The procedure was repeated until the F₃ generation was weaned. Amitraz at 200 ppm decreased the growth, food consumption, fertility, and viability of offspring of the F₀ generation, and this dose was eliminated when the F₁ generation had been weaned, because of the very low survival. No effect was found on the number of litters or mean litter size at 50 ppm; however, a decreased number of young alive at 21 days was observed in all generations. No further effects due to treatment were found in this or the other group. The NOAEL was 50 ppm, equal to 4.4 mg/kg bw per day, for maternal toxicity and 15 ppm, equal to 1.3 mg/kg bw per day, for developmental toxicity (Sutton, 1973b).

(ii) Developmental toxicity

In a study of developmental toxicity, groups of 11–13 female Boots-Wistar rats received amitraz (purity, 99.8%) at a dose of 0, 1, 3, or 12 mg/kg bw per day on days 8–20 of gestation.

Table 2. Results of tests for the genotoxicity of amitraz

End-point	Test system	Concentration or dose	Purity (%)	Results	Reference
<i>In vitro</i>					
Reverse mutation	<i>S. typhimurium</i> TA1535, TA1537, TA1558	31–500 µg/plate	99.9	Negative	Everest & Wilcox (1976)
Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	0, 33, 100, 333, 1000, 3300, 10 000 µg/plate	98.4	Negative	McGregor & Printice (1983)
Chromosomal aberration	Human lymphocytes	0, 5, 10, 20 µg/ml –S9; 0, 3, 5, 30 µg/ml +S9	99.5	Negative	Brooker et al. (1988)
Cell mutation	Mouse L5178Y <i>tk</i> ^{+/-} cells	0.06–33 µg/ml +S9; 0.06–20 µg/ml –S9	98.4	Negative	McGregor & Riach (1983a)
Unscheduled DNA synthesis	Human embryonic fibroblasts	20, 60, 100, 140, 180, 220, 260, 300 µg/ml ±S9	100	Negative	McGregor & Riach (1983b)
DNA damage	Chinese hamster V79 lung fibroblasts	0.01–0.3 mmol/L ±S9	NR	Negative	Petzold et al. (1977)
<i>In vivo</i>					
Reverse mutation (host-mediated, mouse)	<i>S. typhimurium</i> G46, TA1532 TA1964	0, 100, 200, 400 mg/kg bw, single dose	NR	Negative	Everest (1976); Wilcox (1976)
Dominant lethal mutation	Female CFLP mice	0, 12, 50 mg by intra-gastric intubation for 5 days	NR	Negative	Palmer & James (1977a)
Dominant lethal mutation	Male CFLP mice	0, 12, 50 mg by intra-gastric intubation for 5 days	NR	Negative	Palmer & James (1977b)

NR, not reported; S9, 9000 x g microsomal fraction from rodent liver

The rats were killed on day 21, and the uterine contents were examined. There were no deaths or clinical signs of toxicity. Food consumption, body-weight gain, average litter size, fetal viability, and the implantation index were not affected. At 12 mg/kg bw, fetal weight was lower than in the controls, and the calcification of the sternebrae was less advanced. The NOAEL for maternal toxicity was 12 mg/kg bw per day, and that for developmental toxicity was 3 mg/kg bw per day, on the basis of reduced fetal weight and reduced calcification of the sternebrae (Sutton, 1973c).

In another study of developmental toxicity, groups of 24 mated Sprague-Dawley rats weighing 215–280 g were given 0, 7.5, 15, or 30 mg/kg bw per day amitraz (purity, 99.7%) by gavage on days 6–15 of gestation. Immediately after mating, the females were assigned to treatment groups by a randomization process based on stratified body weight. Each female was then individually identified by ear notching. All females were examined once or twice daily for clinical signs of ill health, toxicity, or behavioural changes, and body weights and food intake were recorded on days 0, 6, 10, 15, and 20 of gestation. On day 20 of gestation, the females were killed and their uterine contents examined.

One control female was found dead on day 10 of gestation; there were no other deaths. The principal clinical sign was fur staining, which was slightly more frequent at 30 mg/kg bw per day. At this dose, a slight loss of body weight up to day 10 of gestation was followed by reduced body-weight gain at termination. The body-weight gain of rats at 15 mg/kg bw per day was slightly reduced (10%), but that of animals at 7.5 mg/kg bw per day was not adversely affected by treatment. Food intake of rats at 30 mg/kg bw per day, and to a lesser extent those at 15 mg/kg bw per day, was initially lower than in the control group; there was no effect on food intake at 7.5 mg/kg bw per day. The pregnancy rate was high in all groups, and no

adverse findings were made at necropsy. The treatment did not adversely affect the number of implantations, the incidence of post-implantation loss, or the number or sex ratio of fetuses. Animals at 30 mg/kg bw per day had a statistically significantly increased incidence of dilated ureters and increased bilateral renal pelvic cavitation. Although the incidence of the latter lesion at 15 mg/kg bw per day was statistically significantly higher than that of the concurrent controls ($p < 0.05$), the group percentage increase (5.7%) was comparable to the upper range of the relevant historical control values (5.4%). The NOAEL was 7.5 mg/kg bw per day for both maternal toxicity, on the basis of reduced body-weight gain, and for developmental toxicity, on the basis of increased incidences of dilated ureters and renal pelvic cavitation (McIntyre, 1987a).

Rabbits

In a study of developmental toxicity, groups of 8–10 New Zealand rabbits were treated with amitraz (purity unspecified) at a dose of 0, 1, 5, or 25 mg/kg bw per day on days 6–18 of pregnancy and were killed on day 30. At 25 mg/kg bw per day, the number of litters and mean litter size were decreased and abortions were observed. No increase in the incidence of congenital abnormalities was found. The NOAEL for maternal toxicity was 25 mg/kg bw per day, the highest dose tested, and the NOAEL for developmental toxicity was 5 mg/kg bw per day on the basis of a reduced number of litters and litter size (Sutton, 1973d).

In another study of developmental toxicity, groups of 16 mated female New Zealand white rabbits weighing 3–4 kg were given amitraz (purity, 99.7%) by gavage at a dose of 0, 3, 6, or 12 mg/kg bw per day on days 7–19 of gestation. All females were examined once or twice daily for clinical signs of ill health, toxicity, or behavioural changes, and body weight and food intake were recorded on days 0, 7, 13, 19, 24, and 28 of gestation. On day 28 of gestation, the surviving females were killed and their uterine contents examined.

One female at 12 mg/kg bw per day died, and three females at this dose were killed because of poor clinical condition or abortion. Two of the deaths were considered to be directly related to treatment. Two females at 6 mg/kg bw per day died; at 3 mg/kg bw per day, one female was killed and a further two died. Two control animals also died. Langour, polypnoea, and squinting were observed in all treated groups, and the incidence, severity, and duration of these signs appeared to be dose-related. At 12 mg/kg bw per day, body-weight loss followed by a reduction in body-weight gain were observed during dosing, but the weight gain was similar to that of controls on cessation of dosing. Treatment at 3 or 6 mg/kg bw per day had no effect on body weight. Food intake was reduced during dosing and up to day 24 at 12 mg/kg bw per day, but no adverse effect was seen at 6 or 3 mg/kg bw per day. No adverse effects were seen at necropsy. Three of the surviving females at 12 mg/kg bw per day lost their litters on day 28 of gestation. Litter parameters were not affected at 6 or 3 mg/kg bw per day, as assessed by the numbers of corpora lutea, implantation sites, and viable fetuses. The mean litter and fetal weights and sex ratio of fetuses were not adversely affected by treatment. No major fetal defects were recorded that were considered to be related to treatment, and there were no treatment-related effects on the incidence of minor or variant anomalies. There was no NOAEL for maternal toxicity because of deaths of animals at all doses and in the control group. The NOAEL for developmental toxicity was 6 mg/kg bw per day, on the basis of litter loss (McIntyre, 1987b).

(f) *Special studies*

(i) *Effects on the thymus and hormone concentrations*

Diets containing 400 ppm amitraz (purity, 97.1%), equal to 110 mg/kg bw per day for males and 150 mg/kg bw per day for females, were given to 24 male CFLP mice for up to 18 weeks and to 52 female CFLP mice for up to 33 weeks. A group of 36 males and 64 females given plain diet served as controls. Body weight and food consumption were measured regularly throughout the study, and the animals were examined for overt signs of toxicity at least once a week. Vaginal smears were monitored every morning during weeks 6–9, 15–18, 23–26, and

30–33. Twelve male and 12 female controls were killed during the first week, and 12 males and 12 females from both treated and control groups were killed after 9 and 18 weeks. The remaining animals were killed after 33 weeks. The β -estradiol concentration of the blood of female mice was estimated at weeks 9 and 18, and the thymuses from all animals were weighed at autopsy. Detailed histological examinations were conducted on a selected range of tissues.

Body-weight gain was reduced in amitraz-treated mice, to a slightly greater degree in the males, and food consumption was markedly increased, particularly in the females. Examination of vaginal smears indicated prolongation of estrus in treated mice, but there was no measurable effect on the circulating concentrations of β -estradiol. The thymus weights and histological appearance were not affected by treatment. Histopathological examination revealed two lymphoreticular tumours in the treated females and two in controls. In comparison with the controls, females given amitraz for 33 weeks had a higher incidence of foci of inflammatory cells in the liver (Brown et al. 1978).

(ii) *Effects on the estrus cycle and hormone concentrations*

Mice

The effects of amitraz on the estrus cycle and hormone concentrations were evaluated in groups of 70 female B6C3F₁ mice fed diets containing amitraz (purity, 98–100%) at 0, 25, 100, or 400 ppm, equivalent to 0, 3.8, 15, and 60 mg/kg bw per day, for up to 28 weeks. Permanently stained and mounted vaginal smears were prepared from each animal daily for 30 days after 13 weeks of treatment, and the stage of the oestrus cycle on each day was determined by microscopic examination of the cell population on each slide. Blood samples were taken at necropsy after overnight starvation and assayed for dehydroepiandrosterone sulfate, estradiol, progesterone, testosterone, lutropin, follitropin, prolactin, thyroxine, triiodothyronine, and thyroid hormone uptake, as indicators of hormonal status and routine clinical chemical parameters. The fresh liver weight was recorded at necropsy.

Pro-estrus was prolonged, and a trend towards reduced duration of diestrus was evident in animals at 400 ppm. The blood concentrations of progesterone were lower and the concentrations of dehydroepiandrosterone sulfate were higher in animals at 400 ppm and to a lesser degree in those at 100 ppm when compared with controls. The relative liver weights were increased at 400 (by 4%) and 100 ppm (by 5%). There were no treatment-related effects at 25 ppm. The NOAEL was 25 ppm, equivalent to 3.8 mg/kg bw per day, on the basis of lower blood concentrations of progesterone, higher concentrations of dehydroepiandrosterone sulfate, and increased relative liver weight (Hounsell & Rush, 1984).

Rats

Groups of 20 female 22-week-old Boots-Wistar rats were fed diets containing 0 or 200 ppm (equivalent to 0 and 12 mg/kg bw per day) amitraz (purity unspecified) for 18 weeks. Vaginal smears were taken routinely over 32 days. After fixation in methanol, the smears were stained with 1% aqueous methylene blue and examined for keratinized cells under 60 x magnification. Estrus was characterized by the presence of keratinized cells only, and the cycle length was taken as the interval between the first day of estrus in successive cycles.

The average cycle length of the controls was 4.3 days; two of the controls had prolonged periods in estrus (four and six days), and a third had a period of prolonged diestrus with a cycle lasting 18 days. In the remaining animals, the shortest cycles were two days and the longest six days. The low incidence of prolonged estrus among these animals confirms that the technique of smearing did not affect vaginal cytology. In the treated rats, the average cycle length was 6.1 days, and the range was 2–16 days. Six rats had one or more periods of prolonged diestrus; four of these had been acyclic in a preliminary test. Estrus lasted for three to eight consecutive days in seven rats, two of which had shown a similar tendency in a preliminary test. The second test showed that the cycle length was significantly altered by treatment, either estrus or diestrus being prolonged. Treated rats thus had longer oestrus cycles than controls, resulting from prolonged periods of estrus or diestrus (Merryman & Sutton, 1972).

(iii) *Mechanism of action*

The effects of amitraz and its major metabolite, *N*-methyl-*N'*-(2,4-xylyl)formamidine, given orally or intravenously on the cardiovascular system, pupil diameter, and the respiratory system were studied in conscious and anaesthetized rats, cats, and dogs. Both compounds caused a fall in blood pressure, sometimes preceded by hypertension and bradycardia. The threshold for the effect in conscious rats dosed orally with amitraz was 1 mg/kg bw. Amitraz also caused mydriasis, sedation, and a reduced respiratory rate. The bradycardia, sedation, and mydriatic effect was antagonized by the α 2-blocking agent yohimbine but not by the α 1-blocking agent prazosin, indicating that the effects were caused by stimulation of presynaptic α 2-adrenoceptors. The lethal effect of amitraz in mice and dogs has also been shown to be inhibited by yohimbine (Parkinson & Sim, 1970; Cullen & Reynoldson, 1983; Hsu & Kakuk, 1984; Moser & MacPhail, 1985; Hsu et al., 1986; Hovda & McManus, 1993).

Amitraz and *N*-methyl-*N'*-(2,4-xylyl)formamidine were tested for their ability to potentiate the pressor responses to tyramine in a pithed rat preparation, since a structurally related compound, chlordimeform, inhibited monoamine oxidase *in vitro* in rat liver homogenates and centrally *in vivo* through an effect on brain serotonin concentrations. These effects were obvious only with nearly toxic oral doses of 80 mg/kg bw amitraz and 40 mg/kg bw *N*-methyl-*N'*-(2,4-xylyl)formamidine (Parkinson, 1974).

Amitraz and chlordimeform were tested for their ability to inhibit prostaglandin synthesis after intraperitoneal administration in rats. Both compounds had antipyretic and antiinflammatory effects at doses of 5–80 mg/kg bw. They reduced yeast-induced fever, with potencies intermediate between those of indomethacin and aspirin, and antagonized carrageenan-induced swelling of the hind paw. They also inhibited the synthesis of prostaglandin E2 from arachidonic acid by bovine seminal vesicle microsomes. The potency of amitraz in this assay was the same as that of aspirin (Yim et al., 1978).

(g) *Studies on metabolites*(i) *Acute toxicity*

The acute toxicity of metabolites of amitraz (purity unspecified) has been investigated in several species (Table 3).

(ii) *Short-term toxicity**Rats*

Groups of 10 male and 10 female newly-weaned Boots-Wistar rats were dosed by gastric intubation with *N*-methyl-*N'*-(2,4-xylyl)formamidine (purity unspecified) for 90 days at 0,

Table 3. Acute toxicity of metabolites of amitraz

Species	Route	LD ₅₀ or LC ₅₀ (mg/kg bw or mg/L air)	Reference
<i>N</i>-Methyl-<i>N'</i>-(2,4-xylyl)formamidine			
Mouse	Oral	150	Sutton (1970a)
Rat	Oral	200	Sutton (1970b)
Dog	Oral	>20	Morgan (1973); Morgan & Williams (1974)
<i>4</i>-Amino-meta-toluic acid			
Mouse	Oral	>1600	Shaw & Williams (1973a)
Rat	Oral	>1600	Shaw & Williams (1973b)
<i>Form</i>-2',4'-xylylide			
Rat	Oral	1600	Shaw (1973b)

0.25, 1, 3, or 12 mg/kg bw per day. Body weights were recorded three times per week, and the rats were observed each day for signs of toxicity. At 3 mg/kg bw per day, an initial reduction in body-weight gain was seen in males. At 12 mg/kg bw per day, the rats became nervous and difficult to handle, and two deaths occurred. The growth rate was reduced in animals of each sex but to a greater extent in the males. At the end of experiment, haemoglobin and haematocrit values were decreased in males, and the number of erythrocytes was decreased in females. Slight biochemical changes were seen. At autopsy, the weights of the adrenals, ovaries, uterus, and liver in females and spleen and testes in males were increased, but the only histopathological changes were a slight increase in lymphoid infiltration, some sinusoidal leukocytosis, and loss of glycogen in the livers of most males and slight cellular accumulations in the hearts of some females. The NOAEL was 1 mg/kg bw per day on the basis of the reduced body-weight gain and increased organ weights (Shaw & Williams, 1975).

Groups of five male and five female Wistar rats were dosed by gastric intubation with 4-amino-*meta*-toluic acid (purity unspecified) at a dose of 0, 40, 100, or 250 mg/kg bw per day for 21 days. At 250 mg/kg bw per day, slight decreases in weight gain and in blood urea nitrogen concentration were observed in males and an increased relative weight of the spleen in females. No gross pathological changes due to treatment were found. The NOAEL was 250 mg/kg bw per day, the highest dose tested (Shaw, 1975).

Dogs

Groups of four male and four female beagles were given gelatin capsules containing *N*-methyl-*N'*-(2,4-xylyl)formamidine (purity unspecified) as free base diluted in lactose to 1% at a dose of 0, 0.1, 0.25, or 1 mg/kg bw per day for 90 days. Clinical signs were recorded daily, food consumption twice daily, and body weight once a week. Ophthalmoscopic examination and recordings of temperature and heart rate were carried out before dosing and after 6 and 12 weeks. Haematological, clinical chemical, and urinary analyses were performed, and gross and histopathological examinations were carried out.

At 0.25 and 1 mg/kg bw per day, abnormal quietness and drowsiness and significantly lower body temperature (by up to 16.2 °C) were observed 0.5–4 h after dosing. At 1 mg/kg bw per day, the heart rate was significantly reduced (by up to 40 beats/min) 1–2 h after dosing, and the liver weight and urine volume were increased. A slight reduction in thymus weight was observed at 0.25 and 1 mg/kg bw per day. No histopathological anomalies were found. The NOAEL was 0.1 mg/kg bw per day on the basis of central nervous system depression and lowered body temperature (Chesterman et al., 1973).

Groups of four male and four female beagles were given gelatin capsules containing 4-amino-*meta*-toluic acid (purity unspecified) at a dose of 0, 16, 40, or 100 mg/kg bw per day for 90 days. Slightly increased urinary concentrations of total reducing substances other than glucose were found at the highest dose. No dose-related effects were observed on behaviour, electrocardiogram, heart rate, rectal temperature, body weight, food consumption, haematological or blood chemical parameters, organ weights, or histopathological appearance (Morgan et al., 1974).

(iii) *Genotoxicity*

The results of tests for the genotoxicity of potential metabolites of amitraz are summarized in Table 4. Except for a single positive response to 2,4-dimethylaniline in the assay for forward mutation in mouse lymphoma cells, in the presence of metabolic activation, the results of the tests *in vitro* and *in vivo* were negative.

3. Observations in humans

In a double-blind, randomized cross-over study, six healthy male volunteers aged 18–45 years and weighing 60–70 kg received sequential single oral doses of 0, 0.063, and

Table 4. Results of tests for the genotoxicity of metabolites of amitraz

End-point	Test system	Concentration or dose	Purity (%)	Results	Reference	
<i>In vitro</i>						
Reverse mutation	<i>N-Methyl-N'-(2,4-xylyl)formamidine</i> <i>S. typhimurium</i> TA98, TA100, TA1535, TA1337, TA1538		≤ 5000 µg/plate	NR	Negative	Richold et al. (1983a)
	DNA damage	Chinese hamster V79 lung fibroblasts	0.03–3.0 mmol/L ±S9	NR	Negative	Petzold et al. (1977)
Reverse mutation	<i>Form-2',4'-xylidide</i> <i>S. typhimurium</i> TA98, TA100, TA1535, TA1337, TA1538		≤ 5000 µg/plate	NR	Negative	Richold et al. (1983b)
	DNA damage	Chinese hamster V79 lung fibroblasts	0.01–1.0 mmol/L ±S9	NR	Negative	Petzold et al. (1977)
Forward mutation	<i>Product of acid hydrolysis of N-methyl-N'-(2,4-xylyl)formamidine and form-2',4'-xylidide</i> Mouse L5178Y <i>tk</i> ^{+/−} lymphoma cells		1, 3, 3, 10, 33, 100, 200, 300, 333, 400, 500, 600 µg/ml	NR	Positive + S9 Negative −S9	McGregor & Riach (1984)
	Cell transformation	C3H/10 T1/2 clone 8 mouse embryo fibroblasts	5, 10, 20 µg/ml +S9 100, 200, 400 µg/ml −S9	NR	Negative	McGregoret al. (1984)
DNA damage	Chinese hamster V79 lung fibroblasts	0.03–2.0 mmol/L ±S9	NR	Negative	Petzold et al. (1977)	
DNA damage	<i>4-Amino-meta-toluic acid</i> Chinese hamster V79 lung fibroblasts	0.03–3.0 mmol/L ±S9	NR	Negative	Petzold et al. (1977)	
<i>In vivo</i>						
Micronucleus formation	Mouse bone marrow	56, 113, 225 mg/kg bw twice, 24 h apart	NR	Negative	Hounsell & Walker (1983)	

0.13 mg/kg bw amitraz, two to three weeks apart. Each dose was given with 150 ml water, 30 min after breakfast. Pulse rate, respiration rate, blood pressure, and temperature were measured at −1, −0.5, 1, 3, 6, 12, 24, and 36 h, and electrocardiograms were performed at −1, 1, 3, 6, 12, 24, and 36 h with respect to dosing. Pupil responsiveness and psychomotor performance were evaluated before treatment and at 2.5 and 8 h. Urine was collected at 0–36 h and 36–60 h. There were no clinically significant changes in vital signs or electrocardiographic parameters. Moreover, haematological, blood chemical, and urinary parameters, pupil responsiveness, and psychomotor performance were unaffected by treatment. The NOAEL was 0.13 mg/kg bw, the highest dose tested (Cass, 1992).

Two human volunteers who received a single oral dose of 0.25 mg/kg bw ¹⁴C-amitraz showed drowsiness, disorientation, slurred speech, decreased pulse rate and blood pressure, and other effects (Campbell & Needham, 1984c).

In a double-blind cross-over study, four male and two female volunteers, aged 21–42 years and in normal health, received two doses of 2 mg (about 0.03 mg/kg bw) of the amitraz metabolite, *N*-(2,4-dimethylphenyl)-*N'*-methylformamidine, one week apart in a capsule with 100 ml of water, or placebo. Blood pressure, pulse rate, and temperature were measured at half-hourly intervals over 7 h, and mental alertness was assessed at 0, 3, and 7 h. An electrocardiograph was performed, and urine was collected before dosing and at 0–7 h and 7–24 h. The urine samples were analysed to estimate the amount of the metabolite, 3-methyl-4-aminobenzoic acid, that had been excreted. Mental alertness was estimated at 0, 3, and 7 h.

No difference was seen from those receiving the placebo. The NOAEL was 0.03 mg/kg bw, the only dose tested (Hall et al., 1975).

Symptoms of central nervous system depression ranging from sedation to coma lasting more than 24 h, depressed respiration, hypotension, and bradycardia have been described in 11 reports after accidental or intentional ingestion of uncertain amounts of amitraz. In nine cases, recovery was complete after symptomatic and supportive treatment (Groppi, 1977; Ros & Aken, 1994; Pronczuk et al., 1995).

Comments

Amitraz was well absorbed, extensively metabolized, and rapidly excreted, mainly in the urine, after oral administration to mice, rats, dogs, pigs, hens, cows, baboons, and humans. After oral treatment of mice with ^{14}C -amitraz, 86% of the radiolabelled dose was excreted, 62% in the urine, within the first 24 h. All of it had been excreted by 96 h, with 73% in the urine of animals of each sex. The concentrations of residues were highest in liver, adrenal glands, and eyes. After oral administration of ^{14}C -amitraz to rats, 94% of the dose was recovered within three days, with 82% in urine and 12% in faeces. After oral administration of ^{14}C -amitraz to two humans, 77–87% was recovered within three days. Amitraz is hydrolysed to two components, *N*-methyl-*N*-(2,4-xylyl)formamidine and form-2',4'-xylylide. The former is the pharmacologically active compound and accounted for 5–30% of the total urinary excretion in mice and rats; it was further metabolized to 4-amino-*meta*-toluic acid and the acetyl and formyl conjugates, 4-acetamido- and 4-formamido-*meta*-toluic acids. These five metabolites were also found in plants.

Amitraz has low acute oral toxicity in rats but is more toxic in dogs. The LD_{50} values ranged from 100 mg/kg bw in dogs to > 1600 mg/kg bw in mice, indicating that dogs are more sensitive. The toxic signs after oral administration to mice and rats were hyperexcitability, ataxia, tremor, and ptosis. Amitraz had no sensitizing potential in guinea-pigs, and no local irritation was found in rabbits after a single application to skin or eyes. There was evidence of delayed contact hypersensitivity after application of amitraz either topically or intradermally.

WHO (1996) has classified amitraz as slightly hazardous.

In a 13-week study in which mice were fed diets providing 0, 100, 200, 400, 600, or 800 ppm, the NOAEL was 100 ppm, equal to 17 mg/kg bw per day, on the basis of reduced overall body-weight gain (by 34%).

In a 90-day study, rats were given amitraz at doses of 0, 3, or 12 mg/kg bw per day by gavage. The NOAEL was 3 mg/kg bw per day, on the basis of reduced terminal body-weight gain, absolute liver weight, and relative liver weight.

In a 90-day study in dogs, amitraz was administered at doses of 0, 0.25, 1, or 4 mg/kg per day in gelatin capsules. The NOAEL was 0.25 mg/kg bw per day, on the basis of central nervous system depression and reductions in rectal temperature and pulse rate.

In a two-year study, dogs were given amitraz at doses of 0, 0.1, 0.25, or 1 mg/kg bw per day in gelatin capsules. The NOAEL was 0.25 mg/kg bw per day, on the basis of central nervous system depression.

In two 90-day studies, the amitraz metabolite *N*-methyl-*N*-(2,4-xylyl)formamidine was administered to rats at doses of 0, 0.25, 1, 3, or 12 mg/kg bw per day by gastric intubation or to dogs at 0, 0.1, 0.25, or 1 mg/kg bw per day by gelatine capsules. The NOAEL was 1 mg/kg bw per day in rats, on the basis of reduced body-weight gain and increased organ weights, and 0.1 mg/kg bw per day in dogs, on the basis of central nervous system depression and lowered body temperature.

In a 21-day study in rats and a 90-day study in dogs, the amitraz metabolite 4-amino-*meta*-toluic acid was given at doses of 0, 40, 100, or 250 mg/kg bw per day by gastric intubation to rats or 0, 16, 40, or 100 mg/kg bw per day by gelatin capsules to dogs. The NOAEL was 250 mg/kg bw per day in rats and 100 mg/kg bw per day in dogs, (the highest doses tested).

In an 80-week study, mice were fed diets containing 0, 25, 100, or 400 ppm, equivalent to

0, 3.8, 15, and 60 mg/kg bw per day (wrongly given as 25, 100, or 400 mg/kg bw per day in the 1980 JMPR report). The NOAEL for carcinogenicity was 100 ppm, equivalent to 15 mg/kg bw per day, on the basis of an increased incidence of lymphoreticular tumours in females at 400 ppm.

In a two-year study in which mice were fed diets providing 0, 25, 100, or 400 ppm, the NOAEL for carcinogenicity was 100 ppm, equal to 11 mg/kg bw per day, on the basis of the occurrence of hepatocellular carcinoma in females at 400 ppm. This dose was considered to be greater than the conventional maximum tolerated dose. The NOAEL for toxicity was 25 ppm, equal to 2.3 mg/kg bw per day, on the basis of generalized toxic effects.

In a two-year study, rats were fed diets containing 0, 15, 50, or 200 ppm. The NOAEL for toxicity was 50 ppm, equal to 2.5 mg/kg bw per day, on the basis of effects on the central nervous system and reduced overall body-weight gain in males. There was no evidence of carcinogenicity.

The genotoxic potential of amitraz has been adequately evaluated in a range of assays *in vitro* and *in vivo*. The Meeting concluded that amitraz is not genotoxic.

In view of the lack of genotoxicity and the finding of tumours only in mice and only at concentrations at which severe toxicity was observed, the Meeting concluded that amitraz is not likely to pose a carcinogenic risk to humans.

In a three-generation study of reproductive toxicity in rats at dietary concentrations of 0, 15, 50, or 200 ppm, the NOAEL was 50 ppm, equal to 4.4 mg/kg bw per day, for maternal toxicity and 15 ppm, equal to 1.3 mg/kg bw per day for developmental toxicity. No teratogenic effect was observed.

In two studies of developmental toxicity, pregnant rats were given amitraz at 0, 1, 3, or 12 mg/kg bw per day by gavage on days 8–20 of gestation or 0, 7.5, 15 or 30 mg/kg bw per day by gavage on days 6–15. The NOAEL for maternal toxicity was 7.5–12 mg/kg bw per day, and that for developmental toxicity was 3–7.5 mg/kg bw per day.

In two studies of developmental toxicity in rabbits, amitraz was given at doses of 0, 1, 5, or 25 mg/kg bw per day by gavage on days 6–18 of gestation or 0, 3, 6, or 12 mg/kg bw per day by gavage on days 7–19. The NOAEL for maternal toxicity was 25 mg/kg bw per day in one study, but a NOAEL was not identified in the other because all of the treated animals died. The NOAEL for developmental toxicity was 3–6 mg/kg bw per day.

The effect of amitraz on the estrus cycle and hormone levels was evaluated in mice fed diets containing 0, 25, 100, or 400 ppm for 28 weeks and in rats fed diets containing 0 or 200 ppm for 18 weeks. In mice, pro-estrus was prolonged at 400 ppm; blood levels of progesterone were reduced and those of dehydroepiandrosterone were increased at 100 and 400 ppm. The NOAEL was 25 ppm, equivalent to 3.8 mg/kg bw per day, on the basis of the changed hormone levels. The estrus cycles were longer in treated than in control rats, with no NOAEL.

In a double-blind, randomized, cross-over study of tolerance, six healthy adult male volunteers received sequential single oral doses of 0, 0.063, and 0.13 mg/kg bw amitraz, two to three weeks apart. The NOAEL was 0.13 mg/kg bw, the highest dose tested.

Two human volunteers who received single oral doses of 0.25 mg/kg bw ¹⁴C-amitraz showed effects including drowsiness, disorientation, slurred speech, and decreased pulse rate and blood pressure.

In a double-blind cross-over study of tolerance, six adult volunteers received two single doses of placebo or 2 mg (about 0.03 mg/kg bw) of the amitraz metabolite *N*-methyl-*N*-(2,4-xylyl)formamidine one week apart. The NOAEL was 0.03 mg/kg bw, the only dose tested.

The Meeting established an ADI of 0–0.01 mg/kg bw on the basis of the NOAEL of 1.3 mg/kg bw per day in the study of reproductive toxicity in rats and a safety factor of 100. The pharmacological effects on the central nervous system seen in dogs, with a NOAEL of 0.25 mg/kg bw per day, were considered not to be relevant for setting the ADI because they were reversible and the dogs became tolerant. Moreover, a NOAEL of 0.13 mg/kg bw per day was seen for such effects in humans.

The Meeting established an acute RfD of 0.01 mg/kg bw, on the basis of the NOAEL of 0.13 mg/kg bw per day in the study in humans and a safety factor of 10.

Toxicological evaluation

Levels that cause no toxic effect

Mouse:	25 ppm, equal to 2.3 mg/kg bw per day (toxicity in a two-year study of carcinogenicity)
Rat:	3 mg/kg bw per day (toxicity in a 90-day study of toxicity) 50 ppm, equal to 2.5 mg/kg bw per day (toxicity in a two-year study of toxicity and carcinogenicity) 50 ppm, equal to 4.4 mg/kg bw per day (maternal toxicity in a three-generation study of reproductive toxicity) 15 ppm, equal to 1.3 mg/kg bw per day (developmental toxicity in a three-generation study of reproductive toxicity) 12 mg/kg bw per day (maternal toxicity in a study of developmental toxicity) 3 mg/kg bw per day (developmental toxicity)
Rabbit:	25 mg/kg bw per day (maternal toxicity in a study of developmental toxicity) 5 mg/kg bw per day (developmental toxicity)
Dog:	0.25 mg/kg bw per day (toxicity in a two-year study of toxicity)
Human:	0.13 mg/kg bw (toxicity after single oral doses)

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of acute reference dose

0.01 mg/kg bw

Studies that would provide information useful for continued evaluation of the compound

1. Studies to further characterize the effects on the reproductive system of female rodents
2. Further observations in humans

List of end-points relevant for setting guidance values for dietary and non-dietary exposure

Absorption, distribution, excretion and metabolism in mammals

Rate and extent of absorption	Rapid/complete
Distribution	Liver, adrenals, eyes
Potential for accumulation	Minimal
Rate and extent of excretion	Rapid/complete, 80–100% in 96 h
Metabolism in animals	Metabolites same in rodents, dogs, humans
Toxicologically significant compounds (animals, plants, and environment)	<i>N</i> -Methyl- <i>N'</i> -(2,4-xylyl)formamidine

Acute toxicity

Rat: LD ₅₀ oral	600 mg/kg bw
Rabbit: LD ₅₀ dermal	> 200 mg/kg bw
Rat: LC ₅₀ inhalation	65 mg/L
Skin irritation	Not irritating
Eye irritation	Not irritating
Skin sensitization	Not sensitizing (Buehler test)

<i>Short-term toxicity</i>			
Target/critical effect		Central nervous system depression, dog	
Lowest relevant oral NOAEL		0.25 mg/kg bw per day	
Lowest relevant dermal NOAEL		Rabbit: 50 mg/kg bw per day	
Lowest relevant inhalation NOAEL		Rat: 0.01 mg/L air	
<i>Genotoxicity</i>		Unlikely to be genotoxic	
<i>Long-term toxicity and carcinogenicity</i>			
Target/critical effect		Lymphoreticular tumours, hepatocellular carcinomas	
Lowest relevant NOAEL		Mouse: 11 mg/kg bw per day (80-week and 2-year studies)	
Carcinogenicity		Unlikely to be carcinogenic	
<i>Reproductive toxicity</i>			
Reproduction target/critical effect		Decrease in number of young alive at 21 days	
Lowest relevant reproductive NOAEL		Rat: 1.3 mg/kg bw per day (developmental toxicity)	
Developmental target/critical effect		Reduced fetal weight	
Lowest relevant developmental NOAEL		Rat: 3 mg/kg bw per day (developmental toxicity)	
<i>Neurotoxicity/Delayed neurotoxicity</i>		Acute central nervous system depression	
<i>Other toxicological studies</i>		Prolongation of estrus cycles and reduction of blood concentration of progesterone (mouse, rat)	
<i>Medical data</i>		Central nervous system depression in two volunteers after a single oral dose of 0.25 mg/kg bw	
Summary	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Reproductive toxicity, rat	100
Acute reference dose	0.01 mg/kg bw	Single oral dose in six volunteers	10

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