TRIAZOLE FUNGICIDE METABOLITES

(1,2,4-TRIAZOLE; TRIAZOLE ALANINE; TRIAZOLE ACETIC ACID)

First draft prepared by
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Explanation

1,2,4-Triazole, triazole alanine, triazole acetic acid, triazole pyruvic acid and triazole lactic acid are the common metabolites derived from triazole-containing fungicides that act by inhibiting sterol synthesis. The levels of triazole pyruvic acid and triazole lactic acid found in metabolism studies are low, and no toxicological data on these compounds were available, therefore, they were not considered by the present Meeting.

1,2,4-Triazole, triazole alanine and triazole acetic acid are the commonly used names for IUPAC nomenclatures 1*H*-1,2,4-triazole (CAS No. 288-88-01), 1,2,4-triazolyl-3-alanine (CAS No. 10109-05-4), and 1*H*-1,2,4-triazol-l-ylacetic acid (CAS No. 28711-29-7), respectively. These three metabolites commonly occur as plant or soil metabolites and are collectively known as the "triazole derivative metabolites". Triazole alanine and triazole acetic acid residues are primarily associated with plant commodities, while 1,2,4-triazole is mainly associated with animal commodities, lesser amounts of this compound being found in plant commodities. 1,2,4-Triazole is found in studies of the

metabolism of triazole fungicides in rats, where it may constitute approximately 1–65% of the dose, depending on the parent compound administered.

Triazole alanine was first evaluated by the JMPR in 1989. The Meeting concluded from the available data at that time that residues of triazole alanine arising from the use of triazole fungicides do not present a toxicological hazard. The Meeting has not previously evaluated 1,2,4-triazole and triazole acetic acid. These compounds were reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues (CCPR) and following recommendations made by the JMPR in 2007 (*General consideration 2.3*). A group of manufacturers of these pesticides have formed a taskforce known as the "Triazole Derivative Metabolite Group" (TDMG) and made a joint submission of toxicological data to the JMPR. All pivotal studies with triazole alanine and triazole acetic acid were certified as complying with good laboratory practice (GLP), unless otherwise stated in the toxicological monograph.

The toxicological database for 1,2,4-triazole was sufficient for the evaluation of this compound, while the toxicological databases for triazole alanine and triazole acetic acid were more limited. The Meeting concluded that adequate studies were available to establish an acceptable daily intake (ADI) for 1,2,4-triazole and a group ADI for triazole alanine and triazole acetic acid. This decision was based on the following considerations:

— The chemical structures of triazole alanine and triazole acetic acid are closely related and the two substances have similar physicochemical characteristics.

— Both triazole alanine and triazole acetic acid have the 1,2,4-triazole active (protonated) nitrogen bonded to carbon, which significantly reduces the toxicity of triazole alanine and triazole acetic acid.

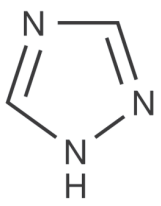
— The available toxicological data suggest that triazole alanine and triazole acetic acid are less toxic than 1,2,4-triazole.

— Triazole alanine and triazole acetic acid have similar toxicokinetic profiles in that they are rapidly eliminated, primarily in the urine and mostly as the parent compound.

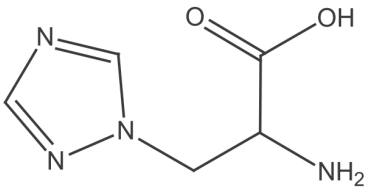
The Meeting recommended that the ADI and acute reference dose (ARfD) values established for these triazole metabolites may be used in risk assessment on a case-by-case basis, depending on the residue and toxicity profile of the parent compound. The Meeting also noted that these values may also be useful in a combined risk assessment, depending on the exposure situation, including whether exposure to these metabolites comes from more than one source of the parent conazoles.

The data for 1,2,4-triazole, triazole alanine and triazole acetic acid are described in the present monograph.

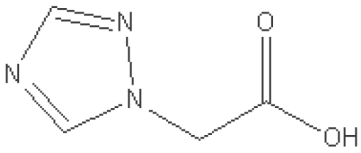
Figure 1. Chemical structures of 1,2,4-triazole, triazole alanine and triazole acetic acid



1,2,4-Triazole



Triazole alanine



Triazole acetic acid

1,2,4-TRIAZOLE

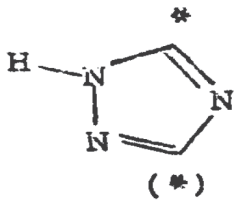
Evaluation for acceptable daily intake

Unless otherwise stated, studies evaluated in this monograph were performed by GLP-certified laboratories and complied with the relevant Organisation for Economic Cooperation and Development (OECD) and/or United States Environmental Protection Agency (US EPA) test guideline(s).

1. Biochemical aspects

1.1 Absorption, distribution, and excretion

Figure 2. Position of radiolabel on 1,2,4-triazole



* Indicates position of radiolabel.

Rats

In a pharmacokinetic study, groups of two male and two female Sprague-Dawley rats were given a single dose of [^{14}C]-labelled 1,2,4-triazole (radiolabelled at positions 3 and 5 of the triazole ring; purity, > 98%) at 0.4, 48.8, or 865.7 mg/kg bw by gavage in deionized water. Treated rats were individually housed in stainless steel metabolism cages. Urine and faeces were collected daily for 7 days, after which the rats were killed. Selected tissues and blood samples were collected and analysed for radioactivity. The study was not conducted in accordance with GLP.

Recovery of total radiolabel was nearly complete (100.2–102.8%); [^{14}C]-ring labelled 1,2,4-triazole was readily absorbed and most of the radiolabel was excreted within 24 h (Table 1). Urine was the predominant pathway of excretion. Excretion of radiolabel in the urine of male rats during 7 days was 93.5%, 80% and 87.6% at the lowest, intermediate and highest dose, respectively. In female rats, urinary excretion of radiolabel in 7 days was 90.6%, 92.4% and 91.9% at the lowest, intermediate and highest dose, respectively. Total excretion of radiolabel in the faeces during 7 days ranged from 6.5% to 19.9% (average, 10.4%) in male and female rats at the doses tested. Absorption was nearly complete based on urinary excretion during 7 days. Most of the absorption occurred within 48 h. Absorption was not saturated at the highest dose tested. The excretion pattern did not exhibit sex-related variability. Total radiolabel in tissues ranged from 0.6% to 1.6% of the administered dose at the doses tested, indicating that 1,2,4-triazole and its metabolites do not undergo significant sequestration (Lai & Simoneaux, 1986).

In a separate pharmacokinetic study, groups of male Sprague-Dawley rats were given a single dose of [^{14}C]-labelled 1,2,4-triazole (radiolabelled at positions 3 and 5 of the triazole ring; purity, 97%) in physiological saline solution. The absorption, distribution, and elimination of radiolabel were studied in groups of five rats that received a single oral dose at 1.0 mg/kg bw by stomach tube or single intravenous doses of 0.1, 1.0, 10.0 or 100 mg/kg bw, and in groups of four male Sprague-Dawley rats with cannulated bile ducts that received an intravenous or intraduodenal dose of 1.0 mg/kg bw. Urine and faeces were collected up to 48 h after dosing. Bile was collected up to 24 h after dosing. Elimination of radiolabel in expired air was also measured. Blood and a number of organs and tissues were collected for analysis from groups of five male Sprague-Dawley rats up to 6 days after a single intravenous dose of 1.0 mg/kg bw. Urine, faeces, bile, blood, tissues and expired air were analysed for radiolabel. These studies were not conducted in accordance with GLP.

Approximately 0.1% of the administered dose was detected in expired air after oral and intravenous administration in 30 h. The main route of excretion after intravenous or oral administration was the urine (92–94% after 48 h), irrespective of dose or route of administration (Table 2). Approximately 3–5% of the administered dose was recovered in the faeces within 48 h after administration by oral

Table 1. Excretion of radiolabel by rats given a single oral dose of [^{14}C]1,2,4-triazole[a]

Sample	Radiolabel (% of total radioactivity)					
	Dose (mg/kg bw)					
	0.4		48.8		865.7	
	Males	Females	Males	Females	Males	Females
Urine, day 1	81.0	78.3	65.2	69.7	43.7	46.9
Urine, day 2	10.2	10.5	11.7	19.2	36.5	38.5
Urine, days 3–7	2.3	1.8	3.1	3.5	7.4	6.5
Urine, subtotal	93.5	90.6	80.0	92.4	87.6	91.9
Faeces, day 1	7.4	6.5	17.9	9.0	3.2	5.9
Faeces, day 2	1.0	0.6	1.5	0.9	2.0	2.8
Faeces, days 3–7	0.3	0.3	0.5	0.5	1.3	0.5
Faeces, subtotal	8.7	7.4	19.9	10.4	6.5	9.2
Tissue residues	0.8	0.6	0.8	0.9	1.6	1.3
Cage wash	0.0	0.5	0.3	0.8	1.0	1.2
Total recovery	103.0	99.1	101.0	104.5	96.7	103.6

From Lai & Simoneaux (1986)

[a] Group size, $n = 2$.

and intravenous routes. Approximately 2% of the administered dose was recovered in the gastrointestinal tract at 48 h. Based on urinary and faecal excretion, the oral absorption and excretion was very rapid. The bioavailability of the oral dose was virtually 100%. In bile duct-cannulated rats, approximately 12% of the administered dose was recovered in the bile at 24 h after intravenous or intraduodenal administration. About 60–65% of the administered dose was eliminated via the urine, and 3.5–4% via the faeces. Approximately 14–18% of the administered dose was recovered in tissues and 6–9% in the gastrointestinal tract of the bile duct-cannulated rats within 24 h. Thirty minutes after an intravenous dose of 1 mg/kg bw, almost 100% of the administered dose was detectable in the body, while the body minus gastrointestinal tract contained about 90% of the administered dose. The concentration of radiolabel in the body declined to 55% of the administered dose at 8 h after intravenous administration, and to about 1.9% of the administered dose at 3 days. The radiolabel was largely uniformly distributed in the rat's body: the highest concentrations at 30 min after administration were measured in muscle and lung (1.2 µg/g), the lowest in renal fat (0.48 µg/g). The decline of radiolabel in plasma and in most tissues was approximately monoexponential up to about day 3 after administration, with an elimination half-life of about 12 h. The concentrations of radiolabel in the body minus gastrointestinal tract and in selected tissues were very low at 6 days after administration. The observed concentrations were at or below the limits of quantitation of 2–7 ng/g. Autoradiography of rats dosed at 1 mg/kg bw via intravenous administration indicates that the radioactivity was detectable in all tissues and organs with the exception of compact body tissues at 5 min (Weber et al., 1978).

1.2 Metabolism

In a separate study of metabolism, 10 male Sprague-Dawley rats were given [^{14}C]-labelled 1,2,4-triazole (radiolabelled at position 3 and 5 of the triazole ring; purity, > 99%) as a single dose at 10 mg/kg bw by gavage in physiological saline solution (0.9% sodium chloride). Urine samples were collected at 0–8 h and 8–24 h. Only urine samples were collected for identification of metabolites since previous studies have shown that greater than 90% of the orally administered dose of 1,2,4-triazole was excreted in the urine in 24 h. Urinary metabolites were separated by three different solvent systems using thin-layer chromatography (TLC). The TLC separations were visualized by extinction of fluorescence induced by ultraviolet (UV) light (250 nm), development of autoradiograms, and a TLC scanner. Radioactivity of the samples was determined by means of liquid scintillation spectrometry. The verification of 1,2,4-triazole, the major urinary elimination product, was done by "reverse isotope dilution analysis" in which 500 mg of unlabelled 1,2,4-triazole was added to a 10 ml sample of urine

Table 2. Excretion of radiolabel by male rats at 48 h after a single oral or intravenous dose of [^{14}C]1,2,4-triazole

Sample	Radiolabel (% of total radioactivity)				
	Dose (mg/kg bw)				
	Intravenous				Oral
	0.1	1	10	100	1
Urine	93.9	92.6	92.1	93.9	91.9
Faeces	3.9	5.0	5.0	3.6	5.4
Total elimination	97.8	97.6	97.1	97.5	97.3
Tissue residues	1.7	2.1	2.4	2.0	2.2
Gastrointestinal tract	0.51	0.44	0.51	0.47	0.47

From Weber et al. (1978)
[a] Group size, $n = 5$.

(collected at 0–8 h) and specific radioactivity was checked after further clean-up steps (12 ethyl acetate extractions and three recrystallizations). This study was not conducted in accordance with GLP.

The TLC chromatographs showed only one major zone (95.3%) representing the parent compound. There were three other zones representing less than 2.8% of the urinary radioactivity. The absence of a change in specific radioactivity in the reverse isotope dilution analysis confirmed the identity of the elimination product (Ecker, 1980).

2. Toxicological studies

(a) Acute toxicity

The acute toxicity of 1,2,4-triazole is summarized in Table 3.

Rats

Groups of three male Crl:CD BD rats were given a single dose of 1,2,4-triazole (purity, 92.8%) at 500 or 5000 mg/kg bw by gavage in 0.5% methyl cellulose. Treated rats were subjected to gross necropsy at the end of a 14-day observation period. Body weights were recorded at initiation and at the end of the study. All rats at 5000 mg/kg bw died within 10 min after dosing. No treatment-related clinical signs were observed at 500 or 5000 mg/kg bw. Gross necropsy of decedents revealed reddened duodenum and reddened glandular portion of the stomach. There was no mortality at 500 mg/kg bw. No treatment-related necropsy findings were observed at termination in the group at 500 mg/kg bw. There were no apparent effects on the body weights of survivors. The oral median lethal dose

Table 3. Acute toxicity of 1,2,4-triazole

Species	Strain	Sex	Route	LD50 (mg/kg bw)	LC50 (mg/l air)	Reference
Rat	Crl:CD BR	Males and females	Oral	> 500 (males) < 5000 (females)	—	Procopio & Hamilton (1992)
	Wistar II albino	Males and females		1650 (males) 1648 (females)	—	Thyssen & Kimmerle (1976)
Rat	Wistar II albino	Males and females	Dermal	4200 (males) 3129 (females)	—	Thyssen & Kimmerle (1976)
Rabbit	New Zealand White	Males and females		>200 (males) < 2000 (females)	—	Procopio & Hamilton (1992)
Rat	Wistar II albino	Male	Inhalation (4 h)	—	Exposure not demonstrated	Thyssen & Kimmerle (1976)
Mice	NMRI	Male	Inhalation (6 h)	—	Exposure not demonstrated	Thyssen & Kimmerle (1976)
Rabbits	New Zealand White	Male	Dermal irritation	Slightly irritating	—	Procopio & Hamilton (1992)
Rabbits	New Zealand White	Not reported	Dermal irritation	Not irritating	—	Thyssen & Kimmerle (1976)
Rabbits	New Zealand White	Male	Ocular irritation	Severely irritating	—	Procopio & Hamilton (1992)
Rabbits	New Zealand White	Not reported	Ocular irritation	Severely irritating	—	Thyssen & Kimmerle (1976)
Guinea-pig	Crl:(HA)BRDunkin Hartley	Male	Dermal sensitization (maximization test)	Not sensitizing	—	Frosch (1998)

(LD_{50}) in rats was greater than 500 mg/kg bw but less than 5000 mg/kg bw (Procopio & Hamilton, 1992).

In a second study, groups of 15 male and 15 female Wistar II albino rats were given a single dose of 1,2,4-triazole (purity, technical grade) at 100, 250, 500, 1000, 1250, 1500, 1750, 1850, 2000 or 2500 mg/kg bw by gavage. The test material was emulsified in distilled water and Cremophor EL in a volume of 10 ml/kg bw. Treated rats were observed for 14 days. Treated rats were subjected to gross necropsy at the end of a 14-day observation period.

Mortality was observed at doses of 1250 mg/kg bw or higher (1 h to 12 days after dosing). The following clinical signs were observed: sedation, breathing disorders, reduction in general well-being, lying in abdominal or side position (at higher doses). The symptoms appeared within an hour of administration and were observed for a maximum of up to 13 days after dosing. No treatment-related necropsy findings were observed at termination. The oral LD_{50} of 1,2,4-triazole in rats was 1650 and 1648 mg/kg bw for males and females, respectively (Thyssen & Kimmerle, 1976).

(b) Dermal administration

Rats

1,2,4-Triazole (purity, technical grade) at doses of 1000, 2000, 2500, 3500, 4000 or 5000 mg/kg bw was applied to the shaved skin of groups of 5–20 male and 5–20 female Wistar-II rats. The test substance was moistened with Cremophor EL. The application site was covered by occlusive dressing for 24 h then washed with water and soap. Treated rats were observed for 14 days.

Mortality (within 1–9 days) was observed at doses of 2500 mg/kg bw or higher. The observed clinical signs were similar to those in the previously described study of acute oral toxicity (i.e. sedation, breathing disorders, reduction in general well-being, abdominal or side position). The dermal LD_{50} of 1,2,4-triazole in rats was 4200 and 3129 mg/kg bw for males and females, respectively (Thyssen & Kimmerle, 1976).

Rabbits

Groups of two male New Zealand White rabbits were given 1,2,4-triazole (purity, 92.8%) at a dose of 200, 2000 or 5000 mg/kg bw in 0.9% saline as a paste applied to the shaved skin. Each application site was covered with an impervious cuff for 24 h. The cuff was removed and the application site was wiped with a paper towel. The rabbits were observed for 14 days. Body weights were recorded at initiation and at the end of the study. All rabbits were subjected to a post-mortem examination at termination. Skin irritation was evaluated according the method of Draize on days 1 to 14.

At 2000 and 5000 mg/kg bw, all treated rabbits died by day 4. The following clinical signs were observed at 2000 and 5000 mg/kg bw: abdominal breathing, ataxia, clear nasal discharge, gasping, iritis, moribundity, salivation, scant droppings, soft faeces, tremors, and yellow nasal discharge. These signs were observed in 1 day and lasted for 3 days. Slight to moderate skin irritation was observed. No deaths occurred at 200 mg/kg bw, no clinical signs were observed, and gross necropsy revealed no visible lesions. There were no apparent body-weight effects in the survivors. Erythema and slight oedema of the skin at the application site were observed during the study. The dermal LD_{50} in rabbits was greater than 200 mg/kg bw but less than 2000 mg/kg bw (Procopio & Hamilton, 1992).

(c) Inhalation

Mice and rats

In a study of acute toxicity after inhalation, groups of five male Wistar II albino rats and 10 male NMRI mice were exposed (whole-body, in a 10 l inhalation chamber) to 1,2,4-triazole (purity,

technical grade) for 4 h or 6 h, respectively. Air was passed at 2 l/min through the test material contained in a dust tower. No substance vaporized or atomized in the 4-h and 6-h experiments. Rats and mice tolerated the inhalation periods without signs of toxicity. No irritant effect on the mucous membrane of the eyes and noses of the animals was observed. The inhalation median lethal concentration (LC_{50}) in mice and rats could not be determined because exposure to the test substance was not demonstrated (Thyssen & Kimmerle, 1976).

(d) Dermal irritation

In a study of primary dermal irritation, two male New Zealand White rabbits were exposed dermally to 0.5 g of 1,2,4-triazole (purity, 92.8%) moistened with 0.9% saline (1 : 1 w/v) and applied on two patches of shaved skin for 24 h. One site was intact and other site was abraded. The treated area was covered with an impervious cuff for 24 h. The application site was wiped clean with a paper towel after 24 h. Skin irritation was scored according to the method of Draize at 24 h, 72 h and 7 days after patch removal. For the intact skin, one application site exhibited moderate erythema at 24 h. For the abraded skin, very slight erythema was observed on two application sites at 24 h and on one application site at 72 h. The primary irritation score (average of values for 24 h and 72 h) was 0.25 for the intact skins and 0.38 for the abraded skin. It was concluded that 1,2,4-triazole was slightly irritating to the skin of rabbits (Procopio & Hamilton, 1992).

In a second study of primary dermal irritation, two New Zealand White rabbits (sex not reported) were exposed dermally to 500 mg of 1,2,4-triazole (purity, technical grade), applied under cellulose patches for 24 h to the hairless skin of the ears using an adhesive dressing. No sign of skin irritation was observed after removal of the dressing or during the 7-day post-treatment observation period. Under the study conditions, 1,2,4-triazole was not irritating to the skin of rabbits (Thyssen & Kimmerle, 1976).

(e) Ocular irritation

In a study of primary ocular irritation, 0.1 g of 1,2,4-triazole (purity, 92.8%), was instilled into the conjunctival sac of one eye of two male New Zealand White rabbits. Irritation was scored by the Draize method at 4, 24, 48, 72 and 96 h and at 7 and 14 days. Corneal, iridal and conjunctival effects were observed at 4 h. Corneal and conjunctival effects were no longer evident on day 7; iridal effects were no longer evident on day 14. 1,2,4-Triazole was considered to be severely irritating (ocular effects were reversible within 21 days but not within 7 days) to the eyes of rabbits (Procopio & Hamilton, 1992).

In a second study of primary ocular irritation, 50 mg of 1,2,4-triazole (purity, technical grade), was instilled into the conjunctival sac of one eye of two New Zealand White rabbits (sex not reported). One h after application, intense reddening and a very intense swelling of the conjunctivae of the treated eyes had developed, which persisted up to 5 days after application in one animal. The conjunctivae of both animals were normal 7 days after application. During the first and second day after application, a slight, dispersed, diffuse opacity of the cornea was observed. The iris was slightly reddened and swollen. Under the study conditions, 1,2,4-triazole was severely irritating to the eyes of rabbits (Thyssen & Kimmerle, 1976).

(f) Dermal sensitization

In a study of dermal sensitization using the maximization method of Magnusson and Kligman, groups of young male Dunkin-Hartley guinea-pigs (5 in the control group, and 10 in the treatment

group) were given 1,2,4-triazole (purity, > 98%) at a concentration of 10% (in water) for intradermal induction, 75% (in Vaseline) for topical induction, and 75% (in Vaseline) for the challenge. The positive-control group was treated with benzocaine under the same experimental conditions.

The test substance caused slight skin irritation with and without Freund complete adjuvant after intradermal injection. No skin reaction was recorded after dermal induction. At 48 h and 72 h after the start of epidermal challenge (i.e. 24 h and 48 h after removal of the dressings) no signs of allergic skin reactions were noted in the test or control groups. The positive controls gave positive responses at 48 h and 72 h after the start of epidermal challenge. Under the study conditions, 1,2,4-triazole was not a skin sensitizer in guinea-pigs as determined by the maximization method (Frosch, 1998).

2.1 Short-term studies of toxicity

Mice

In a 28-day repeat study of oral toxicity, groups of 15 male and 15 female CD-1 {[ICR]/BR} mice were given diets containing 1,2,4-triazole (purity, 99.9%) at a concentration of 0, 50, 250, 500 or 2000 ppm (equal to 0, 9, 47, 90 and 356 mg/kg bw per day for males and 0, 12, 60, 120, and 479 mg/kg bw per day for females). Diets were prepared weekly and stored at room temperature. The stability, homogeneity and dietary concentrations were confirmed analytically. Treated mice were observed at least daily for signs of toxicity and mortality. Detailed clinical examinations were performed weekly. Body weight and food consumption were measured weekly. Blood samples were collected at termination for haematology and clinical chemistry measurements. All mice were subjected to gross pathological examination. Selected organs were weighed. Selected tissues from the mice in the control group and from mice at the highest dose were collected for histopathological examination.

Diets were stable for 7 days at room temperature. The test article homogeneity results were within the acceptable range. The test substance concentration analysis indicated that the measured test concentrations ranged between 96–99% of the target concentration.

No treatment-related effects were observed on survival, clinical signs, body weight, food consumption, haematological or clinical chemistry parameters, organ weights, or on grossly observable lesions. Liver-enzyme analyses were not reported for this study owing to improper storage of the liver samples. Purkinje cell loss, which was noted in a 90-day study in rats (Wahle & Sheets, 2004) and in mice (Wahle, 2004), was not found in any of the cerebellar brain sections from males in the control group or males at 2000 ppm in this study. The only treatment-related effects found included slight

Table 4. Incidence of testicular/epididymal effects in male mice given diets containing 1,2,4-triazole for 28 days

Effect[a]	Dietary concentration (ppm) [b]				
	0	50	250	500	2000
Testicular degeneration	3	ND	ND	ND	5
Apoptotic bodies (testes)	2 (1.0)[a]	4 (1.0)	1 (1.0)	3 (1.0)	5 (1.0)
Spermatid degeneration/depletion/asynchrony (testes)	1 (1.0)	1 (1.0)	1 (1.0)	0	5 (1.4)
Focal tubular atrophy (testes)	1 (1.0)	2 (1.0)	1 (2.0)	2 (2.0)	4 (1.8)
Exfoliated germ cells/debris (epididymides)	0	1 (1.0)	1 (3.0)	0	3 (2.0)

From Wahle (2004a)
ND, not deermined.
[a]Average severity score of lesion is given in parentheses: 1 (minimal) to 5 (severe).
[b] For all groups, $n = 15$

testicular degeneration in 5 out of 15 male mice at 2000 ppm, which was accompanied by apoptotic bodies within the lumen of spermatogenic tubules in stages I–VII (5 out of 15 mice), minimal to slight spermatid degeneration/depletion/asynchrony (5 out of 15 mice), focal tubular atrophy (4 out of 15 mice), and a slight increase in the incidence of exfoliated germ cells/debris in the epididymides (3 out of 15 mice) (Table 4).

The lowest-observed-adverse-effect level (LOAEL) for male CD-1 mice treated with 1,2,4-triazole for 28 days was 2000 ppm (equal to 356 mg/kg bw per day) on the basis of slight testicular effects. A LOAEL for female CD-1 mice was not identified.

The no-observed-adverse-effect level (NOAEL) was 90 mg/kg bw per day and 479 mg/kg bw per day, for male and female CD-1 mice respectively (Wahle, 2004a).

Mice

In a 90-day study of oral toxicity, groups of 20 male and 20 female CD-1 ([ICR]/BR) mice were given diets containing 1,2,4-triazole (purity, 99.9%) at a concentration of 0, 500, 1000, 3000 or 6000 ppm (equal to 0, 80, 161, 487, or 988 mg/kg bw per day for males and 0, 105, 215, 663, or 1346 mg/kg bw per day for females). An additional group of 15 males and 15 females were given diets containing 1,2,4-triazole at a concentration of 0, 3000, or 6000 ppm for 28 days and then killed for hepatic-enzyme analyses. Diets were prepared every 2 weeks and stored at freezer temperature until use. The stability, homogeneity and dietary concentrations were confirmed analytically. Treated mice were observed at least daily for signs of toxicity and mortality. Detailed clinical examination was performed weekly. Body weight and food consumption were measured weekly. Blood samples were collected at 4 weeks and at termination for haematology and clinical chemistry measurements. In addition, activities of selected hepatic enzymes were measured in mice in the control group and in mice at 3000 and 6000 ppm at 4 weeks, and in mice in the control group and mice at 6000 ppm at week 13. All mice were subjected to gross pathological examination. Selected organs were weighed. Selected tissues from mice in the control group and mice at the highest dose were collected for histopathological examination.

Diets were stable for 35 days stored at freezer temperature. The results for test-article homogeneity were within the acceptable range (< 10%). The analysis of test substance concentration indicated that the measured test concentrations ranged between 94–95% of the target concentration.

There were no treatment-related effects on mortality. Treatment-related clinical signs included tremors in males and females at 3000 and 6000 ppm, yellow staining of the ventrum in males at 3000 and 6000 ppm, food spillage in females at 3000 and 6000 ppm, and rough coat in males at 6000 ppm. The tremors were first observed on day 30 of treatment in males and day 35 in females. Male body weights were consistently decreased throughout the study in the groups at 3000 and 6000 ppm (94% and 84% of values for controls, respectively), but the decreases seen at 3000 ppm were not considered to be toxicologically significant. A consistent decrease in food consumption was seen only in the males at 6000 ppm. In females, a treatment-related decrease in body weight was seen throughout the study in the group at 6000 ppm (91% of values for controls), but no significant change in food consumption was noted for this, or any other, treated female group.

No toxicologically relevant changes in haematological and clinical chemistry parameters were observed. Total cytochrome P450 activity was increased in males and females at 6000 ppm compared with controls. In liver tissue, increased activities of 7-ethoxycoumarin deethylase (ECOD), 7-ethoxyresorufin deethylase (EROD), and aldrin epoxide (ALD) were seen after 4 weeks exposure to 1,2,4-triazole at 3000 or 6000 ppm or to 13 weeks exposure to 1,2,4-triazole at 6000 ppm in males and females. These changes in enzyme activities were not correlated with changes in liver weights or histopathology, and were therefore considered to be adaptive changes. There was also a marginal increase in UDP-glucuronyltransferase (GLU-T) activity. Increased activity of GLU-T was observed in males and females compared with controls after 28 days and 90 days at 6000 ppm.

Gross lesions attributable to exposure to 1,2,4-triazole were limited to males at 6000 ppm and included an increased incidence of rough coat and wet/stained ventrum.

Absolute brain weights were reduced in the males at 3000 and 6000 ppm (95% and 91% of control values, respectively) and the females at 6000 ppm (93% of control values; Table 5). This effect was seen in conjunction with decreased numbers of Purkinje cells in the cerebella of groups of males (15 out of 20) and females (10 out of 18) at 6000 ppm (Table 6). These changes were described as involving cell-body loss and, in some cases, degeneration of axons in the white matter of the cerebellar folia. Of males at 6000 ppm, 9 out of 11 mice showing tremors also had Purkinje cell loss; of females at the highest dose, 1 out of 3 of the mice with tremors had Purkinje cell loss.

Absolute testes weights were significantly decreased in males at 6000 ppm (87% of control values) and non-significantly decreased in the groups at 3000 and 1000 ppm (92% each of control values). In conjunction with this decrease in testicular weights, histopathological changes in the testes were observed, including increased incidence of apoptotic-like bodies (4 out of 20, 4 out of 20, 7 out of 20, 11 out of 20, and 12 out of 20 of the males in the control group and the males at 500, 1000, 3000, and 6000 ppm, respectively); spermatid degeneration, depletion, and asynchrony (5 out of 20 and 15 out of 20 of the males at 3000 and 6000 ppm, respectively), and minimal or slight focal tubular atrophy (2 out of 20, 3 out of 20, and 10 out of 20 of the males at 1000, 3000, and 6000 ppm, respectively). A correlated change in the epididymides of the group at 6000 ppm was considered secondary to the testicular effects and consisted of increased germ cells and debris in the luminal duct (10 out of 20) and one male with aspermia. Minimally increased apoptotic-like bodies and tubular atrophy seen at 1000 ppm were considered treatment-related but not adverse. Testicular atrophy at 1000 ppm was considered to be spontaneous due to their limited tissue distribution (focal and/or unilateral) and the lack of accompanying spermatid degeneration/depletion/asynchrony.

Table 5. Mean absolute and relative organ weights of mice fed diets containing 1,2,4-triazole for 90 days

Parameter	Dietary concentration (ppm)				
	0	500	1000	3000	6000
Males (n = 20 per group)					
Terminal body weight (g)	37.3 ± 2.2	337.0 ± 2.2	36.4 ± 2.9	34.9* ± 2.0	31.3* ± 1.7
Absolute brain weight (g)	0.488 ± 0.023	0.491 ± 0.023	0.476 ± 0.025	0.465* ± 0.024 (95)[a]	0.445* ± 0.019 (91)
Relative brain/body (%)	1.328	1.378	1.365	1.376	1.462*
Absolute testes weight (g)	0.253 ± 0.044	0.247 ± 0.038	0.233 ± 0.024 (92)	0.233 ± 0.044 (92)	0.219* ± 0.029 (87)
Relative testes/body (%)	0.688 ± 0.131	0.692 ± 0.110	0.669 ± 0.081	0.687 ± 0.121	0.719 ± 0.103
Females (n = 19–20 per group) [b]					
Terminal body weight (g)	29.1 ± 2.4	28.4 ± 1.8	28.4 ± 2.1	28.7 ± 3.1	26.6* ± 1.6 (93)
Brain weight (g)	0.485 ± 0.031	0.489 ± 0.018	0.483 ± 0.026	0.475 ± 0.016	0.451* ± 0.025 (93)
Relative brain/body (%)	1.737	1.756	1.731	1.717	1.734

From Wahle (2004b)

[a] Value in parentheses is the percentage of the control value.

[b] $n = 19$ for females at 1000 and 3000 ppm; for all other groups of females, $n = 20$.

* Statistically significantly different ($p \leq 0.05$) from the controls.

The LOAEL was 3000 ppm, equal to 487 mg/kg bw per day for males, on the basis of tremors, decreased brain weight, decreased testicular weight and histopathological changes in the testes. The LOAEL was 6000 ppm equal to 1346 mg/kg bw per day for female mice, on the basis of decreased body weight, decreased body-weight gain, decreased brain weight and histopathological findings in the brain (cerebellum).

The NOAEL was 1000 ppm for males and 3000 ppm for females, equal to 161 and 663 mg/kg bw per day, respectively(Wahle, 2004b).

Rats

In a 90-day study of oral toxicity, groups of 15 male and 15 female Wistar rats were given diets containing 1,2,4-triazole (purity, 99.6%) at a concentration of 0, 100, 500, or 2500 ppm (equivalent to 0, 7.8, 37.9, or 212.3 mg/kg bw per day for males and 0, 10.2, 54.2, or 266.7 mg/kg bw per day for females). Dietary concentrations were not measured analytically. Treated rats were observed at least daily for signs of toxicity and mortality. Body weights were measured weekly. Blood samples

Table 6. Incidence of selected histopathology findings in mice given diets containing 1,2,4-triazole for 90 days

Organ/description of finding[a]	Dietary concentration (ppm)				
	0	500	1000	3000	6000
Males (n = 20 per group)					
Testes:					
No abnormality	15	16	12	9	1
Apoptotic-like bodies	4 (1.0)	4 (1.3)	7 (1.1)	11* (1.3)	12** (1.2)
Spermatid degeneration/depletion/asynchrony	1 (1.0)	0	0	5 (1.4)	15** (2.0)
Tubular atrophy	0	0	2 (1.5)	3 (1.0)	10** (1.8)
Epididymides:					
Germ cells and debris in duct lumen	0	0	0	0	10** (2.5)
Aspermia, relative or absolute	0	0	0	0	1 (3.0)
Brain:					
Purkinje cell loss	0	0	0	0	15** (1.7)
Gliosis	0	0	0	0	0
Eye:					
Retinal degeneration	0	0 [b]	0 [c]	0 [c]	2 (2.0)
Erosion	0	0 [b]	0 [c]	1 (3.0) * [b]	2 (1.5)
Females (n = 20 per group)					
Brain:					
Purkinje cell loss	0	0	0	0	10** [c] (1.3)
Gliosis	0	0	0	0	1 (2.0)
Eye:					
Retinal degeneration	0	0 [b]	0 [b]	0 [b]	0
Erosion	0	3 (3.0) * [b]	0 [b]	0 [b]	0

From Wahle (2004b).

[a] Values in parentheses are mean severity, ranging from 1 (minimal) to 5 (severe).

[b] $n = 1$ for males at 500, 1000 and 3000 ppm, n = 4 for females at 500 ppm and n = 2 for females at 1000 and 3000 ppm.

[c] $n = 18$ owing to loss of cerebella of two females at the highest dose.

* Statistically significantly different ($p \leq 0.05$) from the controls, calculated by reviewer.

** Statistically significantly different ($p \leq 0.01$) from the controls, calculated by reviewer.

were collected from five males and five females per group at termination for haematology and clinical chemistry measurements. Rectal temperatures of all rats were measured at 1 month and at termination. Urine analysis was conducted at termination. All rats were subjected to gross pathological examination. Selected organs were weighed. Selected tissues from five males and five females per group were collected for histopathological examination. This study was not conducted in accordance with GLP regulations.

No mortality was observed during the study. Two males and two females at 2500 ppm exhibited temporary slight convulsions. Rectal body temperature was not affected by treatment. Food intake was reduced at 2500 ppm during the first 2 weeks of treatment, but in overall terms food intake was similar in all groups. Body-weight gains were reduced at 2500 ppm, leading to total weight-gain deficits of 12% and 8% for males and females, respectively, relative to values for untreated controls. There were statistically significant changes in erythrocyte parameters after 1 and 3 months in males at 2500 ppm that pointed to slight microcytic hypochromic anaemia. No other alterations in haematological parameters were observed in females at 1 month or at termination. Clinical chemistry demonstrated no deviations of toxicological significance. Urine analysis did not reveal any differences between controls and treated groups at 1 month or at termination. There were no significant differences in protein-bound-iodine (PBI) measurement between controls and treated groups at 1 month or at termination. There were deviations in absolute organ weights at 2500 ppm, particularly in males, that were attributed to lower terminal body weights. Gross findings at necropsy were not considered to be associated with treatment. Histopathological evaluation revealed slight to moderate fat accumulation in liver parenchymal cells in three males at 2500 ppm.

The NOAEL was 500 ppm, equivalent to 37.9 and 54.2 mg/kg bw per day in males and females, respectively, on the basis of retarded body-weight development, temporary slight effects on the central nervous system (CNS), decreases in erythrocyte parameters (males only) and hepatocellular fat accumulation (males only) at 2500 ppm (equivalent to 212.3 and 266.7 mg/kg bw per day for males and females, respectively (Bomhard et al., 1979).

In a combined short-term study of toxicity and neurotoxicity, groups of 20 male and 20 female Wistar (Crl:WI[Glx/BRL/Han]IGS BR) rats were given diets containing 1,2,4-triazole (purity, 99.9%) at a nominal concentration of 0, 250, 500, 3000 or 1000/4000 ppm (1000 ppm for the first 4 weeks and 4000 ppm, thereafter) for approximately 14 weeks. The mean daily intake was 16, 33, 183 and 210 mg/kg bw per day for males and 19, 41, 234 and 275 mg/kg bw per day for females at 250, 500, 3000 and 1000/4000 ppm, respectively. Diets were prepared weekly and stored at room temperature. The stability, homogeneity and dietary concentrations were confirmed analytically. Treated rats were observed at least daily for signs of toxicity and mortality. Detailed clinical examinations were performed weekly. Body weight and food consumption were measured weekly. Ophthalmoscopic examination was performed on all rats before the study and all survivors at termination. Blood samples were collected from 10 males and 10 females per group at termination for haematology and clinical chemistry measurements. Urine samples were collected for urine analysis at termination. Neurobehavioural assessment (functional observational battery [FOB] and motor activity testing) was performed on 12 males and 12 females per group before exposure and during weeks 2, 4, 8 and 13. At study termination, 10 males and 10 females per group were killed and perfused in situ for neuropathological examination. Of the perfused rats, all rats in the control group and those at the highest dose were subjected to histopathological evaluation of brain and tissues of the peripheral nervous system. The remaining 10 males and 10 females per group were killed and standard tissues were weighed and examined for gross and microscopic pathology. In addition, the liver was snap-frozen and analysed for metabolizing enzymes.

Diets were stable for 35 days stored at room temperature. The results for test-article homogeneity were within the acceptable range (< 10%). The analysis of test-substance concentration indicated that the measured test concentrations ranged between 94% and 98% of the target concentrations.

Tremors were observed in one female from the group at 1000/4000 ppm. No other treatment-related clinical signs of toxicity were observed in any rat during exposure, or during daily and weekly examinations. All rats survived to scheduled termination. Beginning on approximately day 42, body weight was significantly or slightly decreased in both sexes at 1000/4000 ppm (92–96% of control value) and at 3000 ppm (93–96% of control value). Final body weight was 7% and 6% lower than values for controls in males and females at 3000 ppm, respectively; while reductions compared to control of 8% and 5% were noted at 1000/4000 ppm in males and females, respectively. Overall (days 0–91), body-weight gain was significantly decreased in males and females (79% of control value) at 1000/4000 ppm and in males (82% of control value) and females (81% of control value) at 3000 ppm. Food consumption in treated rats was comparable to that of the control group for most treated rats, with increased consumption in males and females at the highest dose during the latter half of the exposure period.

Evaluation of haematological parameters provided no treatment-related adverse findings in both sexes at termination. A slight decrease in serum triglyceride and uric acid was observed in males at 3000 ppm and 1000/4000 ppm. A dose-related decrease in thyroid stimulating hormone (TSH) was seen in males at all doses, statistically significant at 500 ppm and above (74–65% of control values). No treatment-related effects were observed in concentrations of thyroxin (T4) and triiodothyronine (T3) in males. A slight decrease in TSH was seen in female rats, but a dose–response relationship was not evident. In the absence of any thyroid histopathology and changes in T3 and T4 concentrations, and overall susceptibility of rat thyroid to chemical perturbation, these decreases in TSH were not considered to be toxicologically relevant.

No treatment-related effects were observed on urine analysis parameters in both sexes. Ophthalmoscopic examinations at termination revealed retinal degeneration in 4 out of 20 males and 2 out of 20 females at 3000 ppm, in 5 out of 20 males and females at 1000/4000 ppm compared with 2 out of 20 males in the control group and no females in the control group. There was a slight increase in numbers of corpora lutea in females at 3000 ppm and 1000/4000 ppm; although not statistically significant, this is consistent with similar findings in a study of reproductive toxicity (Wahle & Sheets, 2004). No other treatment-related effects were observed on gross or microscopic examination at necropsy.

Absolute brain weight was significantly decreased in males and females at 3000 ppm (93–95% of control value), significantly decreased in males at 1000/4000 ppm (94% of control value) and non-significantly decreased in females at 1000/4000 ppm (94% of control value) (Table 7). Analysis

Table 7. Organ changes in in rats fed diets containing 1,2,4-triazole for 14 weeks

Parameter	Dietary concentration (ppm)				
	0	250	500	3000	1000/4000
Males					
Absolute brain weight (g)	2.05	2.02	2.01	1.94*	1.92*
Relative brain weight (%)	0.48	0.48	0.47	0.49	0.50
Females					
Absolute brain weight (g)	1.91	1.89	1.88	1.78*	1.81
Relative brain weight (%)	0.83	0.78	0.81	0.79	0.79
Total corpora lutea	33 ± 9b	NE	33 ± 6	41 ± 9	40 ± 14
Recently cycling corpora lutea	16 ± 5	NE	17 ± 4	21 ± 4	19 ± 6

From TDMG (2008) and Wahle & Sheets (2004).
NE, not evaluated.
[a] $n = 10$.
[b] ± standard deviation

Table 8. Incidence of histopathological findings in brain and peripheral nerves in rats given diets containing 1,2,4-triazole for 14 weeks

Finding [a]	Dietary concentration (ppm)			
	0	500	3000	1000/4000
Males				
Tissues examined	10	10	10	10
Brain, level 7 (cerebellum):				
Degeneration/necrosis	—	—	10* (2.5)	9* (2.8)
Degeneration, nerve fibre	1 (1.0)	2 (1.0)	4 (1.3)	5 (1.0)
Ganglion, dorsal root:				
Vacuolization	1 (1.0)	3 (1.0)	9* (1.1)	7* (1.0)
Chromatolysis	5 (1.0)	3 (1.0)	10* (1.2)	10* (1.0)
Degeneration, nerve fibre	2 (1.5)	5 (1.0)	9* (1.3)	10* (1.1)
Nerve, sural, left: Degeneration, nerve fibre	—	2 (1.0)	6* (1.0)	9* (1.1)
Nerve, sural, right: Degeneration, nerve fibre	—	2 (1.0)	7* (1.1)	8* (1.4)
Nerve, sciatic, left: Degeneration, nerve fibre	2 (1.0)	2 (1.0)	9* (1.3)	9* (1.2)
Nerve, sciatic, right:				
Degeneration, nerve fibre	2 (1.0)	3 (1.0)	9* (1.0)	9* (1.4)
Nerve, tibial, left: Degeneration, nerve fibre	4 (1.0)	5 (1.0)	10* (1.5)	10* (1.5)
Nerve, tibial, right:				
Degeneration, neuronal	—	—	—	1 (1.0)
Degeneration, nerve fibre	2 (1.0)	1 (1.0)	8* (1.5)	10* (1.4)
Females				
Tissues examined	10	10	10	10
Brain, level 7 (cerebellum):				
Degeneration/necrosis	—	—	10* (2.6)	10* (2.3)
Degeneration, nerve fibre	1 (1.0)	—	1 (1.0)	1 (1.0)
Ganglion, dorsal root				
Vacuolization	1 (1.0)	—	—	2 (1.0)
Chromatolysis	3 (1.0)	4 (1.0)	10* (1.0)	10* (1.0)
Degeneration, nerve fibre	8 (1.1)	—	2 (1.3)	5 (1.1)
Nerve, sural, left: Degeneration, nerve fibre	1 (1.0)	1 (1.0)	5 (1.4)	8* (1.4)
Nerve, sural, right: Degeneration, nerve fibre	1 (1.0)	1 (1.0)	4 (1.3)	3 (1.7)
Nerve, sciatic, left: Degeneration, nerve fibre	5 (1.0)	5 (1.0)	7 (1.0)	9 (1.1)
Nerve, sciatic, right: Degeneration, nerve fibre	7 (1.0)	4 (1.0)	6 (1.2)	7 (1.1)
Nerve, tibial, left: Degeneration, nerve fibre	4 (1.0)	3 (1.3)	7 (1.3)	10* (1.4)
Nerve, tibial, right:				
Degeneration, neuronal	—	—	—	—
Degeneration, nerve fibre	3 (1.0)	1 (1.0)	6 (1.0)	7 (1.6)

From TDMG (2008) and Wahle & Sheets, 2004).
—, zero incidence.
[a] The average severity of the lesion, graded from 1 (minimal) to 5 (severe), is shown in parentheses.
* $p < 0.05$.

of the activity of selected hepatic enzymes indicated slightly increased activities in males and females at 3000 and 1000/4000 ppm.

The neurotoxicity part of the study included neurobehavioural (FOB and motor activity) and neuropathology assessments. In the FOB, effects were observed in males and females at 1000/4000 and 3000 ppm with the incidence and severity increased at week 8. Males were more severely affected than females. The effects, which were not observed during pre-treatment testing or in rats in the control group, included ungroomed appearance, red nasal and lacrimal stain, yellow-stained urine, muscle fasciculations, tremors, gait incoordination, decreased activity in the open field, decreased rearing, uncoordinated righting reflex and increased foot-splay. A decrease in motor and locomotor activity was also observed in males at 3000 ppm, during week 4 only. At necropsy, terminal body weight in the neurotoxicity groups was non-significantly decreased in males and females at 1000/4000 (93–94% of control value) and 3000 ppm (94% of control value). Fixed absolute brain weight was significantly decreased in males and females at 1000/4000 ppm (95% of control value) and non-significantly decreased in both sexes at 3000 ppm (96% of control value). No treatment-related effects were observed on macroscopic examination. On microscopic examination, nerve-fibre degeneration was observed in multiple peripheral nerves (sciatic, tibial, sural), in the Gasserian and dorsal root ganglia and in the spinal nerve roots with increased incidence and severity in males and females at 1000/4000 and 3000 ppm as compared with the control group. Males were more severely affected than females. In the brain, lesions were found in the more anterior dorsal cerebellum (level 7) in males and females at 1000/4000 ppm and 3000 ppm. The lesions included mineralization, axonal degeneration, degeneration/necrosis and nerve-fibre degeneration in males but were limited to degeneration/necrosis and nerve-fibre degeneration in females. In females at 1000/4000 ppm, an increase in nerve-fibre degeneration was also reported in brain levels 4 and 5.

The LOAEL for toxicity/neurotoxicity was 3000 ppm, equal to 183 mg/kg bw per day, on the basis of decreased body weight and body-weight gain, FOB changes, decreased absolute brain weight, and increased incidence of neuropathology findings in the peripheral and central nervous system. The NOAEL was 500 ppm, equal to 33 mg/kg bw per day (Wahle & Sheets, 2004).

2.2 Long-term studies of toxicity and carcinogenicity

No studies were submitted.

2.3 Genotoxicity

1,2,4-Triazole has been tested for genotoxicity in a battery of studies in vitro. Two assays for reverse mutation in bacteria, a test for mutation at the *Hgprt* locus and for chromosomal aberration

Table 9. Results of studies of genotoxicity in vitro with 1,2,4-triazole

End-point	Test object	Concentration	Purity (%)	Result	Reference
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	10–5000 µg/ plate ± S9 in water	99.7	Negative[a]	Poth (1989)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537;	100–7500 µg/ plate ± S9 in water	92.8	Negative	Melly & Lohse (1982)
Hgprt forward mutation	Chinese hamster ovary cells	43.2–691 µg/ml ± S9	99.3	Negative	Schisler & Kleinert (2007a)
Chromosomal aberration	Rat lymphocytes	10.8–691 µg/ml ± S9	99.3	Negative	Schisler & Kleinert (2007b)

S9, 9000 × *g* supernatant from rodent liver.
[a] Toxic effects at 1000 and 5000 µg/plate.

gave negative responses in the presence or absence of metabolic activation. No studies on germ cells were available. The results of studies of genotoxicity with 1,2,4-triazole are summarized in Table 9.

2.4 Reproductive toxicity

(a) Multigeneration study

In a two-generation study of reproduction, groups of 30 male and 30 female Wistar Hannover rats were given diets containing 1,2,4-triazole (purity 99.9–101%) at a concentration of 0, 250, 500 or 3000 ppm. For the F_0 and F_1 dams, concentrations of the test article in the diet were reduced to 0, 139/104, 278/207 and 1666/1245 ppm during days 0–7 and 7–21 of lactation, respectively, to maintain a constant intake of the test substance. One litter was produced in each generation; insufficient F_1 pups from the group at the highest dose were produced from which to select parental animals and this dose group was cancelled. Pre-mating doses for the F_0 parental animals in the control group and groups at the lowest dose, intermediate dose and highest dose were 0, 15.4, 30.9, and 188.6 mg/kg bw per day, respectively, for males and 0, 17.5, 36.2, and 217.9 mg/kg bw per day, respectively, for females. Pre-mating doses for the treated F_1 parental rats in the control group and groups at the lowest, and intermediate dose were 0, 16.0, and 32.0 mg/kg bw per day, respectively, for males and 0, 18.9, and 37.5 mg/kg bw per day, respectively, for females. F_0 and F_1 parental rats were given test or control diet for 10 weeks before mating, throughout mating, gestation, and lactation, and until sacrifice.

In addition to the normal end-points for reproductive toxicity, the brains of F_0 adult rats in the control group, and at the intermediate and highest dose, and F_1 adults (non-perfused) in the control group and at the intermediate dose and F_1 and F_2 weanlings (perfused) in the control group and at the intermediate dose were evaluated histopathologically, including morphometric measurement of weanling brains. Stability, homogeneity and dietary concentrations were confirmed analytically. Rats were observed for clinical signs and monitored for changes in body weight and food consumption. All rats placed on study underwent a post-mortem examination, which included documenting and saving all gross lesions, weighing designated organs and collecting representative tissue specimens for histopathological evaluation and sperm analysis.

1,2,4-Triazole was stable at room temperature for 7 days. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage (88.5–105.2%) was acceptable.

No treatment-related deaths or clinical signs of toxicity were observed in any group during the study. Absolute body weight of the F_0 males and females at the highest dose was significantly ($p \leq 0.05$ or 0.01) less than that of controls beginning at days 42 and 21, respectively, and continuing throughout premating (males, 92–95% of control values; females, 95–97% of control values). The most pronounced effect was reduced body-weight gain during the first 2 weeks of premating; during this time, males and females gained 65% and 20%, respectively, of the control value. Overall premating-weight gain was 72% and 63% of controls for males and females at the highest dose, respectively. Absolute body weight and body-weight gain by the F_0 rats at the lowest and intermediate dose were similar to those of controls throughout the pre-mating interval. Absolute body weight was significantly less ($p \leq 0.05$ or 0.01) than that of the controls for the F_1 males at the intermediate dose throughout premating and for the F_1 males at the lowest dose beginning on day 14 of the pre-mating period (Table 10). Absolute body weights and body-weight gains for the treated F_1 females were similar to those of the control group during premating. Food consumption, calculated as g/kg per day, was increased in all treatment groups for most of the treatment period; this increase was statistically significant for F_0 females and for F_1 adults at multiple time-points during the pre-mating treatment period.

The results of gross necropsy were unremarkable. F_0 males and females at the highest dose had significantly reduced terminal body weight and absolute brain weight compared with those of

the controls; several other organs also showed a decrease in weight at the highest dose, including the thyroid in both sexes and spleen in females. In the F_1 adults, males at the lowest and intermediate dose had a significantly reduced final body weight and males at the intermediate dose had decreased absolute brain weight. Absolute spleen weight decreased in F_1 females, statistically significantly at

Table 10. Mean body weight, body-weight gain and food consumption in F_1 adults during the pre-mating interval in a two-generation study in rats fed diets containing 1,2,4-triazole

End-point [a]	Dietary concentration (ppm)		
	0	250	500
Males			
Body weight (g):			
Day 0	266.2 ± 3.98	254.3 ± 4.01	250.6* ± 3.96 (94)[b]
Day 14	328.6 ± 4.54	314.2* ± 4.32 (96)	307.1** ± 4.99 (93)
Day 28	372.3 ± 5.40	350.8** ± 4.64 (94)	342.1** ± 5.55 (92)
Day 42	399.4 ± 6.08	377.8* ± 5.22 (95)	367.3** ± 6.05 (92)
Day 70 (end of premating)	437.7 ± 8.24	418.0 ± 5.90 (95)	405.4** ± 6.65 (93)
Day 98 (termination)	461.9 ± 7.18	435.2* ± 7.32 (94)	428.4** ± 6.89 (93)
Body-weight gain (g):			
Days 0–14[c]	62.4	59.9 (96)	56.5 (90)
Premating[c]	171.5	163.7 (95)	154.8 (90)
After pairing[c]	24.2	17.2	23
Food consumption (g/kg per day):			
Days 0–7	91.0 ± 1.21	92.8 ± 0.96	93.4 ± 1.15
Days 28–35	60.7 ± 0.68	62.2 ± 0.52	63.2* ± 0.67
Days 49–56	52.5 ± 1.54	56.8* ± 0.70	56.8* ± 0.55
Days 63–70	51.7 ± 0.69	53.2 ± 0.83	54.1 ± 0.68
Females			
Body weight (g):			
Day 0	172.3 ± 2.44	166.7 ± 1.82	169.1 ± 2.72
Day 14	193.4 ± 2.39	189.0 ± 2.07	192.0 ± 3.00
Day 28	211.1 ± 2.67	204.4 ± 2.17	205.9 ± 3.28
Day 42	222.0 ± 2.61	214.2 ± 2.33	218.7 ± 3.64
Day 70 (end of premating)	236.2 ± 3.07	227.5 ± 2.64	230.8 ± 3.66
Body-weight gain (g):			
Days 0–14[c]	21.1	22.3	22.9
Premating[c]	63.9	60.8	61.7
Food consumption (g/kg per day):::			
Days 0–7	93.7 ± 1.24	97.7 ± 1.52	98.0 ± 1.46
Days 28–35	73.0 ± 0.86	78.1** ± 1.33	77.0* ± 1.13
Days 49–56	68.6 ± 0.75	69.9 ± 1.27	68.2 ± 1.18
Days 63–70	63.8 ± 0.67	68.9** ± 1.39	69.9** ± 1.20

From Young & Sheets (2006)

[a] ± standard error

[b] Percentage of value for control group, calculated by reviewer, is given in parentheses.

[c] Calculated by reviewer from group mean values.

*$p \leq 0.05$; **$p \leq 0.01$.

both the lowest and intermediate dose. Microscopically in the F_0 rats at the highest dose, mild to moderate degeneration/necrosis was observed in the cerebellum of 30 out of 30 males and 28 out of 30 females. The average severity of the lesion (on a scale of 1–5) was 2.8 and 2.1 for males and females, respectively. Ventricular dilatation was found in one F_1 male adult at the intermediate dose.

The NOAEL for parental systemic toxicity was < 250 ppm, < 16 mg/kg bw per day, and the x LOAEL for parental systemic toxicity was ≤ 250 ppm (≤ 16 mg/kg bw per day) on the basis of decreased body weight and body-weight gain in F_1 males and decreased spleen weight in F_1 females.

No F_1 offspring at the highest dose survived lactation. For litters of both generations at the lowest and intermediate dose, live birth, viability, and lactation indices, mean litter sizes, and sex ratios were similar between the treated and control groups. No treatment-related clinical signs of toxicity

Table 11. Combined findings for male and females in a two-generation study in rats fed diets containing 1,2,4-triazole

Finding	Dietary concentration (ppm)			
	Historical control range	Current study control	250	500
F1 generation				
Pup weight (g):				
Day 0	5.4–6.1	6.2	5.9	6.1
Day 14	24.9–32.6	33.5	32.4	32.1
Day 21	39.3–48.9	50.7	49.1	48.4
Gain (0—21 days)	33.4–42.8	44.5	43.3	42.3
Preputial separation (days):	40.9–44.0	40.7	41.2	41.3
Brain weight (g):				
Absolute	1.378–1.481	1.483	1.463	1.458
Relative	—	2.947	3.047	2.956
Spleen weight (g):				
Absolute	0.209–0.240	0.236	0.236	0.227
Relative	—	0.466	0.479	0.456
F2 generation				
Pup weight (g):				
Day 0	5.4–6.1	6.21[a]	5.8**	5.7**
Day 14	24.9–32.6	32.5	30.8	31.7
Day 21	39.3–48.9	50.2[a]	46.8**	47.6*
Gain	33.4–42.8	44.0[a]	41.0**	41.8**
Preputial separation (days):	40.9–44.0	40.7[a]	41.8*	41.5
Brain weight (g):				
Absolute	1.378–1.481	1.497[a]	1.450*	1.445*
Relative	—	3.006	3.126	3.027
Spleen weight (g):				
Absolute	0.209–0.240	0.246[a]	0.215**	0.221**
Relative	—	0.492	0.46	0.461

From Young & Sheets (2006)
* $p < 0.05$; ** $p < 0.01$ (ANOVA, Dunnett)
[a] Concurrent control results outside range of historical control values.

were observed in the pups during lactation, and gross necropsy was unremarkable. Body weight and body-weight gain of the F_1 pups in the groups at the lowest and intermediate dose were similar to those of the controls throughout lactation (Table 11). F_2 male and female pups from litters at the lowest and intermediate dose had significantly lower ($p \leq 0.05$ or 0.01) body weight at birth and on day 21 of lactation compared with the controls (Table 12). Body-weight gain by the pups in both treated groups was significantly less than that of the controls during days 14–21 of lactation. For the F_1 pups, absolute and relative organ weights were similar between the treated and control groups. For F_2 pups in the groups at the lowest and intermediate dose (sexes combined) absolute weight of the brain and spleen was significantly less ($p \leq 0.05$ or 0.01) than that of the controls; in F_2 female pups, spleen weight was also statistically significantly decreased at both doses. These changes in body weights and organ-weight changes were minor and were toxicologically significant but within the higher bound of the range for historical controls. During the neuropathology assessment, no effects were seen on qualitative neuropathology, and there were no treatment-related differences in brain morphometric measurements between the treated and control groups of either generation.

The NOAEL for developmental/systemic toxicity in offspring was 500 ppm, the highest dose tested (30.9 mg/kg bw per day). No LOAEL for developmental/systemic toxicity in offspring was identified.

The fertility index was significantly reduced ($p \leq 0.01$) in F_0 rats at the highest dose compared with the controls (7.1% vs 76.7% for the controls). Only two litters containing one female pup each were produced by the F_0 dams at the highest dose. These two dams at the highest dose were the only females in the group with implantations and both dams and their pups were sacrificed before weaning. The mean number of implantations was 1.5 per dam for the females at the highest dose with litters, compared with 11.2 and 12.4 per dam for the other treated and control groups. Other end-points of reproductive performance were not affected by treatment in the F_0 generation. No treatment-related differences in mating, fertility, or gestation indices, number of days to mating, or duration of gestation were seen between the treated and control groups of the F_1 parental rats during litter production. Body weight, body-weight change, and food consumption by the F_0 and F_1 dams at the lowest and intermediate dose were similar to those of the control group during gestation and lactation.

No treatment-related differences in the in-life evaluations of the estrous cycle or estrous-cycle length were observed between the treated and control females of either generation; there was an apparent shift in the staging of estrus at sacrifice in F_1 females only. No treatment-related differences in sperm motility were found between the treated and control males of either generation. F_0 males at

Table 12. Micropathology observations in the adult parental generation in a two-generation study in rats fed diets containing 1,2,4-triazole

Observation	Dietary concentration (ppm)							
	Males				Females			
	0	250	500	3000	0	250	500	3000
Brain – tissues examined:	30	0	30	30*	30	6	30	30
Degeneration/necrosis	—	—	—	30 * (2.8)	—	—	—	28 * (2.1)
Ovaries – tissues examined:	—	—	—	—	7	5	5	10
Total corpora lutea count	—	—	—	—	24.9	23.0	15.6	41.3*
Uterus - tissues examined:	—	—	—	—	30	6	5	30
Dilatation	—	—	—	—	4 (2.3)	2 (2.5)	2 (2.0)	14* (1.8)

From Young & Sheets (2006)

[a] Average severity of lesions, graded 1 (minimal) to 5 (severe), is shown in parentheses.

* $p < 0.05$.

the highest dose had a significantly lower ($p \leq 0.05$; 74% of control) epididymal sperm count compared with the controls (Table 13). F_0 males at the intermediate and highest dose had a significantly ($p \leq 0.05$) lower percentage of normal (95.7–97% vs 98.7% for controls) sperm with concomitant increases in the percentage of abnormal (1.4–1.5% vs 0.8% for controls) and detached sperm (1.6–2.8% vs 0.5% for controls). F_1 males at the intermediate dose had slightly fewer epididymal and testicular sperm numbers and a slightly greater percentage of abnormal sperm than did the control group, but statistical significance was not attained.

F_0 females at the highest dose had significantly increased left and right ovarian weights, and an increase in the number of corpora lutea. The total numbers of corpora lutea for F_0 females in the control group and groups at the lowest, intermediate and and highest dose were, respectively, 24.9 ± 7.1, 23.0 ± 6.8, 15.6 ± 8.3, and 41.3 ± 6.5 ($p \leq 0.05$, see Table 14). For F_1 females, the total number of corpora lutea per female was significantly decreased at the intermediate dose (total numbers of corpora lutea in the control group and group at the intermediate dose were 48.9 ± 7.9 and 39.3 ± 7.4 [$p \leq 0.05$], respectively); females at the lowest dose were not evaluated. Dilatation of the uterus was seen in 14 out of 30 F_0 females at the highest dose with a severity of 1.8 compared with 4 out of 30 rats in the control group with a severity of 2.3. F_0 rats at the intermediate dose were not examined but the incidence of dilatation of the uterus was not increased in the F_1 females at the intermediate dose. There was also delay in vaginal opening in females of both generations, which was statistically significant for F_1 females at both doses ($p \leq 0.05$ or 0.01). Anogenital distance was not affected by treatment, for either sex, in F_2 pups.

The NOAEL for reproductive toxicity was 250 ppm (15.4–16 and 17.5–18.9 mg/kg bw per day for males and females, respectively) and the LOAEL for reproductive toxicity was 500 ppm (30.9–32 and 36.2–37.5 mg/kg bw per day for males and females, respectively) on the basis of an increase in abnormal sperm in F_0 and F_1 males and decreases in corpora lutea count in F_1 females.

Table 13. Sperm parameters in a two-generation study in rats fed diets containing 1,2,4-triazole[1]

Parameter	Dietary concentration (ppm)			
	0	250	500	3000
Parental F_0 males				
Motility (%)	76.2	78.9	78.9	78.9
Progressive (%)	55.9	56.5	56.4	57.3
Epididymis sperm count	58.2	57.0	65.7	43.2* (74)
Testis sperm count	72.0	63.1* (88)	64.4 (89)	61.2* (85)
Normal (%)	98.7	98.1	97.0*	95.7*
Abnormal (%)	0.8	1.0	1.4*	1.5*
Detached (%)	0.5	0.8	1.6*	2.8*
F_1 males				
Motility (%)	87.1	87.8	89.5	—
Progressive (%)	63.9	65.7	67.6	—
Epididymis sperm count	49.2	—	48.6	—
Testis sperm count	69.2	—	68.3	—
Normal (%)	98.1	—	97.9	—
Abnormal (%)	1.1	—	1.4	—
Detached (%)	0.8	—	0.7	—

From Young & Sheets (2006)

[a] Values are means; standard deviations were not given. n = 27–30 per group.

* $p \leq 0.05$.

The NOAEL for paternal toxicity was < 250 ppm (equivalent to < 16.0 mg/kg bw per day) on the basis of retarded body-weight gain at 250 and 500 ppm in F_1 males. The NOAEL for maternal toxicity was 500 ppm (equivalent to 36.2 mg/kg bw per day) on the basis of lower body weights, degenerative findings in the cerebellum, increased number of corpora lutea, and uterine horn dilatation at 3000 ppm in parental females.

The study author identified the NOAEL for reproductive toxicity was 500 ppm (equivalent to 34.4 mg/kg bw per day) on the basis of reduced fertility and decreased implantation sites at 3000 ppm.. This value was different than the NOAEL for reproductive toxicity was 250 ppm (15.4–16 and 17.5–18.9 mg/kg bw per day for males and females, respectively) established by the Meeting.

The NOAEL for developmental toxicity was > 500 ppm (equivalent to > 35.8 mg/kg bw per day) on the basis of lack of treatment-related effects in F_1 and F_2 pups at 250 and 500 ppm (Young & Sheets, 2006).

(b) Developmental toxicity

Rats

In a non-guideline, non-GLP study, 10 pregnant rats (Alpk: AP [Wistar-derived]) were given 1,2,4-triazole (purity not reported, vehicle not reported) at a dose of 0, 25 or 100 mg/kg bw per day during days 7–17 of gestation using the Chernoff-Kavlock assay. Maternal observations were restricted to body weights on days 1, 7–17, and 22. Offspring observations: litter weights of live pups on postnatal days 1 and 5, and the number of live and dead pups on these days. No specific examination for malformation was conducted.

Under the study conditions, 1,2,4-triazole had no effect on maternal weight gain, the number of viable litters, litter size, survival or postnatal-weight gain. 1,2,4-Triazole was not teratogenic in rats as determined by a modified Chernoff-Kavlock assay (Wickramaratne, 1987).

In a non-guideline, non GLP, study conducted in vitro, 1,2,4-triazole (purity not reported; vehicle, ethanol; together with flusilazole and fluconazole) was evaluated for malformations. Rat embryos (Crl:CD), aged 9.5 days (one to three somites), were exposed to the test substance at concentrations of 500 to 5000 µmol/l in vitro. After 48 h in culture, the embryos were examined morphologically using a dissecting microscope. The visceral yolk-sac diameter and the crown–rump and head length were measured, the somite number recorded, and the developmental degree evaluated according to the scoring method described by Brown & Fabio. After examination of abnormalities, a few embryos were fixed in 4% buffered formaldehyde and processed for histological examination. The remaining embryos were evaluated for total protein content and DNA content.

A significant reduction in visceral yolk-sac diameter, crown–rump length, somite number, and total score was found in embryos at 5000 µmol/l. No effects on embryonic DNA and protein content were observed. The study authors concluded that only slight developmental retardation and blood discoloration were observed with 1,2,4-triazole at the highest concentration, suggesting no teratogenic activity (Menegola et al., 2001).

In a study of developmental toxicity, groups of 25 pregnant female rats [Bor:WISW (SPF Cpb)] were given 1,2,4-triazole (purity, 95.3%) at a dose of 0, 10, 30 or 100 mg/kg bw per day by gavage in aqueous 0.5% (w/w) Cremophor EL on days 6 to 15 of gestation. Stability and concentrations were confirmed analytically. Treated rats were observed daily for clinical signs and mortality. Body weights were measured on days 0, 6–15, and 20. On day 20 of gestation, all rats underwent caesarian section. Observations included number of nidations, number of fetuses (live and dead), sex of surviving fetuses, weight of each fetus, average fetal weight per litter, runts, total and average placental weight per litter, examination of all fetuses for external malformations, investigation of a number

of fetuses (approximately 30% of total) for visceral malformations (modified Wilson technique), remaining fetuses assigned to skeletal and soft tissue evaluations.

The test substance was stable for 8 days. The analytical data indicated that the variance between nominal and actual dosage (within 10%) was acceptable.

No mortality was observed. No treatment-related clinical signs were observed. Mean body-weight gain was statistically significantly reduced at 100 mg/kg bw (79.8 g vs 92.9 g for the controls). There were no treatment-related effects on pregnancy parameters. There were no treatment-related effects on fetuses at doses of up to 30 mg/kg bw per day. A significantly lower fetal weight and simultaneously greater number of runts were observed at 100 mg/kg bw per day (Table 15). The observed malformations at 100 mg/kg bw per day affected only one fetus each and were considered to be spontaneous in nature (Table 16).

The NOAEL for maternal toxicity was 30 mg/kg bw per day on the basis of decrease in body-weight gain seen at 100 mg/kg bw per day, the LOAEL. The NOAEL for developmental toxicity was 30 mg/kg bw per day on the basis of an increased incidence of runts and lower fetal weights seen at the LOAEL of 100 mg/kg bw per day (Renhof, 1988c).

Table 14. Fetal effects in a study of developmental toxicity in rats given 1,2,4-triazole by gavage

Parameter	Dose (mg/kg bw per day)			
	0	10	30	100
No. of implantations per dam	11.6	10.5	11.4	10.6
No. of males per dam	6.5	5.1 *	6.0	5.0 *
No. of females per dam	4.5	5.0	4.6	4.5
No. of males and females per dam	11.0	10.1	10.6	9.5
No. of losses per dam	0.6	0.4	0.8	1.1
Mean weight of fetuses (g)	3.58	3.59	3.53	3.25**
Mean weight of placenta (g)	0.56	0.56	0.57	0.56
No. of fetuses per litter with minor skeletal deviations	2.00	2.41	2.84	2.42
No. of fetuses per litter with malformations	0.05	0.05	0.05	0.17
No. of runts per litter	0.33	0.23	0.53	2.21**

From Renhof (1988c) and TDMG (2008).
* $p \leq 0.05$. * * $p \leq 0.01$

Table 15. Fetal malformations in a study of developmental toxicity in rats given 1,2,4-triazole by gavage

Type of malformation	Dose (mg/kg bw per day)			
	0	10	30	100
Microphthalmia, bilateral	1	0	0	0
Microphthalmia, right side	0	1	0	1
Microphthalmia, left side	0	0	0	1
False posture of right hind leg	0	0	1	0
Anophthalmia	0	0	0	1
Dysplasia and asymmetry of body of vertebrae and vertebral arches of thoracic spine and abnormal position of one rib	0	0	0	1

From Renhof (1988c) and TDMG (2008).

In a second study of developmental toxicity, groups of 25 pregnant female rats [Bor:WISW (SPF Cpb)] were given 1,2,4-triazole (purity, 94%) at a dose of 0, 100 or 200 mg/kg bw per day by gavage in aqueous 0.5% (w/w) Cremophor EL on days 6 to 15 of gestation. Stability and concentrations of the test substance in the diet were confirmed analytically. Treated rats were observed daily for clinical signs and mortality. Body weights were measured on days 0, 6–15, and 20. Food consumption was determined from days 0–6, 6–11, 11–16, and 16–20. On day 20 of gestation, all rats underwent caesarian section. Observation included number of nidations, number of fetuses (live and dead), sex of surviving fetuses, weight of each fetus, average fetal weight per litter, runts, total and average placental weight per litter, examination of all fetuses for external malformations, investigation of a number of fetuses (approximately 30% of total) for visceral malformations (modified Wilson technique), remaining fetuses assigned to skeletal and soft tissue evaluations.

The test substance was stable for 8 days. The analytical data indicated that the variance between nominal and actual dosage (within 10%) was acceptable.

No mortality was observed. No treatment-related clinical signs were observed. Mean body-weight gain was slightly (non-significantly) reduced at 100 mg/kg bw per day. Marked reduction in body-weight gain was observed at 200 mg/kg bw per day (60.4 g vs 96.9 g in the control group). Food consumption was not affected by the treatment. There were no treatment-related effects on pregnancy parameters.

Fetal weight and placental weight were reduced at 100 and 200 mg/kg bw per day. The incidence of runts was higher at 100 and 200 mg/kg bw per day (Table 16). The incidence of fetuses with minor skeletal deviations was higher at 100 mg/kg bw per day. The number of surviving fetuses per dam was reduced at 200 mg/kg bw per day. The incidence of fetuses with malformations (cleft palates and hind legs) was higher at 200 mg/kg bw per day (Table 17).

The NOAEL for maternal toxicity was < 100 mg/kg bw per day on the basis of reduced body-weight gain at 100 and 200 mg/kg bw per day. The NOAEL for developmental toxicity was < 100 mg/kg bw per day on the basis of an increased incidence of runts, lower fetal and placental weights, and a higher incidence of minor skeletal deviations at 100 mg/kg bw. Teratogenic effects (cleft palate, hind-leg malformations) were observed at 200 mg/kg bw per day (Renhof, 1988d).

Table 16. Fetal effects in a study of developmental toxicity in rats given 1,2,4-triazole by gavage

Parameter	Dose (mg/kg bw per day)		
	0	100	200
No. of corpora lutea per dam	13.6	13.9	14.2*
No. of implantations per dam	12.5	12.2	11.8
No. of males per dam	5.9	6.0	3.1**
No. of females per dam	6.1	5.9	2.4**
No. of males and females per dam	12.0	11.9	5.5**
No. of losses per dam	0.5	0.3	6.3**
Mean weight of fetuses (g)	3.55	3.06**	2.35**
Mean weight of placenta (g)	0.59	0.52*	0.49**
Fetuses per litter with minor skeletal deviations	2.67	4.32*	2.24
Fetuses per litter with malformations	0.29	0.63	0.80*
No. of runts per litter	0.24	2.84**	4.96**

From Renhof (1988d); TDMG (2008)
* $p < 0.05$; ** $p < 0.01$

Rabbits

In a study of developmental toxicity, groups of 25 timed-mated female New Zealand White [Hra:(NZW)SPF] rabbits were given 1,2,4-triazole (purity, 99.9%) at a dose of 0, 5, 15, 30 or 45 mg/kg bw per day by gavage in aqueous 0.5% (w/w) carboxymethylcellulose on days 6 to 28 of gestation. Dosing solutions were prepared weekly and stored at refrigerator temperature. Homogeneity, stability and concentrations were confirmed analytically. Treated rabbits were observed twice per day for clinical signs and mortality. Body weight and food consumption were measured daily during the dosing period and at termination. On day 29 of gestation, all surviving does were killed and necropsied, and all fetuses were weighed, examined externally, sexed internally, and subjected to a visceral examination by gross dissection. Heads from approximately half of the fetuses in each litter were examined by serial sections, and brains from the remaining fetuses were examined in situ. All fetuses were examined for skeletal alterations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage (within 10%) was acceptable.

Five females at the highest dose were killed in a moribund condition during days 16–24 of gestation after exhibiting decreased food consumption and body-weight loss beginning as early as day 7 of gestation. Gravid uterine weights were significantly reduced in the group at the highest dose. Other abnormal clinical signs at the highest dose included the following: decreased motor activity and ptosis (5 out of 5); scant, soft, and/or liquid faeces (4 out of 5); a clear perinasal substance (3 out of 5); excessive salivation (3 out of 5); hyperpnea (2 out of 5); lacrimation (1 out of 5); head tilt (1 out of 5); and feeling cold to the touch (1 out of 5). Most of these signs occurred in does that were killed in a moribund condition. One rabbit at the highest dose delivered on day 29 of gestation, before scheduled sacrifice, and this rabbit also exhibited excess salivation on day 29. Among surviving rabbits, treatment-related clinical signs were seen in four additional animals at the highest dose and included the following: clear perinasal substance from three rabbits; decreased motor activity in two rabbits; head tilt in one rabbit; and hyperpnea in one rabbit. Among survivors, there were no treatment-related effects on body weight, body-weight gain, or food consumption, and no treatment-related gross pathology was noted.

There were no total litter losses; a single dead fetus was noted in the group at 30 mg/kg bw per day. The pre- and postimplantation losses and mean numbers of corpora lutea, implantations,

Table 17. Fetal malformations in a study of developmental toxicity in rats given 1,2,4-triazole by gavage

Type of malformation	Number of affected fetuses		
	Dose (mg/kg bw per day)		
	0	100	200
Total number of fetuses examined	253	226	138
Microphthalmia, left side	2	0	0
False posture of hind legs	0	0	1
Undescended testicle	2	11	6
Hydronephrosis	1	1	7
Multiple malformations	1	0	0
Cleft palate	0	0	4
Humeral dysplasia	0	0	1
General oedema	0	0	1
Long-bone displacia	0	0	2
Diaphragmatic hernia	0	0	1

From Renhof (1988d) and TDMG (2008).

viable fetuses, and resorptions of the treated does were similar to those of controls. There were no treatment-related effects on the fetal sex ratio. Mean fetal weight was decreased in both sexes at 45 mg/kg bw per day (males, 88% of controls, $p < 0.01$; females, 90% of controls, $p < 0.05$; Table 18). The total numbers of live fetuses (and litters) evaluated in the control group and groups at the lowest, low-intermediate, high-intermediate, and highest dose were 217 (25), 207 (24), 199 (24), 218 (25), and 157 (19), respectively. No treatment-related external or skeletal malformations/variations were observed. Treatment-related urinary tract malformations were noted in four fetuses from two litters at the highest dose. These included the following (none of which were seen in controls): low set and small kidney(s) in three fetuses from the same litter; absent left kidney and absent left ureter in one of these same three fetuses; and an absent kidney in one fetus from a different litter. No visceral malformations were seen in any other group.

The NOAEL for maternal toxicity was 30 mg/kg bw per day on the basis of mortality, decreased body weight and body-weight gains, decreased food consumption, and clinical signs (decreased motor activity, ptosis, scant, soft, and/or liquid faeces, a clear perinasal substance, excessive salivation, hyperpnea, lacrimation, head tilt, and/or feeling cold to the touch) seen at the LOAEL of 45 mg/kg bw per day. The NOAEL for developmental toxicity was 30 mg/kg bw per day on the basis of decreased fetal weight and increased incidences of urinary-tract malformations (small kidneys, absent kidney, absent ureter) seen at the LOAEL of 45 mg/kg bw per day (Hobermann, 2004).

2.5 Special studies

(a) Neurotoxicity

Neurotoxicity parameters were evaluated as a part of a short-term study of toxicity in rats (described under short-term studies of toxicity).

(b) Estrogen biosynthesis

In a non-guideline, non-GLP study conducted in vitro, 1,2,4-triazole (purity not reported) was evaluated for its action on estrogen biosynthesis. Triplicate cultures containing 0.25–0.63 $\times 10^5$ viable immature rat granulosa cells were incubated in the presence of human follicle-stimulating hormone (FSH) (100 ng/ml), testosterone (10^{-7} mol/l) and the test substance (10^{-5} mol/l) for 48 h at 37 °C

Table 18. Fetal body weights in a study of developmental toxicity in rabbits given 1,2,4-triazole by gavage

Mean body weight (g/litter ± standard deviation)	Dose (mg/kg bw per day)[a]				
	0	5	15	30	45
Total fetuses	44.35 ± 3.37	43.42 ± 5.85	43.82 ± 5.70	42.48 ± 4.22	39.46 ± 5.20**
Male fetuses [a]	44.92 ± 3.78	43.91 ± 6.14	44.25 ± 5.72	42.39 ± 4.22 [24][b]	39.65 ± 4.73**
Female fetuses [a]	42.92 ± 3.95	42.79 ± 5.51 [23][c]	43.64 ± 6.17	42.40 ± 4.34	38.70 ± 5.90*

From Hobermann (2004)

[a] The number of values averaged is shown in square brackets.

[b] Litter 8081 contained no male fetuses

[c] Litter 8039 contained no female fetuses.

* $p \leq 0.05$; ** $p \leq 0.01$.

in a humidified tissue-culture incubator with 95% air/5% carbon dioxide. The medium was collected at the end of this period and stored at −20° C until analysis for estradiol and progesterone content by radioimmunoassays.

The results of the study indicated that 1,2,4-triazole had no suppressive effect on aromatase activity. The ratio of 20α-hydroxy-4-pregnen-3-one to progesterone (4 : 1 in control cultures) did not change in the presence of increasing concentrations of 1,2,4-triazole. The levels of estradiol and progesterone in rat granulosa-cell cultures were unaffected by treatment with 1,2,4-triazole. Therefore, it appeared that 1,2,4-triazole does not modulate ovarian estrogen biosynthesis in vitro (Wickings et al., 1987).

(c) Studies on metabolites

No studies on metabolites were submitted. On the basis of results of the metabolite-identification study, no studies are necessary since the major compound identified was 1,2,4-triazole itself.

3. Observations in humans

No observations in humans were submitted.

TRIAZOLE ACETIC ACID

Explanation

Triazole acetic acid (CAS No. 28711-29-7; 1,2,4-triazole-1-yl- acetic acid) is one of the three common metabolites derived from the parent triazole fungicide compounds belonging to the sterol demethylation inhibitors (the other two being 1,2,4-triazole and triazole alanine).

JMPR has not previously evaluated triazole acetic acid.

Evaluation for acceptable daily intake

Unless otherwise stated, studies evaluated in this monograph were performed by GLP-certified laboratories and complied with the relevant OECD and/or US EPA test guideline(s).

4. Biochemical aspects

4.1 Absorption, distribution, and excretion

Rats

In a pharmacokinetic study, groups of two male and female Sprague-Dawley rats were given [^{14}C]-ring labelled triazole acetic acid (purity, > 99%) as a single gavage dose at 0.58, 58.63 or 1034.69 mg/kg bw. The test substance was administered in water. Treated rats were individually housed in stainless-steel metabolism cages. Urine and faeces were collected daily for 7 days. Seven days after dosing, the rats were killed. Selected tissues and blood samples were collected and analysed for radioactivity. The study was not conducted in accordance with GLP regulations.

Total radiolabel recovery was nearly complete (100.1–107.2%). [^{14}C]-Ring labelled triazole acetic acid was readily absorbed and most of the radiolabel was excreted within 24 h. Urine was the predominant pathway of excretion. Excretion of radiolabel in the urine of male rats in 7 days was 91%; 101.7%; and 87.3% at the lowest, intermediate and highest dose, respectively. In female rats, urinary excretion of radiolabel during 7 days was 90.3%; 103.7%; and 98.7% at the lowest, intermediate, and highest dose, respectively. Total excretion of radiolabel in the faeces during 7 days ranged from 1.2% to 7.4% in male and female rats at the doses tested. Absorption was nearly complete (96.3–111.6%) on the basis of urinary excretion during 7 days. Most of the absorption occurred within 24 h. Absorption was not saturated at the highest dose tested. The excretion pattern did not exhibit sex-related variability. Total radiolabel in tissues ranged from 0.8% to 3.1% at the doses tested, indicating that triazole acetic acid and its metabolites do not undergo significant sequestration (Lai et al., 1986a).

4.2 Metabolism

A separate study of absorption and excretion was conducted to qualitatively characterize the metabolic profile of excreted triazole acetic acid. The study design and dosing was similar to the first study by Lai et al. (1986a). Urinary metabolites were identified by various TLC systems, column chromatography and GC/MS (gas chromatography/mass spectroscopy).

Most of the orally-administered dose of triazole acetic acid was rapidly eliminated in the urine within 24 h, regardless of the dose or sex. Only one single major zone with a very similar migration to the parent compound was separated by the TLC systems. After isolation and purification, derivatization and GC/MS analysis, it was concluded that the major urinary zone was unaltered parent compound. No other significant zones were detectable by autoradiography of TLC plates developed in multiple solvent systems (Lai et al., 1986b).

5. Toxicological studies

5.1 Acute toxicity

The acute toxicity of triazole acetic acid is summarized in Table 19.

Rats

Three male and three female young adult Tif:RAIf(SPF) rats were given triazole acetic acid (purity, > 99%) as a single dose at 5000 mg/kg bw by gavage in distilled water containing 0.5% carboxymethylcellulose and 0.1% polysorbate 80. Treated rats were monitored daily for mortality and clinical signs and symptoms and body weights were evaluated on days 1, 7, 14 and at death. Treated rats were subjected to gross necropsy at the end of a 14-day observation period.

No rats died during the study. Slight to moderate incidences of dyspnea, exophthalmos, ruffled fur, and hunched posture were observed after dosing. All clinical signs disappeared within 10 days after dosing. No effects on body weights were observed. No abnormalities were observed at necropsy. The LD_{50} was > 5000 mg/kg bw in male and female rats (Thevenaz, 1984).

Table 19. Acute toxicity of triazole acetic acid

Species	Strain	Sex	Route	LD50 (mg/kg bw)	Reference
Rats	Tif:RAIf(SPF)	Males and females	Gavage	> 5000	Thevenaz (1984) [a]

[a] This study was not conducted in accordance with GLP.

5.2 Short-term studies of toxicity

Rats

In a 14-day study of oral toxicity, groups of five male and five female Tif:RAIf (SPF) rats were given diets containing triazole acetic acid, (purity, 99%) at a concentration of 0, 100, 1000 or 8000 ppm, equivalent to 0, 10.6, 102.8 or 788.3 mg/kg bw per day in males and 0, 10.1, 97.2 or 703.5 mg/kg bw per day in females. Stability and dietary concentrations were confirmed analytically. Rats were inspected daily for signs of toxicity and mortality. Body weights were measured during the acclimatization period and weekly thereafter. Food and water consumption were determined weekly for each cage. Urine analysis was not performed. Ophthalmoscopic and auditory perception examinations were conducted on the control group and group at the highest dose during the acclimatization period and on day 12 of the study. At termination, blood was taken for haematological and clinical chemistry analysis. All rats sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed. Selected tissues from animals in the control group and group at the highest dose were collected for histological examination. This study was conducted in accordance with GLP.

Samples tested 50 days after preparation (stored at 22° C) were within a range of 86–99% of nominal concentrations. Test substance concentrations were 95.1, 93.1 and 89.9% of nominal, for the groups at the lowest, intermediate and highest dose, respectively. Homogeneity was not determined.

All rats survived to terminal sacrifice. It was stated that no clinical symptoms and signs of systemic toxicity were observed in the course of the study. However, no data were provided in the study report. It was stated that ophthalmic and hearing examinations did not reveal any treatment-related effects. Again, however, no data were provided in the study report. There were no treatment-related changes in body weight, food consumption, water consumption, haematology, or clinical chemistry parameters for any group of rats. Absolute liver weight in males at the highest dose was 86% of control values. The liver/body weight and liver/brain weight ratios were also decreased in males at the highest dose, to 90% and 86% of control values, respectively. A statistically-significant ($p < 0.01$) dose-related trend of decreasing liver/body weight ratios was seen in all treated groups of males. Absolute adrenal weights were 72% and 78% of control values in males at the intermediate and highest dose, respectively. Adrenal/body weight and adrenal/brain weight ratios were decreased to approximately 72% and 78% of control values in the groups at the intermediate and highest dose, respectively. Organ weights in females were comparable to those of the controls. No treatment-related gross or microscopic findings were seen in any rats.

Table 20. Results of studies of genotoxicity with triazole acetic acid in vitro

End-point	Test system	Concentration	Purity (%)	Result	Reference
Reverse mutation (Ames test)	*S. typhimurium* strains TA98, TA100, TA153 and TA1537 *E. coli* WP2P; WP2P *uvrA*	0, 20, 80, 320 1280 and 5120 µg/0.1 ml	> 99	Negative	Deparade (1984) [a]
Forward mutation	L5178Y mouse lymphoma cells	0.63, 1.25, 2.5, 5 and 10 mmol/l (±S9)	96.95	Negative	Clare (2002)
Chromosomal aberration	Human lymphocytes	2.5, 5 and 10 mmol/l (±S9)	96.95	Negative	Pitchard (2002)

S9, 9000 × *g* supernatant from rodent liver.
[a] This study was not conducted in accordance with good laboratory practice.

The NOAEL was 8000 ppm, equivalent to 703.5 mg/kg bw per day, the highest dose tested. It is difficult to interpret the data on organ weights confidently owing to the high variability and the small number of animals evaluated (Thevenaz, 1986).

5.3 Long-term studies of toxicity and carcinogenicity

No studies were submitted.

5.4 Genotoxicity

The results of studies of genotoxicity with triazole acetic acid are summarized in Table 20.

5.5 Reproductive toxicity

No studies were submitted.

5.6 Special studies

No studies were submitted.

Studies on metabolites were not submitted. On the basis of results of a metabolism-identification study, no studies are necessary since the major metabolite was triazole acetic acid itself.

6. Observations in humans

Observations in humans were not submitted.

TRIAZOLE ALANINE

Explanation

Triazole alanine (or triazolyl alanine, CAS No. 10109-05-4; 1,2,4-triazolyl-3- alanine-IUPAC; alpha-amino-1*H*-1,2,4-triazole-3-propanoic acid) is one of the three common metabolites derived from the parent triazole fungicide compounds belonging to the sterol demethylation inhibitors, the other two being 1,2,4-triazole and triazole acetic acid. It is commonly present as plant or soil metabolite.

Triazole alanine was first evaluated by the JMPR in 1989. The Meeting at that time concluded from the available data that residues of triazole alanine arising from the use of triazole fungicides do not present a toxicological hazard.

For the present evaluation, no new studies were submitted, except a pharmacokinetic study in rats.

Evaluation for acceptable daily intake

Unless otherwise stated, studies evaluated in this monograph were performed by GLP-certified laboratories and complied with the relevant OECD and/or US EPA test guideline(s).

7. Biochemical aspects

7.1 Absorption, distribution, and excretion

Rats

In a pharmacokinetic study, two groups of four male and female Tif: RAI f (SPF) rats were given [^{14}C]-labelled triazole alanine (at position 3 and 5 of the triazole ring; purity, > 99%) as a single gavage dose at 0.5 or 50 mg/kg bw. The test substance was given in water. Treated rats were individually housed in stainless-steel metabolism cages. Urine, faeces and expired air were collected at 24-h intervals for 7 days. Seven days after dosing, the rats were killed. Selected tissues and blood samples were collected and analysed for radioactivity.

Total radiolabel recovery was nearly complete (98.99–109.47%). [^{14}C]-Ring labelled triazole alanine was readily absorbed and excreted, mainly via the urine. Excretion of radiolabel in the urine of male rats in 24 h was 96.06% and 97.67% at the lowest and highest dose, respectively. In female rats, urinary excretion of radiolabel in 24 h was 92.01% and 98.96% at the lowest and highest dose, respectively. Approximately 3–7% of the administered doses were recovered in the faeces after 7 days. Less than 0.5% of the administered dose was excreted in the expired air. No tissue residues were found after 7 days at 0.5 mg/kg bw. At 50 mg/kg bw, only minute residues, not exceeding 22 ppb D,L-triazole alanine equivalents were found, mainly in the liver, kidneys and blood. The total amount remaining in the rats after 7 days did not exceed 0.01% of the administered dose. Up to 86% of the administered dose was excreted unchanged in the urine. TLC analysis (0–24 h) of the urinary metabolites revealed one major (U1) and one minor metabolite (U2), accounting for 72–86% and 8–19% of the recovered radiolabel, respectively (Hamboeck, 1983a).

In a separate non-GLP study of pharmacokinetics, groups of two male and two female Sprague-Dawley rats received radiolabeled D,L-triazole alanine (at position 3 and 5 of the triazole ring; purity, > 99%) as a single oral dose at 0.56, 54.4, or 993.7 mg/kg bw by gavage in polyethylene glycol (PEG). Urine and faeces samples were collected at 24-h intervals for 7 days. Rats were killed after 7 days and blood and various tissues were collected for analysis.

Recovery of radiolabel was nearly complete (90.7–101.2%). Urine was the main route of excretion. Within 24 h, 66.1–79.7% of the administered doses were recovered in the urine. An average of 97.4%, 87.35 and 88.2% of the total dose was excreted after 48 h at the lowest, intermediate and highest dose, respectively. Approximately 6–18% of the administered dose was recovered in the faeces after 7 days. The concentrations of radiolabel in selected tissues after 7 days were low at all doses (0.004 ppm, 0.40 ppm and 8.5 ppm at the lowest, intermediate and highest dose, respectively (Lai & Simoneaux, 1986a, 1986b).

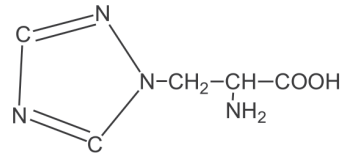
7.2 Biotransformation

In a study of metabolism identification, excreta obtained from rats given a single oral dose of [^{14}C]-D,L-triazolyl alanine at 0.5 or 50 mg/kg bw in the previously described study by Hamboeck (1983a) were used for isolation of metabolites. The excretory products in the urine and in faeces were subjected to metabolite identification using high-voltage electrophoresis (HVE), high-performance liquid chromatography (HPLC), NMR and MS analysis techniques. Approximately 69–86% of the administered dose in urine and 1–2% of the administered dose in faeces was identified as the parent compound, D,L-triazole alanine. Approximately 8–19% of the excreted dose in the

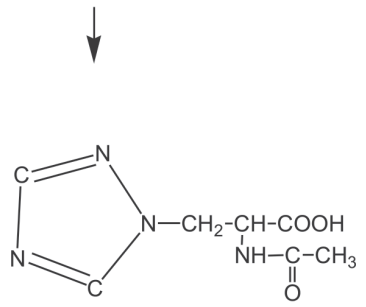
urine and < 1% of the administered dose in faeces was identified as *N*-acetyl-D,L-triazole alanine (Hamboeck, 1983b).

In a second study of metabolism identification that did not comply with GLP, excreta obtained from rats given a single oral dose of [^{14}C]-D,L-triazolyl alanine at 0.5 or 50 mg/kg bw in the previously described studies by Lai & Simoneaux (1986a, 1986b) were used for isolation of metabolites. Urine samples were subjected to TLC for metabolic identification using five different solvent systems for metabolic separations. The TLC analysis revealed that D,L-triazole alanine, represented 82–93% of the radiolabel in 0–24-h urine and *N*-acetyl-D,L-triazole alanine represented 13–30% of the radiolabel in 0–24-h urine (Lai & Simoneaux, 1986a, 1986b). Figure 3 shows the metabolic pathway of triazole alanine in rats.

Figure 3. Metabolic pathway of triazole alanine in rats



α-Amino-1,2,4-triazole-1-propanoic acid



2-Acetylamino-3-(1H-1,2,4-triazol-1-yl)-propanoic acid

Table 21. Acute toxicity with triazole alanine

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Reference
Rat	Wistar	Males and females	Oral	> 5000	Mihail (1982)
	Bor:WISW (SPF-Cpb)				
Rat	Alderley Park (SPF)	Males and females	Oral	> 2000	Henderson & Parkinson (1980)
Mouse	NMRI (SPF-Hah)	Males and females	Oral	> 5000	Mihail (1982, 1986)

8. Toxicological studies

8.1 Acute toxicity

The acute toxicity of triazole alanine is summarized in Table 21.

(a) Lethal doses

Rats

Triazole alanine (analytically pure) was given to groups of 10 male and 10 female Bor:WISW (SPF-Cpb) Wistar rats as a single dose at 500, 1000, 2500 and 5000 mg/kg bw for fasted males and 5000 mg/kg bw for fasted females by gavage in 2.0% Cremophor EL in distilled water. A single gavage dose of 2500 and 5000 mg/kg bw was given to fed males and 5000 mg/kg bw to fed females. The rats were examined daily for clinical signs and mortality. Treated rats were subjected to gross necropsy at the end of a 14-day observation period. Body weights were recorded at initiation and at the end of the study.

Fasted male rats at 5000 mg/kg bw exhibited polyuria on the day of treatment. In some cases, the lungs were mottled, had dark discoloration, and were distended. Some findings, e.g. firm zones of the lungs, were interpreted as the result of infection. There were no indications of treatment-related gross necropsy findings. No findings were reported in fasted females at 5000 mg/kg bw. No mortality was observed in fasted male and female rats. One fed male rat of the group at 5000 mg/kg exhibited the following signs starting on day 7 of the study: piloerection, tachypnea (accelerated breathing], stiff/spastic gait, and staggering. The first two signs lasted 1 day. The impaired activity persisted up to day 11 of the study. These clinical signs were not considered to be treatment-related because they were not observed in other fed rats or in fasted rats. No treatment-related effects were observed at necropsy. No mortality was observed in fed male and female rats. The oral LD_{50} in rats (fasted and fed) was > 5000 mg/kg bw (Mihail, 1982).

In a second study, groups of five male and five female Alderley Park (SPF) rats were given triazole alanine (purity not reported) as a single dose at 2000 mg/kg bw by gavage in 20% (w/v) suspension in distilled water. The rats were examined daily for clinical signs and mortality for 14 days. No mortality or clinical signs of toxicity were observed. The oral LD_{50} in rats was > 2000 mg/kg bw (Henderson & Parkinson, 1980).

Mice

Groups of five male and five female NMRI (SPF-Hah) fasted mice were given triazole alanine (purity, 92.8%) as a single dose at 5000 mg/kg bw by gavage in 2.0% Cremophor EL in distilled water. The rats were examined daily for clinical signs and mortality. Treated mice were subjected to gross necropsy at the end of a 14-day observation period. Body weights were recorded at initiation and at the end of the study.

No treatment-related clinical signs, body-weight changes, necropsy findings or mortalities were observed. The oral LD_{50} in mice was > 5000 mg/kg bw (Mihail, 1982).

(b) Administration dermally or by inhalation

No studies were submitted.

(c) Dermal and ocular irritation or sensitization

No studies were submitted.

8.2 Short-term studies of toxicity

Rats

In a 2-week study of oral toxicity, which did not comply with GLP or relevant guidelines, groups of 10 male Bor:WISW (SPF-Cpb) rats were given drinking-water containing triazole alanine (purity, approximately 100%) at a concentration of 0, 3000 or 10 000 ppm. The average daily doses received by the rats were equal to 0, 448 and 1491 mg/kg bw per day, respectively. Cage-side observations were made twice daily, body weights were recorded weekly while food and water consumption were recorded for the entire study period. No opthalmoscopic examinations, urine analysis or haematology and clinical parameter measurements were conducted. All necropsied animals were examined for gross pathology. The weights of the thyroid, thymus, liver, spleen, kidneys, adrenals and brain were determined during gross examination.

Appearance, behaviour, body weight, food consumption, water intake, organ weight, mortality and necropsy findings were unaffected by treatment with drinking-water containing triazole alanine at concentrations up to 10 000 ppm. On the basis of the results of this range-finding study, the study author recommended that concentrations of 1000, 3000 and 10 000 ppm in drinking-water should be tested in short-term study of toxicity.

The NOAEL was > 10 000 ppm, equal to 1491 mg/kg bw per day, the highest concentration tested (Bomhard, 1982).

In a 28-day study of toxicity, which did not comply with GLP, groups of 20 male and 20 female Bor:WISW (SPF-Cpb) rats were given triazole alanine (analytically pure) at a dose of 0, 25, 100 and 400 mg/kg bw per day by gavage suspended in distilled water with added Cremophor EL. After treatment, half the rats from each group were observed for an additional 28 days (recovery period). The rats were inspected daily for signs of toxicity and mortality, with clinical examinations conducted at the end of the 28-day treatment period and at the end of the 4-week recovery period. Body weight and food consumption were measured weekly. At termination, blood was taken for haematological and clinical chemistry analysis. No ophthalmoscopic examination was performed. Urine analysis was performed at the end of the 28-day treatment period and at the end of the 4-week recovery period. At the end of 4 weeks of treatment and on completion of the 4-week recovery period, 10 males and 10 females from each group were sacrificed and subjected to gross pathological examination. Selected tissues from five males and five females in each group were examined histologically and selected organs were weighed.

The test suspension was prepared before each treatment and it was stable for at least 7 h. Results of concentration analysis were not provided.

No biologically or toxicologically significant treatment-related effects were found on food or water consumption, body weight or body-weight gain, and haematology, clinical chemistry or urine-analysis parameters. There were no macroscopic and microscopic changes in tissues and organs. Blood urea and creatinine concentrations (Table 22) were lower in males at 400 mg/kg bw per day and lower urea concentrations were also observed in males at 400 mg/kg bw per day at the end of the recovery period; however, there was no kidney pathology associated with these findings nor were any other clinical parameters affected. Therefore, these effects were not considered to be adverse. Organ-weight analysis revealed lower absolute and relative liver weights for females at 400 mg/kg bw per day (Table 23). This finding was considered spurious in the absence of a corroborating histopathology and clinical chemistry. No induction of total cytochrome P450 was found and the concentration of hepatic triglycerides was not increased.

The NOAEL was > 400 mg/kg per day (the highest dose tested) for males and females exposed to triazole alanine by gavage daily for 28 days (Mihail. & Vogel, 1983).

In a 90-day study of toxicity, groups of 20 male and 20 female Bor:WISW(SPF_Cpb) rats were given diets containing triazole alanine (purity, 97.5%) at a concentration of 0, 1250, 5000 or 20 000 ppm. The average daily doses received by males were 0, 90, 370 and 1510 mg/kg bw per day, respectively and 0, 160, 400 and 1680 mg/kg bw per day for females, respectively. Diets were prepared and stored at room temperature (frequency of preparation not reported). Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice daily for signs of toxicity and mortality, with clinical examinations done after 1 month of treatment and at the end of the study. Body weight and food consumption were measured weekly. Blood was taken for haematological and clinical chemistry analysis after 1 month of treatment and at the end of the study. Urine analysis and ophthalmoscopic examinations were performed before the start of the study and at the end of the study. The selected tissues from all males and females in the control group and the group at 20 000 ppm, and from all animals that died during the study, were examined histologically. In addition, the liver, lung and kidneys and all grossly altered organs or possible target organs from all rats in the groups at 1250 and 5000 ppm were weighed and examined histologically.

Diets were stable for up to 14 days at room temperature. The test article homogeneity results were within the acceptable range (± 12% of nominal). The test substance concentration analysis indicated that the measured test concentrations ranged between 88–116% of the target concentrations.

Males at 20 000 ppm had slightly reduced body weights (approximately 8%) throughout the study and an approximately 11% decrease in total body-weight gain by the end of the study. Minor decreases in leukocyte counts were observed at 20 000 ppm. Clinical chemistry demonstrated changes of no toxicological significance, i.e. statistically significant decreases in triglyceride, bilirubin and urea concentrations in males at 20 000 ppm and in triglyceride concentrations in females at 5000 and 20 000 ppm (Table 24). These changes in haematological and clinical chemistry parameters were considered of no toxicological significance since the changes were small in magnitude, were not seen throughout the study, and were likely to be due to decreases in body-weight gains. No significant treatment-related effects were found for organ weight, haematology, clinical chemistry, urine analysis, or macro- and microscopic tissue examinations.

Table 22. Clinical chemistry parameters (mean values) in male rats given triazole alanine by gavage for 28 days

Parameter	Dose (mg/kg bw per day)							
	0		25		100		400	
	Treatment	Recovery	Treatment	Recovery	Treatment	Recovery	Treatment	Recovery
Urea (mmol/l)	6.65	7.00	6.39	7.25	6.64	5.95	5.35*	5.78*
Creatinine (µmol/l)	61	47	66	48	60	49	49**	52

From TDMG (2008)
* $p \leq 0.05$, ** $p \leq 0.01$.

Table 23. Mean liver weights for female rats given triazole alanine by gavage for 28 days

Liver weight	Dose (mg/kg bw per day)			
	0	25	100	400
Absolute (g)	6.79	6.80	6.52	5.88**
Relative (% of body weight)	3.98	3.88	3.80	3.59**

From TDMG (2008)
** $p < 0.01$

The NOAEL was 5000 ppm, equal to 370 mg/kg bw per day, on the basis of decreased body-weight gains seen at the LOAEL of ≥ 20 000 ppm, equal to 1510 mg/kg bw per day, the highest dose tested (Maruhn & Bomhard, 1984).

Dogs

In a 90-day study of toxicity, four male and four female dogs were given diets containing triazole alanine (purity, 97.5%) at a concentration of 0, 3200, 8000 or 20 000 ppm (equal to 0, 144, 322, and 850 mg/kg bw per day for males and 0, 150, 345, and 902 mg/kg bw per day for females, respectively). Diets were prepared daily. Stability, homogeneity and dietary concentrations were confirmed analytically. The dogs were inspected daily for signs of toxicity and mortality, with clinical observations conducted before the start of the study and in weeks 2, 4, 7 and 13. Body weights were measured weekly and food consumption was measured daily. Before the start of the study and on weeks 2, 4, 7 and 13 blood was taken for haematological and clinical chemistry analysis. Urine analysis was performed before the start of the study and on weeks 2, 4, 7 and 13, while ophthalmoscopic examinations were performed before the start of the study and during weeks 7 and 13. All dogs that died and those that were sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed. Selected tissues were collected for histological examination.

The test-article homogeneity and stability results were within the acceptable range (± 15% of nominal). The analysis of test-substance concentration indicated that the measured test concentration was within 12% of nominal.

No treatment-related deaths or changes in appearance or behaviour occurred during the study. Body temperature, pulse rates, neurological examinations and ophthalmoscopic examinations were not affected by treatment. At the highest dose, female body-weight gain (20% of control) and food consumption (90% of control) were slightly decreased. No haematology or clinical chemistry parameters, gross/histopathology observations, or organ weights showed treatment-related effects. The LOAEL for female dogs was 20 000 ppm, equal to 902 mg/kg bw per day, on the basis of decreased body-weight gains and decrease in food consumption. The corresponding NOAEL for female dogs was 8000 ppm, equal to 345 mg/kg bw per day. The NOAEL for male dogs was > 20 000 ppm, equal to 850 mg/kg bw per day, the highest dose tested (von Keutz & Gröning, 1984).

8.3 Long-term studies of toxicity and carcinogenicity

No studies were submitted.

Table 24. Clinical chemistry parameters (mean values) in male and female rats given diets containing triazole alanine for 90 days

Parameter	Dietary concentration (ppm)															
	0				1250				5000				20 000			
	Males		Females		Males		Females		Males		Females		Males		Females	
	Week 4	Week 13	Week 4	Week 13	Week 4	Week 13	Week 4	Week 13	Week 4	Week 13	Week 4	Week 13	Week 4	Week 13	Week 4	Week 13
Bilirubin (µmol/l)	2.7	4.4	1.7	3.2	2.5	4.2	1.5	3.2	2.5*	4.1	1.4	2.7	2.0**	3.8**	1.6	3.1
Urea (mmol/l)	8.0	7.6	8.5	7.9	7.6	7.2	8.1	7.6	7.7	6.9	7.3**	7.6	7.7	6.8*	7.5*	7.9
Triglycerides (mmol/l)	1.00	1.22	0.90	1.30	0.98	1.03	0.87	1.24	1.05	1.11	0.61*	0.77**	0.82	0.72**	0.58**	0.85**

From TDMG (2008)
* $p < 0.05$; ** $p < 0.01$

8.4 Genotoxicity

Overall, triazole alanine gave negative results in an adequate battery of assays for genotoxicity in vivo and in vitro. The results of studies of genotoxicity with triazole alanine are summarized in Table 25.

8.5 Reproductive toxicity

(a) Multigeneration studies

In a preliminary study of reproductive toxicity, which did not comply with GLP or relevant guidelines, groups of six male and 12 female Alderley Park rats were given diets containing triazole alanine (purity > 90%) at a concentration of 0, 150, 625, 2500 or 10 000 ppm 6 weeks before mating on a two-

Table 25. Results of studies of genotoxicity with triazole alanine

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Gene mutation[a]	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	20–5 000 µg/0.1 ml ± S9 in DMSO	97.4	Negative	Deparade (1986)
	S. typhimurium TA98, TA100, TA1535 and TA1537	312.5–5 000 µg/plate ± S9 in DMSO	> 96	Negative	Hertner (1993)
	E. coli strain *WP2uvrA*				
	S. typhimurium TA98, TA100, TA1535, TA1537 and TA1538	20–12 500 µg/plate ± S9 in DMSO	Not stated	Negative	Herbold (1983a) [a]
Gene mutation	Chinese hamster V79 cells	500–10 000 µg/0.1 ml ± S9 in water	97.4	Negative	Dollenmeier (1986)
Bacterial DNA repair	*E. coli* pol A+ and pol A1-	62.5–1 000 µg/plate ± S9 in DMSO	NS	Negative	Herbold (1983b) [a]
DNA repair test (rec assay)	*Bacillus subtilis* H17 (rec+) and M45 (rec-)	20–1 000 µg/disk ± S9 in water	> 96	Negative	Watanabe (1993)
Mammalian DNA repair	Rat hepatocytes	80–10 000 µg/ml ± S9 in culture medium	97.4	Negative	Puri (1986)
Mammalian cell transformation	BALB/3T3, clone A31-1-1 mouse fibroblasts	62.5–1 000 µg/ml ± S9 in distilled water	97.4	Negative	Beilstein (1984)
Mammalian cell transformation	Baby hamster kidney (BHK 21 C13) cells	500–8 000 µg/ml −S9 1 000–16 000 µg/ml +S9	NS	Positive	Richold (1981)
In vivo					
Micronucleus formation	Mouse (NMRI)	8 000 mg/kg bw, single oral dose in Cremophore solution	Not stated	Not clastogenic or aneugenic	Herbold (1983c)
	Mouse (CBC F1)	2 500 and 5 000 mg/kg bw, intraperitoneal injection in 0.5% Tween 80	97.4	Not clastogenic or aneugenic	Watkins (1982)
	Chinese hamster	5 000 mg/kg bw, single oral dose in 0.5% carboxymethylcellulose	97.4	Not clastogenic or aneugenic	Strasser (1986)

DMSO, dimethyl sulfoxide; S9, 9000 × *g* supernatant from rodent liver.
[a] Study did not comply with good laboratory practice.
NS = not stated

females-to-one-male basis. The purity of the first batch of triazole alanine was 48% and not > 90% as was originally specified. The dietary concentrations of the second batch using a purer compound were adjusted to the first batch in order to maintain continuous exposure. Male rats were killed after mating and females were allowed to continue on treated diets throughout pregnancy, lactation and weaning of offspring. All parents and selected offspring were subjected to necropsy and selected tissues were examined. Parameters that were recorded or derived during the study included clinical observations, body weight, food consumption and food use during the pre-mating period, body-weight gain in females during pregnancy and in litters from birth to weaning, male and female fertility indices, gestation length, pre-coital interval, live-born index, survival index, litter size and sex distribution.

Diets were stable for up to 10 weeks and homogeneity was satisfactory. The mean achieved dietary concentrations were within 14% of the nominal concentrations except for the second occasion for the dietary concentration of 10 000 ppm (within 21% of the nominal).

No treatment-related clinical signs or mortality were observed. No treatment-related effects on body weight and food consumption were observed. A prolonged pre-coital interval (statistically significant) was noted in rats at 10 000 ppm. No other reproductive parameters were affected by the treatment. At 10 000 ppm, the group-mean litter weight of both male and female pups was significantly reduced on day 1. No other abnormalities in the offspring were noted.

The NOAEL for parental toxicity was 10 000 ppm, equivalent to 1000 mg/kg bw per day, the highest dose tested. The NOAEL for reproductive and offspring toxicity was 2500 ppm, equivalent to 250 mg/kg bw per day, on the basis of increases in pre-coital interval (statistically significant) and the slight reductions in neonatal weights of males and females at 10 000 ppm (Birtley, 1983).

In a two-generation study of reproduction, groups of 15 male and 30 female Alpk:AP(Wistar-derived, SPF) rats were given diets containing triazole alanine (purity, 97.8% w/w) at a concentration of 0, 500, 2000, or 10 000 ppm. The diets were given during premating, mating, gestation and lactation for two successive generations. Two litters were produced in each generation. The F_0 parental rats, aged 4 weeks at the start of premating, received test diets for 12 weeks before they were paired to produce the F_{1a} litters. The F_1 parental animals, aged 5 weeks old when selected at the start of premating, received the test diets for 11 weeks before they were paired to produce the F_{2a} litters. The F_0 and F_1 parents were aged about 16 weeks at mating. After a brief rest period after weaning the first litters, the F_0 and F_1 were re-mated to produce the F_{1b} and F_{2b} generations, respectively. Premating doses of the compound, estimated from graphs, averaged 50, 213 and 1098 mg/kg bw per day, respectively, for F_0 males; 51, 223 and 1109 mg/kg bw per day, respectively, for F_0 females; 47, 192 and 929 mg/kg bw per day, respectively, for F_1 males and 49, 199 and 988 mg/kg bw per day, respectively, for F_1 females. Stability, homogeneity and dietary concentrations were confirmed analytically. Body weights and food consumption were determined on days 1, 8, 15 and 22 of gestation and on days 1, 5, 11, 22 and 29 of lactation. Litter size, number of live and dead pups, individual sexes, weights, and external observations were recorded for pups on the same lactation days. At each dose, rats were randomly selected to continue on treatment as parents for the F_2 generation. They underwent the same study phases as F_0 rats. All F_1 weanlings that were not selected to be parents and all F_2 weanlings were examined as indicated for the culled pups. Adults were necropsied and reproductive tissues were examined histologically.

The test substance was evenly distributed and chemically stable in the diet for approximately 2–3 months. The mean concentrations of test substance were within 10% of the nominal concentrations except for one diet prepared for the group at 10 000 ppm, which was 13% lower than target. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage was acceptable.

A few parental rats died or were killed because of conditions not related to administration of triazole alanine and there were no treatment-related effects on clinical signs, body weight, food

consumption, food use, gross necropsy, microscopic findings, and any measures of reproductive performance. No adverse effects were observed on clinical signs and food consumption, parental body weight, and necropsy findings. No effects were noted on reproductive parameters. There were no treatment-related effects on the following offspring parameters of this study: percentage live-born pups, pup viability, sex ratio, mean litter size, live-birth index, and survival index to day 22. Litter weights and body weights of the F_{1a} group at the highest dose are reduced approximately 10% at all time points, while the F_{1b} offspring were comparable to control values. The F_{2a} litter weights were significantly lower than controls at the intermediate and highest dose; however, the effects were not dose-related. The F_{2b} offspring exhibited decreased mean litter weights in the group at the highest dose. The decreases were statistically and biologically significant at all time points ($p < 0.01$ to $p < 0.05$; decreased 16.3–19.8% in comparison to control values).

The NOAEL for parental systemic toxicity and reproductive toxicity was > 10 000 ppm, equal to 929 mg/kg bw per day, the highest dose tested. The NOAEL for offspring systemic toxicity was 2000 ppm, 192 mg/kg bw per day, on the basis of reduced mean litter weights seen in males and females of both generations at 10 000 ppm, equal to 929 mg/kg bw per day (Milburn et al., 1986).

(b) Developmental toxicity

In a study of developmental toxicity, groups of 24 Alderley Park Alpk/AP (Wistar-derived) female rats were given triazole alanine (purity, 94.8% active ingredient) at a dose of 0, 100, 300 or 1000 mg/kg bw per day by gavage on days 7 to 16 of gestation. Dams were observed for clinical observations, body weight and food consumption. Dams were sacrificed on day 22 of gestation and examined grossly. Each fetus was examined for external malformations including cleft palate. Two thirds of each litter were examined for visceral abnormalities and were subsequently processed for skeletal examination. The remaining fetuses were fixed and decalcified in Bouin fluid for soft tissue examination and serial sectioning of the head.

There were no treatment-related effects on survival, clinical signs, body weight or body-weight gain, food consumption or caesarian parameters. The number of fetuses (litters) examined externally and viscerally was 279 (24), 301 (24), 264 (24) and 293 (24) and the number examined skeletally was 185 (24), 201 (24), 178 (24) and 195 (24) in the control group and groups at the lowest, intermediate and highest dose, respectively (Table 26 and Table 27). A statistically significant increase in the fetal incidence of several skeletal anomalies was found in the group at the highest dose. The fetal (litter) incidence rates in the control group and groups at the lowest, intermediate, and highest dose were 95 (23), 95 (23), 92 (23) and 122 (23), respectively, for total minor skeletal findings, 1 (1), 3 (2), 2 (2) and 12 (7), respectively, for partially ossified transverse processes of the seventh cervical vertebra (bilateral), 0 (0), 0 (0), 1 (1) and 6 (2), respectively, for unossified fifth sternebra, and 1 (1), 4 (2), 4 (4) and 7 (6), respectively, for partially ossified 13th thoracic centrum. In addition, the fetal incidence of unossified odontoid process was increased in groups at the intermediate and highest dose, 12 (9), 6 (6), 24 (13) and 29 (15), respectively. In the absence of historical control data, these skeletal anomalies were considered to be adverse.

The NOAEL for maternal toxicity was 1000 mg/kg bw per day on the basis of the absence of adverse findings at this dose, the highest tested. The NOAEL for developmental toxicity was 100 mg/kg bw per day on the basis of an increased incidence of non-ossification of the odontoid process (delayed ossification) at 300 mg/kg bw per day. Triazole alanine did not induce teratogenicity at up to the highest dose of 1000 mg/kg bw per day (Clapp et al., 1983).

8.6 Special studies

No studies were submitted.

Studies on metabolites were not submitted. On the basis of results of the metabolism-identification study, no studies were necessary since the major compound identified was triazole alanine itself.

9. Observations in humans

Observations in humans were not submitted.

Table 26. Incidence of external and visceral effects in a study of developmental toxicity in rats given triazole alanine by gavage

Observations	Dose (mg/kg bw per day)			
	0	100	300	1000
Fetuses (litters) examined	279 (24)	301 (24)	264 (24)	293 (24)
Fetuses (litters) affected—minor defects	13 (7)	5 (5)	12 (8)	7 (5)
Fetuses (litters) affected—major defects	0	0	0	1 (1)
Unilateral increased renal pelvic dilatation-slight (minora)	10 (5)[b]	4 [c] (4)	8 (6)	4 (3)
Severely malformed fetus (major)	0	0	0	1 (1)
Bilateral increased renal pelvic dilatation-slight (minor)	2 (2)	0	2 (2)	1 (1)
Left gonad vestigial (minor)	0	0	0	1 (1)

From Clapp et al. (1983)

[a] Moderate dilatation was seen in one pup at the intermediate dose.

[b] Fetal (litter) incidence

[c] The summary table indicated that five fetuses had this finding; however, this reviewer could only find four.

Table 27. Skeletal effects in a study of developmental toxicity in rats given triazole alanine by gavage

Observations	Dose (mg/kg bw per day)			
	0	100	300	1000
Fetuses (litters) examined	185 (24)	201 (24)	178 (24)	195 (24)
Fetuses (litters) affected—minor defects	95 (23)	95 (23)	92 (23)	122* (23)
Fetuses (litters) affected—major defects	0	0	0	1 (1)
Fetuses (litters) affected—variants	170 (23)	197** (24)	169 (24)	186 (24)
Transverse processes of seventh cervical vertebra-bilateral- partially ossified (minor)	1 (1)[a]	3 (2)	2 (2)	12** (7)
Fifth sternebra not ossified (minor)	0	0	1 (1)	6[b] (2)
Odontoid process not ossified (minor)	12 (9)	6[c] (6)	24* (13)	29** (15)
Thirteenth thoracic centrum partially ossified (minor)	1 (1)	4 (2)	4 (4)	7* (6)

From Clapp et al. (1983)

[a] Fetal (litter) incidence.

[b] The summary table indicates that seven fetuses had this finding and based on this incidence it was statistically higher than the control group; however, this reviewer could only find six fetuses with this finding which is also likely to be significantly higher. It is possible this finding was observed in the fetus that was severely malformed; however, from the description of that fetus, it cannot be confirmed.

[c] This reviewer could only find six fetuses with odontoid process not ossified. The report indicated that the incidence was 10.

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

Comments

1,2,4-TRIAZOLE

Biochemical aspects

In rats treated orally, radiolabelled 1,2,4-triazole was rapidly and completely absorbed and excreted mostly unchanged and mainly in the urine (80–94%) in the first 24 h, irrespective of dose or route of administration. Approximately 0.1% of the administered dose was recovered within 30 h in expired air after oral and intravenous administration. Approximately 3–5% of the administered dose was recovered in faeces in 48 h. Approximately 2% of the administered dose was recovered in the gastrointestinal tract at 48 h. In bile duct-cannulated rats, approximately 12% of the dose was recovered in the bile at 24 h after intravenous or intraduodenal application.

Toxicological data

1,2,4-Triazole is of moderate toxicity when administered orally. The LD_{50} in rats treated orally was 1648 mg/kg bw. The LD_{50} in rats treated dermally was 3129 mg/kg bw. 1,2,4-Triazole appears to be more toxic dermally in rabbits than in rats. The dermal LD_{50} in rabbits was > 200 and < 2000 mg/kg bw. It is slightly irritating to the skin and severely irritating to the eyes of rabbits. It is not a skin sensitizer as determined by Magnusson & Kligman (maximization) test in guinea-pigs. The following clinical signs were observed after oral dosing: sedation, breathing difficulties, reduction in general well-being, hunched posture (at higher doses). These signs appeared within 1 h of administration and were observed for a maximum of 13 days after administration. Similar clinical signs were observed in rats treated dermally.

In short-term studies in mice and rats, neurotoxicity was seen in number of studies. In a 28-day study of toxicity in mice, the only treatment-related effects were slight testicular degeneration accompanied by apoptotic bodies at 2000 ppm, equal to 356 mg/kg bw per day (the LOAEL). No effects were observed in females at doses up to and including 2000 ppm, equal to 479 mg/kg bw per day. The NOAEL in mice was 500 ppm, equal to 90 mg/kg bw per day.

In a 90-day study of toxicity in mice, decreased body weight, tremors (observed from day 30) and loss of cerebellar Purkinje cells were observed in males and females at 6000 ppm, equal to 988 mg/kg bw per day. At 6000 ppm (the highest dose), 9 out of 11 males showing tremors also had Purkinje cell loss, while in females at this highest dose one out of three mice with tremors had Purkinje cell loss. Decreased testicular weights and histopathological findings in testes similar to the 28-day study were observed in males at 3000 and 6000 ppm. The NOAEL was 1000 ppm, equal to 161 mg/kg bw per day, on the basis of tremors, decreased brain weights, decreased testicular weights and histopathological changes in the testes seen in males at the LOAEL of 3000 ppm, equal to 487 mg/kg bw per day.

In a 90-day dietary study of toxicity in rats, retarded body-weight development, transient effects on the central nervous system, lower erythrocyte parameters (microcytic hypochromic erythrocytes, in males only) and hepatocellular fat accumulation (males only) were observed at 2500 ppm, equivalent to 212.3 mg/kg bw per day. The NOAEL was 500 ppm, equivalent to 37.9 mg/kg bw per day. In a combined short-term study of toxicity and neurotoxicity in rats, FOB effects were observed at 3000 ppm and 1000/4000 ppm (equal to 183 and 210 mg/kg per day, respectively) and with increased incidence and severity at week 8. Males were more severely affected than females. Other effects observed were ungroomed appearance, red nasal and lachrymal stain, yellow urine stain, muscle fasciculations, tremors, gait incoordination, decreased activity in the open field, decreased rearing, uncoordinated righting reflex and increased foot splay. A decrease in motor and locomotor activity was also observed in males at 3000 ppm during week 4 only. Decreases in absolute brain weights and degenerative lesions were seen in the cerebellum, the lumbar dorsal root ganglion and

other peripheral nerves at 3000 ppm and at 1000/4000 ppm. The brain lesions were limited to the anterior, dorsal cerebellum and were coded overall as an increased incidence of cellular degeneration and necrosis. Findings were characterized by extensive loss of Purkinje cells, variable white-matter degeneration and gliosis. Subtle atrophy of the molecular layer, primarily at the cerebellar surface, or loss of granule cells was occasionally present. The NOAEL was 500 ppm, equal to 33 mg/kg bw per day, on the basis of decreased body weight and body-weight gain, tremor and incoordination, decreased absolute brain weight, and increased incidence of neuropathology findings in the peripheral and central nervous system at the LOAEL of 3000 ppm, equal to 183 mg/kg bw per day.

1,2,4-Triazole gave negative results in a battery of assays for genotoxicity, including the Ames test in vitro, an assay for forward mutation, and a test for chromosomal aberration.

The Meeting concluded that 1,2,4-triazole is unlikely to be genotoxic.

No studies of carcinogenicity were submitted. However, the Meeting considered that 1,2,4-triazole is unlikely to be carcinogenic at anticipated levels of exposure since it does not accumulate in the body, it is non-mutagenic, and because of the absence of pre-neoplastic changes with 1,2,4-triazole at high doses.

In a two-generation study of reproductive toxicity in rats, decreased body weights were observed in F_1 males at 250 ppm, equal to 16 mg/kg bw per day, the lowest dose tested. These changes in body weight were minor and were seen only in males and in only one generation and were not seen in short-term studies in rats given similar doses. At 3000 ppm, parental animals (F_0) had statistically significantly reduced terminal body weights, and decreased absolute brain weights associated with mild to moderate degeneration/necrosis in the cerebellum. No F_1 offspring at the highest dose survived the lactation period. No offspring toxicity was observed at doses up to 500 ppm, equal to 30.9 mg/kg bw per day. The NOAEL for reproductive toxicity with 1,2,4-triazole was 250 ppm, equal to 16 mg/kg bw per day, on the basis of an increase in abnormal sperm in F_0 and F_1 males seen at the LOAEL of 500 ppm.

In two studies of developmental toxicity in rats, there was maternal toxicity (retarded weight gain) at 100 mg/kg bw per day or higher, developmental toxicity (decreased body weights, lower fetal and placental weights, and a higher incidence of minor skeletal deviations) at 100 mg/kg bw per day or higher, and an increased incidence of malformations (hydronephrosis, cleft palate, long-bone dysplasia, diaphragmatic hernia) at 200 mg/kg bw per day. The NOAEL for maternal toxicity and for developmental toxicity in rats was 30 mg/kg bw per day. In a study in rabbits, however, lower body-weight gain and clinical signs of systemic toxicity such as excess salivation, hyperpnoea and ptosis were evident at 45 mg/kg bw per day. Five out of 25 dams at this dose were sacrificed in a moribund condition. Developmental effects included lower body weights of fetuses at 45 mg/kg bw per day, and there were a few alterations in the urogenital system, which occurred in several fetuses. The NOAEL for maternal toxicity and for developmental toxicity was 30 mg/kg bw per day in rabbits.

The Meeting concluded that 1,2,4-triazole is teratogenic in rats and rabbits at maternally toxic doses.

No study of acute neurotoxicity was submitted. Clinical signs of neurotoxicity were observed in studies of acute toxicity in which very high doses were given dermally or orally. Neurotoxic effects observed in a short-term study of combined toxicity/neurotoxicity are described above.

The Meeting concluded that 1,2,4-triazole is neurotoxic.

The Meeting concluded that the existing database on 1,2,4-triazole was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.2 mg/kg bw based on a NOAEL of 250 ppm, equal to 16 mg/kg per day, on the basis of testicular effects (sperm abnormalities, sperm counts) seen at

500 ppm, equal to 30.9 mg/kg bw per day, and using a safety factor of 100. At 250 ppm, reduced body weights and body-weight gains were observed in F_1 males; however, the Meeting noted that the reductions in body weight observed at 250 ppm were marginal (< 6%) and were seen only in one sex and in only one generation and were not seen in short-term studies with similar doses. The Meeting therefore concluded that it was not necessary to use an additional safety factor. This ADI is protective for neurotoxic effects seen at 3000 ppm, equal to 183 mg/kg bw per day, in a short-term study of toxicity/neurotoxicity in rats in which the NOAEL was 500 ppm, equal to 33 mg/kg bw per day. The Meeting considered that it was not necessary to add an additional safety factor to allow for the lack of studies of carcinogenicity because 1,2,4-triazole is unlikely to be carcinogenic at anticipated levels of exposure since it does not bioaccumulate in the body, it is non-mutagenic, and because of the absence of pre-neoplastic changes at high doses.

The Meeting established an ARfD of 0.3 mg/kg bw based on a NOAEL of 30 mg/kg bw per day, identified on the basis of alterations of the urogenital system that occurred in several fetuses at the LOAEL of 45 mg/kg bw per day and clinical signs of neurotoxicity in the dams in a study of developmental toxicity in rabbits, and using a safety factor of 100.

Levels relevant to risk assessment for 1,2,4-triazole

Species	Study	Effect	NOAEL	LOAEL
Mouse	Ninety-day study of toxicity[a]	Toxicity	1000 ppm, equal to 161 mg/kg bw per day	3000 ppm, equal to 487 mg/kg bw per day
Rat	Ninety-day study of toxicity[a]	Toxicity	500 ppm, equal to 33 mg/kg bw per day	3000 ppm, equal to 183 mg/kg bw per day
	Multigeneration study of reproductive toxicity[a]	Parental toxicity	250 ppm, equal to 16.0 mg/kg bw per day[d]	500 ppm, equal to 31 mg/kg bw per day[c]
		Offspring toxicity	500 ppm, equal to 31 mg/kg bw per day[c]	—
	Developmental toxicity[b]	Maternal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	30 mg/kg bw per day	45 mg/kg bw per day[c]
		Embryo and fetal toxicity	30 mg/kg bw per day	45 mg/kg bw per day[c]

[a] Dietary administration.
[b] Gavage administration.
[c] Highest dose tested.
[d] Marginal effects on body weight, only seen in F_1 males.

Estimate of acceptable daily intake for humans

0–0.2 mg/kg bw per day

Estimate of acute reference dose

0.3 mg/kg bw

Information that would be useful for continued evaluation of the compound

Results from epidemiological and other such observational studies of human exposures

Critical end-points for setting guidance values for exposure to 1,2,4-triazole

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid and nearly complete absorption
Distribution	Widely distributed in tissues
Potential for accumulation	Low, no evidence of significant accumulation
Rate and extent of excretion	Approximately 80–94% of the administered dose excreted in urine in first 24 h
Metabolism in animals	No significant metabolism
Toxicologically significant compounds (animals, plants and environment)	1,2,4-Triazole

Acute toxicity

Rat, LD_{50}, oral	1650 mg/kg bw
Rat, LD_{50}, dermal	3129 mg/kg bw
Rat, LC_{50}, inhalation	No adequate data
Rabbit, dermal irritation	Slight irritation
Rabbit, ocular irritation	Severe irritation
Guinea-pig, dermal sensitization	Not a sensitizer (Magnusson & Kligman test)

Short-term studies of toxicity

Target/critical effect	Nervous system, brain
Lowest relevant oral NOAEL	500 ppm, equal to 33 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEL	No data

Genotoxicity

	Unlikely to be genotoxic

Long-term studies of toxicity and carcinogenicity

Target/critical effect	No data
Lowest relevant NOAEL	No data
Carcinogenicity	Unlikely to be carcinogenic

Reproductive toxicity

Reproduction target/critical effect	Sperm abnormalities, decrease in body weight
Lowest relevant reproductive NOAEL	250 ppm, equal to 16 mg/kg bw per day
Developmental target/critical effect	Urogenital alterations in rabbits
Lowest relevant developmental NOAEL	30 mg/kg bw per day (rats and rabbits)

Neurotoxicity/delayed neurotoxicity

Neurotoxicity	Evidence of clinical signs of neurotoxicity and cerebellar lesions

Mechanistic data

No studies were submitted

Medical data

No data

Summary

	Value	Study	Safety factor
ADI	0–0.2 mg/kg bw per day	Rat, two-generation studies of reproductive toxicity	100
ARfD	0.3 mg/kg bw	Rabbit, study of developmental toxicity	100

TRIAZOLE ALANINE AND TRIAZOLE ACETIC ACID

Biochemical aspects

In rats given a single dose of radiolabelled triazole alanine (up to 994 mg/kg bw) by gavage, almost all the administered dose was absorbed on the basis of urinary excretion (69–98%). Approximately 3–18% of the administered dose was recovered in the faeces after 7 days. Less than 0.5% of the administered dose was recovered in the expired air. No significant bioaccumulation of triazole alanine was observed. Approximately 8–30% of the excreted dose in the urine and < 1% of the dose in faeces was identified as *N*-acetyl-D,L-triazole alanine, the remainder was parent compound.

In rats given a single dose of radiolabelled triazole acetic acid by gavage, almost all the administered dose (96–112%) was absorbed on the basis of urinary excretion. Triazole acetic acid was rapidly absorbed and excreted mainly via the urine (87–104% after 7 days). Approximately 1.2–7.4% of the administered dose was recovered in the faeces after 7 days. Total radiolabel in tissues after 7 days ranged from 0.8% to 3.1% of the administered dose. Only the parent compound was found in the urine.

Toxicological data

Triazole alanine is of low acute toxicity when administered orally. The oral LD_{50} in mice and rats was > 5000 mg/kg bw. No treatment-related clinical signs or mortalities were observed in these studies.

Triazole acetic acid is of low acute toxicity when administered orally. The oral LD_{50} in rats was > 5000 mg/kg bw. A slight to moderate increase in the incidence of dyspnoea, exophthalmos, ruffled fur, and hunched posture were observed after dosing and subsided within 10 days.

For triazole alanine, no target organ or any treatment-related toxicity was observed in short-term studies in rats and dogs, except for reduced body-weight gains observed in 90-day studies of toxicity in rats and dogs (females only). No long-term studies were submitted.

For triazole acetic acid, no target organ or any treatment-related toxicity was observed in a short-term study in rats. No long-term studies were submitted.

No treatment-related toxicity was observed in a 14-day study in rats given drinking-water containing triazole alanine at concentrations up to 10 000 ppm, equal to 1491 mg/kg bw per day. Haematological and clinical chemistry parameters were not measured in this study. No treatment-related effects were seen in the 28-day study of oral toxicity in which rats were given triazole alanine at doses of up to 400 mg/kg bw per day by oral gavage. In this study, haematological, clinical chemistry and histopathological analyses were incomplete.

In a 90-day dietary study of toxicity in rats fed triazole alanine, decreased body-weight gains was observed at the highest dose of 20 000 ppm, equal to 1510 mg/kg bw per day. Small decreases in concentrations of leukocytes, triglycerides and bilirubin were observed, but were considered to be of no toxicological significance since the changes were small and may have been secondary to the decreased body weights. The NOAEL was 5000 ppm, equal to 370 mg/kg bw per day.

In a 90-day dietary study of toxicity in dogs fed triazole alanine, decreased body-weight gain and food consumption was observed in females at the highest dose of 20 000 ppm, equal to 902 mg/kg bw per day. The NOAEL was 8000 ppm, equal to 322 mg/kg bw per day.

No treatment-related toxicity was observed in a 14-day study in rats given diets containing triazole acetic acid at doses of up to 8000 ppm, equal to 703.5 mg/kg bw per day.

Triazole alanine gave negative results in a adequate battery of tests for genotoxicity in vivo and in vitro.

Triazole acetic acid gave negative results in an Ames test in vitro, and in assays for mutation or cytogenotoxicity in mammalian cells.

The Meeting concluded that triazole alanine and triazole acetic acid are unlikely to be genotoxic.

No studies of carcinogenicity were available; however, triazole alanine and triazole acetic acid are unlikely to be carcinogenic at anticipated levels of exposure since they do not bioaccumulate in the body, are non-mutagenic, are not chemically reactive, and no specific target-organ toxicity was identified in the available toxicological studies with doses of up to 1510 mg/kg bw per day.

In a non-guideline, one-generation study of reproductive toxicity in rats given triazole alanine, no systemic toxicity was seen in parental animals at doses of up to 10 000 ppm, equivalent to 1000 mg/kg bw per day. In this study, a statistically significant increase in pre-coital interval and slight reductions in neonatal weights of males and females were observed at 10 000 ppm. The NOAEL for reproductive and developmental toxicity was 2500 ppm, equal to 250 mg/kg bw per day. In a two-generation study of reproductive toxicity in rats, no systemic toxicity was observed in the parental animals at doses of up to and including 10 000 ppm. No reproductive toxicity was observed at doses of up to and including 10 000 ppm, equal to 929 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 2000 ppm, equal to 192 mg/kg bw per day, on the basis of reduced mean litter weights seen at the LOAEL of 10 000 ppm, equal to 929 mg/kg bw per day. In a study of developmental toxicity in rats given triazole alanine, no systemic toxicity was observed with triazole alanine at doses of up to and including 1000 mg/kg bw per day given by oral gavage. Increased incidences of skeletal findings were seen in the offspring at the intermediate and highest doses. These skeletal findings included unossified odontoid processes at 300 and 1000 mg/kg bw per day, with partially ossified transverse processes of the seventh cervical vertebra (bilateral), unossified fifth sternebra, and partially ossified 13th thoracic centrum observed only at 1000 mg/kg bw per day. The NOAEL for developmental toxicity was 100 mg/kg bw per day.

The Meeting concluded that triazole alanine was not teratogenic. Triazole acetic acid is unlikely to be teratogenic on the basis of its structural and toxicological similarity with triazole alanine.

No studies of neurotoxicity with triazole alanine were submitted. However, there was no evidence that exposure to triazole alanine results in neurotoxicity in the short-term studies in rats and dogs, the study of developmental toxicity in rats, or studies of reproductive toxicity in rats.

No studies of neurotoxicity with triazole acetic acid were submitted. In a study of acute lethality, a slight to moderate increase in the incidence of dyspnoea, exophthalmos, ruffled fur, and curved body position were observed after dosing, and subsided within 10 days. These clinical signs were considered to be non-specific and attributable to bolus dosing with a very high dose (5000 mg/kg bw) by gavage rather than specific neurotoxicity.

The Meeting concluded that triazole alanine and triazole acetic acid are unlikely to be neurotoxic on the basis of the available data.

The Meeting concluded that the existing database on triazole alanine was adequate to characterize the potential hazards to fetuses, infants and children. This conclusion was also applicable to triazole acetic acid for the reasons described above.

Toxicological evaluation

The Meeting established a group ADI for triazole alanine and triazole acetic acid (alone or in combination) of 0–1.0 mg/kg bw based on a NOAEL of 100 mg/kg bw per day for developmental toxicity in a study of developmental toxicity in rats given triazole alanine, on the basis of delayed ossification seen in rats at the LOAEL of 300 mg/kg bw per day, and using a safety factor of 100. The Meeting concluded that it was not necessary to use an additional safety factor for the lack of studies of carcinogenicity because the compounds are unlikely to be carcinogenic at anticipated levels of exposure, do not bioaccumulate in the body, are non-mutagenic, are not chemically reactive, and no specific target-organ toxicity was identified in the available toxicological studies with doses of up to 1510 mg/kg bw per day.

The Meeting concluded that it was unnecessary to establish an ARfD for triazole alanine and triazole acetic acid because no toxicity could be attributed to a single exposure in the available database, including a study of developmental toxicity in rats.

Levels relevant to risk assessment for triazole alanine and triazole acetic acid (based on data for triazole alanine

Species	Study	Effect	NOAEL	LOAEL
Rat	Multigeneration study of reproductive toxicity[a]	Parental toxicity	10 000 ppm, equal to 929 mg/kg bw per day[c]	—
		Offspring toxicity	2 000 ppm equal to 192 mg/kg bw per day	10 000 ppm, equal to 929 mg/kg bw per day[c]
	Developmental toxicity[b]	Maternal toxicity	1 000 mg/kg bw per day[c]	—
		Embryo and fetal toxicity	100 mg/kg bw per day	300 mg/kg bw per day
Dog	Ninety-day study of toxicity[b]	Toxicity	8 000 ppm, equal to 345 mg/kg bw per day	20 000 ppm, equal to 850 mg/kg bw per day[c]

[a] Dietary administration.
[b] Gavage administration.
[c] Highest dose tested.

Estimate of acceptable daily intake for humans

Group ADI for triazole alanine and triazole acetic acid: 0–1 mg/kg bw per day

Estimate of acute reference dose

Unnecessary

Information that would be useful for continued evaluation of the compound

Results from epidemiological and other such observational studies of human exposure.

Critical end-points for setting guidance values for exposure to triazole alanine and triazole acetic acid

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid and nearly complete absorption
Distribution	Widely distributed in tissues
Potential for accumulation	Low, no evidence of significant accumulation
Rate and extent of excretion	Approximately 96–99% of the administered dose excreted in urine in first 24 h
Metabolism in animals	Limited, about 8–19% excreted as N-acetyl triazole alanine in the urine. No metabolism of triazole acetic acid.
Toxicologically significant compounds (animals, plants and environment)	Triazole alanine; triazole acetic acid

Acute toxicity

Rat, LD_{50}, oral	> 5000 mg/kg bw for triazole alanine and triazole acetic acid
Rat, LD_{50}, dermal	No data
Rat, LC_{50}, inhalation	No data
Rabbit, dermal irritation	No data
Rabbit, ocular irritation	No data
Dermal sensitization	No data

Short-term studies of toxicity

Target/critical effect	Decreased body-weight gain
Lowest relevant oral NOAEL	5000 ppm, equal to 370 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEL	No data

Genotoxicity

	Unlikely to be genotoxic (triazole alanine and triazole acetic acid)

Long-term studies of toxicity and carcinogenicity

Target/critical effect	No data
Lowest relevant NOAEL	No data
Carcinogenicity	Unlikely to be carcinogenic (triazole alanine and triazole acetic acid)

Reproductive toxicity

Reproduction target/critical effect	No toxicologically relevant effects
Lowest relevant reproductive NOAEL	10 000 ppm, equal to 929 mg/kg bw per day (rats; highest dose tested)
Developmental target/critical effect	Delayed ossifications
Lowest relevant developmental NOAEL	100 mg/kg bw per day (rats)

Neurotoxicity/delayed neurotoxicity

Acute neurotoxicity	No indication of neurotoxicity from other studies

Mechanistic data

No data

Medical data

No data

Summary

	Value	Study	Safety factor
ADI	0–1 mg/kg bw per day	Rat, study of developmental toxicity	100
ARfD	Unnecessary	—	—

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