TRIADIMENOL AND TRIADIMEFON

First draft prepared by
Jürg Zarn,[1] Les Davies[2] and Alan Boobis[3]
[1] Food Toxicology Section, Swiss Federal Office of Public Health, Zurich, Switzerland;
[2] Science Strategy and Policy, Office of Chemical Safety, Australian Government Department of Health and Ageing, Woden, ACT, Australia; and
[3] Experimental Medicine & Toxicology, Division of Medicine, Faculty of Medicine, Imperial College London, London, England

Explanation

The toxicity of triadimenol ((1RS,2RS;1RS,2SR)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol), a triazole fungicide, was evaluated by the 1989 JMPR, when an acceptable daily intake (ADI) of 0–0.05 mg/kg bw was established based on a no-observed-adverse-effect level (NOAEL) of 5 mg/kg bw per day in a two-generation study in rats. As currently manufactured, triadimenol is an 80 : 20 mixture of the diastereoisomers A (1RS,2SR) and B (1RS,2RS). Older studies of toxicity in the database were performed with 60 : 40 mixtures.

Triadimefon is closely chemically related to triadimenol, with which it shares some similar metabolic pathways in animals. The toxicity of triadimefon ((RS)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-one) was evaluated by the JMPR in 1981, 1983 and 1985. An ADI of 0–0.03 mg/kg bw was established based on a NOAEL of 50 ppm, equivalent to 2.5 mg/kg bw per day, in a 2-year dietary study in rats.

Although triadimenol and triadimefon are independent active ingredients, on the basis of their close chemical and toxicological relationship they were re-evaluated together by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues. Triadimenol and triadimefon act as systemic fungicides by blocking fungal ergosterol biosynthesis. The mechanism of action of these fungicides is inhibition of demethylation.

TRIADIMENOL

Evaluation for acceptable daily intake: triadimenol

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The metabolism and the excretion of [phenyl-UL-^{14}C]triadimenol in Sprague-Dawley rats was investigated in a pre-good laboratory practice (GLP) study and no statement of quality assurance (QA) was provided. To study the pattern of excretion, two male and two female rats were given either isomer A or isomer B (specific activity, 19.8 mCi [732.6 MBq]/mmol) as a single dose at 4 mg/kg bw by gavage. For isomer A, urine and faeces were collected at 4, 8, 12 and 24 h and then daily until termination on day 6. For isomer B, collection times of 4 h and 8 h were replaced by a time-point at 6 h. Samples of expired air, blood and organs were collected for analysis of radiolabel. For tissue and metabolite analyses, 10 animals of each sex were given [^{14}C]phenyl-labelled triadimenol A (9.9 mCi [366.3 MBq]/mmol) at a dose of 25 mg/kg bw by gavage. At 1, 2, 4, 8 and 24 h after administration, two animals per sex were killed and samples of blood and organs were collected for radiolabel analyses.

No radioactivity was detected in the expired air. In the faeces, males excreted 55% of the administered dose of isomer A and 78% of isomer B, while females excreted 37% and 44%, respectively. In the urine, males excreted 31% and 14%, and females excreted 52% and 47% of the administered dose of isomer A and B, respectively. Thus, males in particular eliminated a significantly greater proportion of isomer B than isomer A in the faeces. Maximal residue concentrations in organs and blood were <0.01 ppm, with the exception of the liver, which contained residue at 0.01–0.06 ppm. Tissue concentrations peaked at 1–4 h, with highest concentrations in fat, skin, liver and kidney. The estimated average elimination half-life for the radiolabel was 24 h for both isomers in both sexes.

The pattern of metabolites was not affected by the sex of the animals. Major metabolic reactions were hydroxylation of one of the *t*-butyl methyl groups of triadimenol with subsequent oxidation to the carboxylic acid. Additionally, limited oxidation of triadimenol to triadimefon was observed. Conjugate formation was of minor significance. Particularly in males, unchanged isomer B was excreted to a greater extent than was unchanged isomer A (Puhl & Hurley, 1978).

In a whole-body autoradiography study, male Wistar rats were given [phenyl-UL-^{14}C]triadimenol (specific activity, 29.2 mCi/mmol i.e. 3.66 MBq/mg; A : B = 80 : 20) as a single dose at 3 mg/kg bw by gavage. Expired air, urine and faeces were collected individually and single animals were killed at intervals of between 1 h and 168 h after dosing. This study complied with the Organisation for Economic Co-operation and Development (OECD) requirements for GLP. Triadimenol was absorbed rapidly and peak conecntrations were reached at 1 h in most organs. In the eyes and the urinary bladder, peak concentrations were reached at 4 h and 8 h, respectively. Within 24 h after dosing, 79–90% of the radioactivity was excreted, and excretion was virtually complete by 96 h. Only 0.01% of the administered dose was expired. Radiolabel was found (in decreasing order of activity) in perirenal and brown fat, urinary bladder, liver, adrenals, lachrymal glands, kidney and nasal mucosa. These activities corresponded to concentrations of between 2.4 and 0.4 µg/g. At 120 h after administration, elimination from most organs resulted in concentrations of triadimenol that were below the limit of quantification or detection (Justus, 2002a). In a study of biokinetics and metabolism, groups of male and female Wistar rats were given [phenyl-UL-^{14}C]triadimenol (specific activity, 29.2 mCi/mmol i.e. 3.66 MBq/mg; A : B = 80 : 20) as a single oral dose at 1 mg/kg bw. Additional groups dosed in this way included a group of male rats that had been pretreated with unlabelled triadimenol for 14 days before dosing, and a group of bile-duct cannulated male rats. A group of male rats was given a single dose of [phenyl-UL-^{14}C]triadimenol at 100 mg/kg bw. Another group of male rats received single low doses of [phenyl-UL-^{14}C]triadimenol as individual isomers A and B at 1 mg/kg bw. All test groups consisted of four animals (except the group of bile-duct cannulated rats, which comprised six animals) and the duration of investigation was 120 h. In these studies, radioactivity was measured in urine, faeces, plasma, organs and the whole body after termination. This study complied with OECD requirements for GLP. Triadimenol was rapidly absorbed, with peak plasma concentrations occurring at 1.5 h. The elimination half-lives of the radiolabel were in the range of 6 h to 15 h, with the longest half-lives for males at the high dose and females at the low dose. In animals at the low dose, males and females excreted 14–21% and 48% of the dose via urine, respectively. The rest of the administered dose was excreted in the faeces, with very low levels remaining in the whole body (0.02–0.06% of the administered dose). In bile-duct cannulated males, 6% of the radiolabel was recovered in the urine and 93% in the bile, indicating enterohepatic recycling. Pretreatment of the animals with unlabelled triadimenol for 14 days did not affect the results (Justus, 2002b).

In a study of dermal absorption, groups of 24 Charles River Crl:CD rats were given ^{14}C ring-labelled triadimenol (15.78 mCi [583.9 MBq]/mmol per l) at a dose of 0.01, 0.1, 1.0 or 10 mg (equivalent to 0.04–40 mg/kg bw) applied to an area of shaved skin of 15 cm^2. The test material was covered by a gauze patch after application. Four animals per group were bled and then terminated at 0.5, 1, 2, 4, 8 and 24 h after dosing. Urine and faeces were collected from each animal. Radioactivity was also determined in whole carcass, skin wash and excised skin. Dermal absorption was rapid. It was estimated that 50% of the test material administered was absorbed, and that absorption was somewhat slower at higher concentrations (with half-lives for elimination of radiolabel ranging from 27 h at the lowest dose to 86 h at the highest dose), suggesting that transport was saturated (Leeling et al., 1988, quoted from JMPR 1989).

1.2 Metabolism

Triadimenol was extensively metabolized (see Figure 1), mainly by oxidation of one of the *t*-butyl methyl groups to the hydroxyl or carboxy group. Non-conjugated metabolites predominated in the urine and the faeces, while bile metabolites were extensively glucuronidated. The most abundant metabolite in the urine and faeces was carboxytriadimenol (KWG 1640 [M02], 45–64% of the administered dose), followed by hydroxy triadimenol (KWG 1342 [M10], 18–33% of the administered dose). Levels of each of the other metabolites were <8% of the administered dose. The most abundant metabolite in the bile was hydroxytriadimenol glucuronide (M23, 52% of the administered dose), followed by triadimenol glucuronide (M24, 21% of the administered dose) and carboxytriadimenol glucuronide (M30, 17% of the administered dose), respectively. Levels of each of the other metabolites were <3% of the administered dose. Cleavage of the triazole or 4-chlorophenyl moiety was found only to a minor extent, producing triadimenol-ketocarboxyglucuronide (M34) and a conjugate of 4-chlorophenol (M07) (maximum, 3% and 0.5% of the administered dose, respectively). Female rats excreted more carboxytriadimenol in the urine than did males, but less in the faeces (Justus, 2002b).

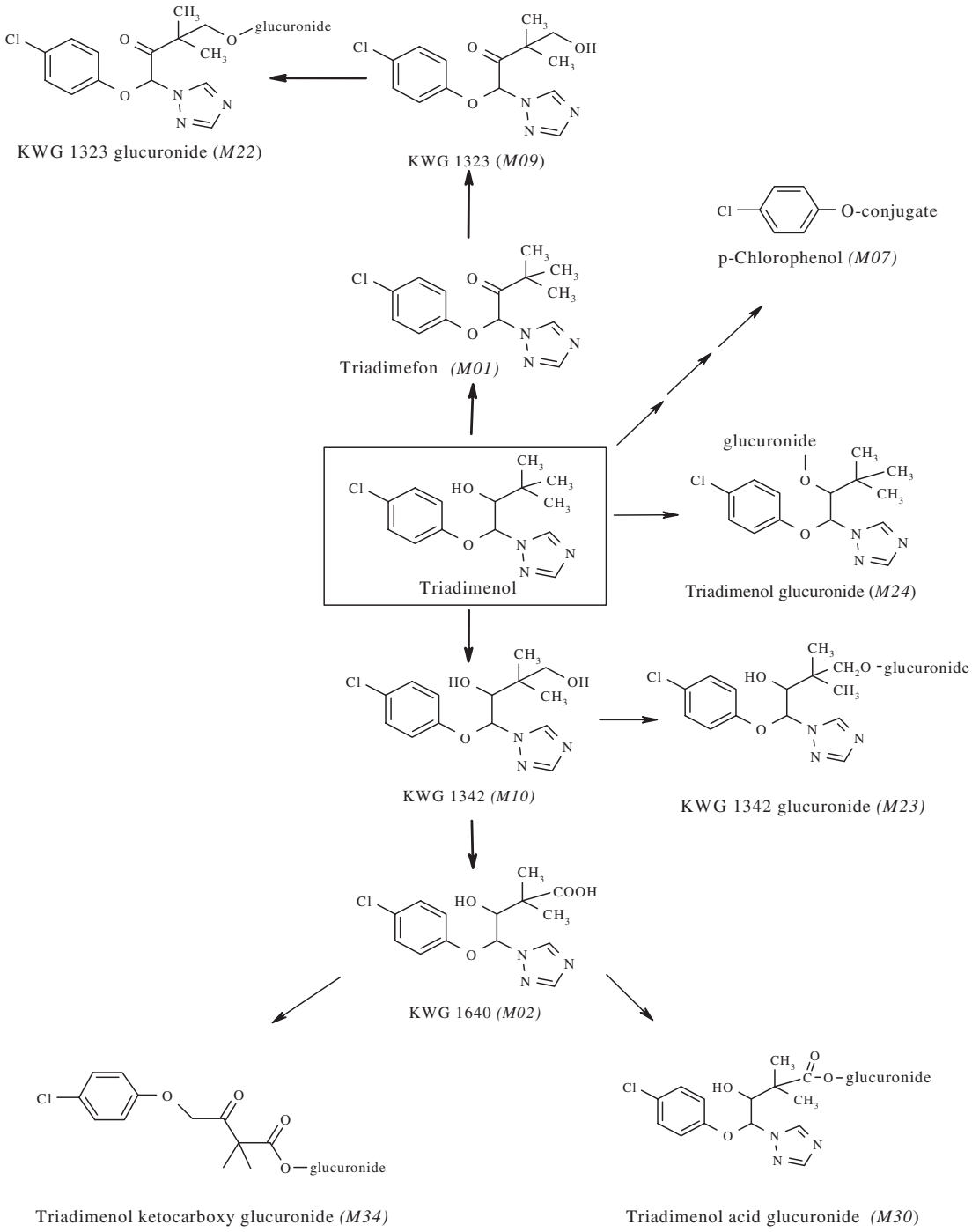
1.3 Effects on enzymes and other biochemical parameters

In a comparative assay for enzyme inhibition in vitro, the effects of triadimenol and triadimefon and other pesticides on aromatase activity were studied. Aromatase converts androgens to estrogens and is therefore important for the balance of sex steroids. Half maximal inhibition of human placental microsomal aromatase was observed for triadimenol and triadimefon at 21 µmol/l and 32 µmol/l, respectively. This was judged to be weak inhibition when compared with that of other azole compounds such as prochloraz (Vinggaard et al., 2000).

Both compounds were found to be weak agonists of the estrogen receptor in MCF7 breast cancer cells (triadimefon and triadimenol at 10 µmol/l induced a 2.4 and 1.9-fold increase in cell proliferation, respectively) but not in estrogen receptor α-transfected yeast cells. Other azole compounds, such as prochloraz and imazalil, were either negative in these assays or had very low activity (Vinggaard et al., 1999).

In a review of azole compounds including triadimenol and triadimefon, a possible relationship between inhibition of mammalian lanosterol demethylase and aromatase and developmental and reproductive effects in laboratory animals was considered (Zarn et al., 2003).

Figure 1. Proposed metabolism of triadimenol in rats



From Bayer CropScience AG (2003); the scheme is reproduced from the manufacturer's evaluation.

2. Toxicological studies

2.1 Acute toxicity

The acute toxicity of triademenol is summarized in Table 1.

(a) General toxicity

In the studies of acute toxicity, signs of intoxication in rats included impairment of the general health condition, piloerection, drowsiness, laboured breathing, cramps, decreased and increased mobility, aggressiveness, self-mutilation and lying on their sides (Thyssen & Kimmerle, 1976b; Mihail & Thyssen, 1980). With the exception of subcutaneous application in mice, there was very little difference in acute toxicity between the sexes; the reason for this is not known. In a study of acute oral toxicity in rats, the median lethal dose (LD_{50}) of isomer A was an order of magnitude lower than that of isomer B. Since the vehicle was the same in both studies (Cremophor EL), stereochemical differences in kinetics and metabolism might explain this finding. A study with the isomer composition A : B = 80 : 20 suggests an influence of feeding status on the acute oral toxicity of triadimenol.

(b) Dermal and ocular irritation

In New Zealand White rabbits and in a unspecified rabbit strain, incidental and not treatment-related very slight dermal and ocular irritation reactions were observed in a few

Table 1. Acute toxicity of triadimenol

Isomer ratio	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity (%)	Reference
A : B = 60 : 40	Mouse	NMRI	Male	Oral	1300		NS	Thyssen & Kimmerle (1976b)
	Mouse	NMRI	Female	Oral	1267			
	Mouse	NMRI	Male	Subcutaneous	1580			
	Mouse	NMRI	Female	Subcutaneous	2441			
	Rat	Wistar	Male	Oral	1161			
	Rat	Wistar	Female	Oral	1105			
	Rat	Wistar	Male	Intraperitoneal	367			
	Rat	Wistar	Female	Intraperitoneal	352			
	Rat	Wistar	Male	Dermal	>5000			
	Rat	Wistar	Female	Dermal	>5000			
	Rat	Wistar	Males and females	Inhalation		>0.315		
Isomer A	Rat	NS	Male	Oral	579		99.9	Flucke (1979a)
Isomer B	Rat	NS	Male	Oral	5000		99.0	Flucke (1979b)
A : B = 60 : 40	Rat	NS	Male	Oral (not stated whether fed or fasted)	819–895		NS	Flucke (1979c)
A : B = 80 : 20	Rat	Wistar	Male	Oral (fasted)	689		92.4	Mihail & Thyssen (1980)
			Female	Oral (fasted)	752			
	Rat	Wistar	Male	Oral (fed)	1098			
			Female	Oral (fed)	1037			
	Rat	Wistar	Male	Intraperitoneal (fed)	371			
			Female	Intraperitoneal (fed)	286			
	Rat	Wistar	Males and females	Dermal	>5000			
	Rat	Wistar	Males and females	Inhalation (4 h)		>0.954		

NS, not stated

cases. In humans, there was no evidence of primary skin irritation (Thyssen & Kimmerle, 1976b; Mihail & Thyssen, 1980; Nagashima, 1982a; Nagashima, 1982b; Kroetlinger, 1993).

(c) Dermal sensitization

In a Magnusson-Kligman maximization test, 10 male and 10 female guinea-pigs were treated intradermally with 0.1 ml of a 2.5% formulation of triadimenol and, additionally, 1 week later by topical application of a 25% formulation of triadimenol. The challenge with a 25% formulation was performed 2 weeks after the topical application. Triadimenol was not a skin sensitizer (Flucke, 1981).

2.2 Short-term studies of toxicity

Mice

Groups of 10 male and 10 female Crl:CD-1(ICR)BR mice were fed diets containing triadimenol (purity, 97.4%; A:B = 80:20) at a concentration of 0, 160, 500, 1500 or 4500 ppm for 13 weeks. The average daily intakes of triadimenol were 0, 24.9, 76.8, 235 and 872 mg/kg bw per day for males and 0, 31.4, 94.1, 297 and 797 mg/kg bw per day for females. This study complied with OECD requirements for GLP. A slightly increased rate of mortality was observed in males at 4500 ppm. In this group, the males also showed higher intake of food, piloerection and squatting position. Mean body weights were reduced at 1500 ppm in males and at 4500 ppm in females. Erythrocyte volume fraction was decreased and mean corpuscular haemoglobin concentration was increased in females at 4500 ppm. Lower leukocyte counts were found in males receiving triademenol at concentrations of ≥1500 ppm. Hypertrophy of liver cells was found in males given triademenol at ≥500 ppm and in females at ≥1500 ppm, with increased liver weights, cytoplasmic vacuolation and single cell necrosis at ≥1500 ppm in both sexes. These liver effects were accompanied by several changes in clinical chemistry. In animals of both sexes, increased activity of aspartate and alanine aminotransferases (AST and ALT) and glutamate dehydrogenase at ≥500 ppm was observed (Table 2). At ≥1500 ppm, decreased total protein concentrations, increased triglyceride concentrations and lipid storage were found in both sexes. In males at ≥1500 ppm and in females at 4500 ppm, decreased albumin and cholesterol concentrations were observed. Bilirubin concentrations were decreased at dietary concentrations of of ≥500 ppm in males and ≥1500 ppm in females. In both sexes, hepatic aminopyrine-*N*-demethylase activity was

Table 2. Enzyme activities in mice fed with diets containing triademenol for 13 weeks

Dietary concentration (ppm)	AST (U/l)	ALT (U/l)	Glutamate dehydrogenase (U/l)
Males			
0	26.0	29.7	5.8
160	31.1	31.7	13.6*
500	39.2**	47.3*	46.0*
1500	56.3**	75.9**	78.1**
4500	77.7**	158.1**	84.3**
Females			
0	30.2	27.9	9.1
160	34.1	30.5	9.6
500	41.3*	38.4*	26.1*
1500	88.4*	85.8**	81.1**
4500	133.1*	218.6**	110.4**

From Schladt & Sander (1998)
ALT, alanine aminotransferase; AST, aspartate aminotransferase
* $p = 0.05$
** $p = 0.01$

increased at ≥160 ppm. In animals of both sexes at 4500 ppm, adrenal weights were increased, and in females the adrenal cortical X-zone was lacking vacuoles; in mice, the X-zone of the adrenals usually shows vacuoles with a strain dependent incidence—the significance of this finding is not clear, neither is the role of the X-zone in general). The significance of this finding was unresolved (the study author concluded that this was not an unusual finding, being seen after dosing with several compounds from different chemical classes). The NOAEL was 500 ppm, equal to 76.8 mg/kg bw per day, on the basis of microscopic changes in the liver and clinical chemistry findings at 1500 ppm (Schladt & Sander, 1998).

Rats

Groups of 20 male and 20 female Wistar rats were given technical-grade triadimenol (purity, 98.5%; A : B = 60 : 40) at a dose of 0, 1.5, 5, 15 and 45 mg/kg bw per day by gavage for 28 days. One-half of all the animals in each group was then terminated and the remaining animals were kept for another 28 days as a recovery group. This is a pre-GLP study and no statement on QA was provided. In females, the only noteworthy findings were increased absolute (50 mg in the control group, 57, 57 and 60 mg at the lowest, intermediate and highest dietary concentrations) and relative (28 mg in the control group, 33, 32 and 33 mg at the lowest, intermediate and highest dietary concentrations) ovary weight in all treated groups at the end of the dosing period, but not at the end of the recovery period. These effects on ovary weight were without an apparent dose–response relationship and were judged to be incidental. In males at the highest dose only, there was minimally but significantly increased absolute and relative weights of the thyroid. No other treatment-related changes were observed regarding behaviour, appearance, weight gain, blood chemistry, liver and kidney functions, organ weights, gross pathology and histopathology. The NOAEL was 45 mg/kg bw per day, the highest dose tested (Thyssen & Kaliner, 1976).

To study the toxicological effects of the two different isomer compositions, groups of 20 male and 20 female Wistar rats were given either technical-grade triadimenol (A : B = 80 : 20; purity, 98.3%) at 15, 45 or 100 mg/kg bw per day, or technical-grade triadimenol (A : B = 60 : 40; purity, 84.7%) at 45 or 100 mg/kg bw per day. Half of the animals of each group were terminated and the remaining animals were kept for another 28 days as a recovery group. This is a pre-GLP study and no statement on QA was provided. No treatment-related changes were observed regarding appearance, weight gain, blood chemistry, liver and kidney functions, gross pathology or histopathology. The only effects observed with both isomer compositions were slightly and transiently increased liver weights in females at the highest dose. Additionally, at doses of ≥45 mg/kg bw per day with both batches very minor induction of hepatic *N*- and *O*-demethylases and cytochrome P450 was found in both sexes. However, these changes lacked a clear dose–response relationship. At doses of ≥45 mg/kg bw per day, a slightly increased motor activity was observed with both isomer mixtures in both sexes, persisting for the first 2 h after dose application. On the basis of the slight behavioural changes observed at 45 mg/kg bw per day with both mixtures, the NOAEL for the triadimenol isomer composition A : B = 80 : 20 was 15 mg/kg bw per day and for A : B = 60 : 40 it was <45 mg/kg bw per day (Mihail & Vogel, 1981).

Groups of 15 male and 15 female Wistar rats were fed diets containing technical-grade triadimenol (purity, 98%; A : B = 60 : 40) at a concentration of 150, 600 or 2400 ppm for 3 months. The control group consisted of 30 animals of both sexes. Average daily compound intakes were 12.2, 49.2 and 203 mg/kg bw per day for males and 17.1, 71.3 and 287 mg/kg bw per day for females. This is a pre-GLP study and no statement of QA was

provided. Behaviour, appearance and survival were not affected in any group. At the highest dose, body-weight gain was slightly but significantly reduced in both sexes, while liver weights in both sexes and kidney and ovary weights were significantly increased in females. However, histopathology on all organs did not indicate any damage. At the highest dose, minor changes in blood parameters consisted of reduced erythrocyte volume fraction, and relative eosinophil counts in females and reduced mean corpuscular volume and mean corpuscular haemoglobin in males. The NOAEL was 600 ppm, equal to 49.2 mg/kg bw per day, on the basis of minor changes in body weight and organ weights and effects on blood parameters at 2400 ppm (Loeser & Kaliner, 1977).

Groups of 20 male and 20 female Sprague-Dawley Crj:CD rats were fed diets containing technical-grade triadimenol (purity, 94%; A:B = 80:20) ata a concentration of 0, 120, 600 or 3000 ppm for 3 months. The average daily intakes of triadimenol were 0, 8.0, 39.6 and 209 mg/kg bw per day for males and 0, 9.4, 46.4 and 221 mg/kg bw per day for females. There was no report on compliance of this study with any GLP standards and no statement of QA was provided. At the highest dietary concentration, piloerection and depilation were observed in animals of both sexes in the first month of exposure. Body-weight gain was reduced in both sexes, concomitant with initial (males) and continuing (females) reduced food intake and initially reduced food efficiency. At dietary concentrations of ≥600 ppm, absolute and relative liver weights were increased and the livers were enlarged in both sexes. Additionally, the livers of females showed a pronounced lobular structure and in both sexes at the highest dietary concentration, histopathology on the liver revealed fatty changes and eosinophilic degeneration of hepatocytes. At the highest dietary concentration, haemoglobin and erythrocyte volume fraction were reduced in both sexes. Clinical chemistry showed reduced triglyceride and free fatty acid concentrations in both sexes at the highest dietary concentration and females had increased total cholesterol, phospholipid and total protein concentrations and a decreased albumin/globulin (A/G) ratio and decreased albumin concentrations.

The NOAEL was 600 ppm in the diet, equal to 39.6 mg/kg bw per day, on the basis of effects on the liver observed at 3000 ppm (Nishimura, 1983).

Groups of 10 male and 10 female Wistar rats were exposed to technical-grade triadimenol (purity not stated) at a concentration averaging 0.030, 0.068 or 0.229 mg/l as a liquid aerosol in ethanol/polyethylene glycol solvent by inhalation for 6 h per day for 3 weeks. Measurements were gravimetric and concentrations reported are actual rather than nominal. Animals in the control group were exposed only to the ethanol/polyethylene glycol solvent. This is a pre-GLP study and no statement on QA was provided.

No treatment-related findings were recorded in behaviour, appearance, body and organ weights, clinical chemistry, haematology or histopathology.

The NOAEC was 0.229 mg/l, the highest concentration tested (Kimmerle, 1976).

Rabbits

Groups of six male and six female New Zealand White rabbits were given triadimenol (purity, 98%; A:B = 80:20) at a dose of 0, 50 or 250 mg/kg bw per day applied dermally on either intact or abraded skin for 6 h per day, 5 days per week, for 3 weeks. There was no report on compliance with any GLP standards and no statement of QA was provided.

No treatment-related findings related to behaviour, appearance, body and organ weights, clinical chemistry, haematology or histopathology were recorded.

The NOAEL was 250 mg/kg bw per day, the highest dose tested (Heimann & Schilde, 1984).

Dogs

Groups of four male and four female beagle dogs were fed diets containing technical-grade triadimenol (purity, 98.5%; A : B = 60 : 40) at a concentration of 0, 150, 600 or 2400 ppm for 13 weeks. The average daily intakes of triadimenol were 0, 4.5, 17.8 and 71 mg/kg bw per day (calculated from the daily intakes per animal of 0, 44.5, 178.8 and 709.5 mg and a default body weight of 10 kg. This is a pre-GLP study and no statement of QA was provided.

The body-weight gain of animals at the highest dose was slightly reduced (statistically not significant) and the relative weights of their liver and kidneys (males only) were increased. Alkaline phosphatase (ALP) activity was elevated in all treated animals without any dose–response relationship. At the highest dose, cytochrome P450 levels and aminopyrine *N*-demethylating activity were increased. At 6 weeks, but not at the end of the study, animals at ≥600 ppm showed increased serum glutamate transaminase activity and cholesterol concentrations. At the end of the study, elevated concentrations of cholesterol were statistically significant only at the highest dose.

The NOAEL was 600 ppm, equivalent to 17.8 mg/kg bw per day, on the basis of effects on organ weightss and changes in clinical chemistry parameters at 2400 ppm (Hoffmann & Kaliner, 1977).

Groups of six male and six female beagle dogs were fed diets containing technical-grade triadimenol (purity, 98%; A : B = 80 : 20) at a concentration of 0, 10, 30 or 100 ppm for 6 months. The average daily intakes of triadimenol were 0, 0.4, 1.2 and 4 mg/kg bw per day (calculated from the daily intakes per animal of 0, 4.03, 12.26 and 40.2 mg and a default body weight of 10 kg). There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

There were no treatment-related findings related to appearance, behaviour, ophthalmology, clinical chemistry, haematology or gross pathology. No microscopic histopathology was performed on the organs.

The NOAEL was 100 ppm, the highest concentration tested, equivalent to 4.0 mg/kg bw per day (Hoffmann, 1984).

Groups of four male and four female beagle dogs were fed diets containing technical-grade triadimenol (purity, 94.9%; A : B = 60 : 40) 0, 150, 600 and 2400 ppm for 2 years. The average daily intakes of triadimenol were 0, 5.6, 21.1 and 85.9 mg/kg bw per day (calculated from the daily intakes per animal of 0, 55.7, 211.3 and 859.0 mg and a default body weight of 10 kg). There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

The control group showed an unusually large body-weight increase. Therefore, the body-weight gains in all groups deviated considerably from the control group in a non-dose-

related manner. The study author concluded that there were no treatment-related body weight effects. At the highest dose, cytochrome P450 levels and aminopyrine *N*-demethylating activity were increased. The NOAEL was 600 ppm, equivalent to 21.1 mg/kg bw per day, on the basis of clinical chemistry changes at 2400 ppm (Hoffmann & Vogel, 1984).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female CF_1/W 74 mice were fed diets containing triadimenol (purity, 95%; A : B = 60 : 40) at a concentration of 0, 125, 500 or 2000 ppm for 2 years. The average daily intakes of triademenol were 0, 30, 140 and 620 mg/kg bw per day for males and 0, 50, 200 and 810 mg/kg bw per day for females. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

Males and females at ≥500 ppm showed reduced body-weight gain, which was statistically significant at the end of the study only for males at the highest dose. Food consumption was not affected. At the highest dose, the absolute and relative weights of the liver of males and females were increased, as were the weights of testes in males. The weights of ovaries were not reported. The only findings in haematology were Jolly bodies in all females of all dosed groups and in one male in each of the groups at 125 and 500 ppm and in nine males at 2000 ppm. This observation could not be explained and was judged by the study author to be of no biological significance. At 24 months, ALT and AST activities were increased in each sex at ≥500 ppm, as was ALP at 2000 ppm (Table 3). After 12 months at the highest dose, cholesterol concentrations were decreased in both sexes. At the end of the study, this effect was only significant for males at the highest dose. At 2000 ppm, the livers of both sexes showed more hyperplastic nodules and females in the groups receiving the intermediate and highest doses had increased incidences of adenomas (intermediate dose: 4 (8%); and highest dose: 6 (12.2%); versus 0 in the control group) (Table 3). In studies of historical controls, liver adenomas were found in 3.9% (mean value from 14 studies) of females, with a range of 0–12%. It is therefore questionable whether triademenol has carcinogenic potential. In two males at the highest dose, a carcinosarcoma in the urinary bladder was identified. Additionally, a slightly increased incidence of cystic alterations was found in the thyroid (more pronounced in males).

Table 3. Liver enzyme activities and incidence of liver adenomas in mice fed diets containing triademenol for 2 years

Dietary concentration (ppm)	AST (U/l)	ALT (U/l)	Adenoma
Males			
0	37.5	53.7	6
125	54.0	71.4	4
500	58.2	88.0*	5
2000	97.8**	159.4**	8
Females			
0	40.0	30.7	0
125	48.6	42.8	0
500	53.6*	48.0*	4
2000	185.7**	283.5**	6

From Bomhard & Loeser (1982)
ALT, alanine aminotransferase AST, aspartate aminotransferase
* $p = 0.05$
** $p = 0.01$

The NOAEL was 500 ppm, equal to 140 mg/kg bw per day, on the basis of increased incidence of adenoma at 2000 ppm (Bomhard & Loeser, 1982).

Groups of 50 male and 50 female Crl:CD-1(ICR)BR mice were fed diets containing triadimenol (purity, 96.8%) at a concentration of 0, 80, 400 or 2000 ppm for 80 weeks. The average daily intakes of triadimenol were 0, 11.3, 60.2 and 340 mg/kg bw per day for males and 0, 17.2, 91.3 and 472 mg/kg bw per day for females. This study complied with OECD requirements for GLP.

Males and females at 2000 ppm had reduced body-weight gain (16% in males, 12% in females) and males of this group had slightly increased feed intake. At the highest dose, absolute and relative liver weights of males and females and absolute and relative brain weights of males were increased. Statistical significant increases in hepatocellular hypertrophy and single cell necrosis were found in males at ≥400 ppm and hepatocellular hypertrophy was found in females at 2000 ppm (Table 4). Males and females at 2000 ppm showed more yellow-brown pigmentation in the liver, while females of this group also had fatty changes and intracytoplasmic hepatocellular vacuolation and males had increased inflammatory infiltration, respectively. At doses of ≥80 ppm, significantly more males showed basophilic foci in the liver. At 2000 ppm, animals of both sexes showed reduced cerebral mineralization. This effect might be caused by reduced ageing-related normal arteriosclerotic changes. At the highest dose, males showed increased erythrophagocytosis in the mesenteric lymph node.

An increase in liver adenomas and carcinomas in males at 400 ppm was observed, reaching statistical significance for carcinomas in animals in the groups receiving the lowest and intermediate doses (adenomas, 7/50, 5/50, 10/50 and 5/49; carcinomas, 0/50, 3/50, 4/50 and 2/49, in order of increasing dose). Since there is no dose–response relationship for the incidences of adenoma, the Meeting considered that they were of no concern for humans. Two females at 2000 ppm had luteomas, while there were none in the other groups. Animals in the historical control groups had these tumours at incidences ranging from of 0.9% to 10%. The NOAEL was 400 ppm, equal to 60.2 mg/kg bw per day, on the absis of effects on the liver at 2000 ppm (Schladt, 1998).

Rats

Groups of 60 male and 60 female Bor:WISW rats were fed diets containing triadimenol (purity, 94.9%; A:B = 60:40) at a concentration of 0, 125, 500 or 2000 ppm for 2 years. The average daily intakes of triademenol were 0, 7, 25 and 105 mg/kg bw per day for

Table 4. Histopathological findings in the liver of mice fed diets containing triademenol for 80 weeks

Finding	Dietary concentration (ppm)							
	0	80	400	2000	0	80	400	2000
	Males				Females			
Hepatocellular hypertrophy	5	8	34**	49**	2	2	2	45**
Pigmentation	7	7	10	36**	29	33	25	40**
Single cell necrosis	6	9	20**	42**	8	5	8	25**
Basophilic foci	0	3*	2	5**	0	1	0	2

From Schladt (1998)
*$p = 0.05$
**$p = 0.01$

Table 5. Relative mean weights of organs (mg/100 g) in rats fed diets containing triademenol for 2 years

Dietary concentration (ppm)	Lung	Liver	Spleen	Kidney	Testes or ovaries
Males					
0	436	3795	197	703	923
125	414	3916	306	766	963
500	434	3861	195	715	901
2000	419	3867	187	709	942*
Females					
0	513	3962	271	751	50.9[a]
125	528	3829	231	764	56.0[a]
500	540	4139	244	765	57.0[a]
2000	540*	4781**	239*	811*	75.1**[a]

From Kroetlinger et al. (1982)

[a] Statistical analysis of the raw data was performed by the Meeting, in view of inappropriate values in the statistics presented in the study

* $p = 0.05$

** $p = 0.01$

males and 0, 9, 35 and 144 mg/kg bw per day for females. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

Males and females at 2000 ppm showed reduced body-weight gain throughout the study, while consumption of food was not affected. The relative weights of the spleen and ovaries were reduced, but increases in weights of lungs, liver, kidneys and ovaries in females at 2000 ppm were seen (Table 5). In males at 2000 ppm, relative weights of the testes were increased. Statistically significant observations in the blood of animals treated with triademenol at 2000 ppm were reduced erythrocyte counts for both sexes and reduced haemoglobin and erythrocyte volume fraction for females at 6 months, as well as reduced eosinophilic granulocyte counts in females at 500 and 2000 ppm. Although statistically significant, these findings were mostly within the physiological range. At doses of 2000 ppm, transaminase activities (ALT and AST) were increased by less than twofold in both sexes, and glutamate dehydrogenase activity was increased by nearly three-fold in males. In males at 2000 ppm, reduced protein concentrations were found. In males at ≥125 ppm, lower creatinine values were found, while in females at ≥500 mg/kg, higher urea values in the plasma were found.

There was no histopathological evidence for treatment related non-neoplastic or neoplastic changes.

The NOAEL was 500 ppm, equal to 25 mg/kg bw per day, on the basis of effects on organ weights at 2000 ppm (Kroetlinger et al., 1982).

2.4 Genotoxicity

The results of studies of genotoxicity with triademenol are summarized in Table 6.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a three-generation study, groups of 10 male and 20 female Long Evans FB 30 rats were fed diets containing triadimenol (purity not reported) at a concentration of 0, 125, 500

Table 6. Studies of genotoxicity with triadimenol

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation[a]	*S. typhimurium* TA1535, TA1537, TA100, TA98	4–2500 µg/plate +S9 2500 µg/plate –S9, in DMSO	93.7	Negative Negative	Herbold (1979a)
Reverse mutation[b]	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98 and *E. coli* WP2 hcr	5–5000 µg/plate ±S9, in DMSO	97.5	Negative	Tanahashi & Moriya (1982)
Reverse mutation[b]	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98 and *E. coli* B/r WP2 try$^-$ hcr$^-$	5–5000 µg/plate ±S9, in DMSO	97.5	Negative	Nagane et al. (1982)
Forward mutation[c]	Mouse lymphoma L5178Y *Tk*$^{+/-}$ cells	25–150 µg/ml ±S9	97.5	Negative	Cifone (1982)
Rec assay	*Bacillus subtilis* H17 (rec$^+$) and M45 (rec$^-$)	50–10 000 µg/disk	97.5	Negative	Tanahashi & Moriya (1982)
Rec assay	*B. subtilis* NIG17 (rec$^+$) and NIG45 (rec$^-$)	200 µg/disk	97.5	Negative	Nagane et al. (1982)
Unscheduled DNA synthesis[d]	Primary hepatocytes from male F344 rats	0.25–50 µg/ml	97.5	Negative	Myhr (1982)
DNA damage	*E. coli* (K12)p 3478 (pol A$_1^-$) and W3110 (pol A$^+$)	62.5–1000 µg/plate	97.5	Negative	Herbold (1981)
Sister chromatid exchange	Chinese hamster ovary K1 cells (CHO)	38–300 µg/ml –S9 100–200 µg/ml +S9[e] 125–225 µg/ml +S9	93.0	Negative Positive Negative	Putman (1987)
In vivo					
Micronucleus formation	Bone-marrow erythroblasts of male and female NMRI mice	Two oral doses at 175 or 350 mg/kg bw	93.7	Negative	Herbold (1978a)
Micronucleus formation	Bone marrow erythroblasts of male and female NMRI mice	Two oral doses at 350 or 500 mg/kg bw	96.5	Negative	Herbold (1979b)
Dominant lethal mutation	Male NMRI mice	Single oral dose at 500 mg/kg bw	93.7	Negative	Herbold (1978b)

DMSO, dimethylsulfoxide; S9, 9000 × *g* rat liver supernatant

[a] Bacteriotoxicity observed at doses of > 500 µg/plate.

[b] Bacteriotoxicity observed at doses of > 1000 µg/plate.

[c] Cytotoxicity observed at 250 µg/ml.

[d] Reduced survival (53%) at 50 µg/ml.

[e] The first assay with S9 gave positive results (14.66–15.62 sister chromatid exchanges (SCEs) per cell in treated cells and 12.90 SCEs per cell in non-treated cells) at all doses tested, without dose dependency. The confirmatory assay gave negative results.

or 2000 ppm. Although an old study, it had not been evaluated previously. The pretreatment period before the first mating was 70 days. In each generation, 10 male and 20 female pups of the second of two matings (F_{1b}, F_{2b}, F_{3b}) were used to produce the next generation. All female animals were kept consecutively for a period of longer than one estrus cycle with each of three males. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

The fertility in the F_0 generation decreased at dietary concentrations of ≥125 ppm (statistically significant at 2000 ppm) with fewer pregnant rats and fewer F_1 pups born, and a non dose-dependent increase in the male : female ratio in the F_{1b} litters at dietary concentrations of ≥125 ppm and reduced viability of pups at 2000 ppm (Table 7). The body weights of F_1 pups at birth were not affected. Fertility in the F_1 generation decreased at doses of ≥500 ppm, with fewer pregnant rats and fewer F_2 pups born, and significantly reduced viability of pups was observed in the first mating at doses of ≥125 ppm and in the second mating at 2000 ppm at 4 weeks. Although reduced pup viability was statistically significant also at 125 ppm, the Meeting considered it to be marginal. No changes in the sex ratio were seen in F_2 animals. Reduced body-weight gains at ≥125 ppm and reduced fertility was seen in F_2 animals at dietary concentrations of ≥500 ppm with fewer pregnant rats and fewer F_3

Table 7. Reproductive parameters[a] in a three-generation study in rats fed with diets containing triademenol

Parameter	Dietary concentration (ppm)			
	0	125	500	2000
F_0 1st mating	17/20 (85)	16/20 (80)	14/19 (73.7)	4/20 (20**)
F_0 2nd mating	16/18 (88.9)	16/20 (80)	14/19 (73.7)	13/19 (68.4)
F_1 1st mating	20/20 (100)	20/20 (100)	14/20 (70*)	4/8 (50**)
F_1 2nd mating	16/14 (84.2)	17/20 (85)	6/20 (30**)	4/7 (57.1)
Viable F_{2a} pups at 4 weeks	98.9	93.9*	70.3**	63.6**
Viable F_{2b} pups at 4 weeks	97.3	91.3	95.8	73.3**

From Loeser & Eiben (1982)

[a] The values given are No. of pregnant rats/No. of mated rats (%)

* $p = 0.05$

** $p = 0.01$

pups born. The birth body weights of F_2 pups were decreased only at dietary concentrations of ≥500 ppm in the first mating and at 2000 ppm in the second mating. All pups of the first F_2 mating of animals fed diets containing triademenol at dietary concentrations of ≥500 ppm died within the first 4 weeks. This effect was not seen in the second mating. On gross pathological examination, no malformations in the pups were detected. Histopathology was not performed. Organ weight analyses of all F_{2b} parents revealed significantly increased testes weights at dietary concentrations of ≥500 ppm. In all three generations, body-weight gain was reduced in animals of both sexes, in the F_0 and F_1 at 2000 ppm only and in the F_2 at dietary concentrations of ≥125 ppm.

The NOAEL for maternal toxicity was 2000 ppm, the highest dietary concentration tested. The NOAEL for reproductive toxicity was 125 ppm, equivalent to 12.5 mg/kg bw, on the basis of reduced viability of pups at dietary concentrations of ≥500 ppm (Loeser & Eiben, 1982).

In a two-generation study, groups of 10 male and 20 female Bor:WISW rats were fed diets containing triadimenol (purity, 97.5%; A:B = 80:20) at a concentration of 0, 20, 100 or 500 ppm. The pretreatment period before the first mating was 70 days. In each generation, 10 males and 20 females of the second of two matings (F_{1b}, F_{2b}) were used to produce the next generation. All female animals were kept consecutively for a period of longer than one estrus cycle together with each of three males. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

Males of the F_{1b} generation at all doses had significantly decreased body-weight gains (<10%). The insemination indices (ratio of inseminated:not inseminated female rats) of both F_1 matings were decreased at 500 ppm (70% and 80% versus 95% and 100% in control groups). This effect was not commented on by the study authors. F_{2a} pups of both sexes showed reduced body-weight gains at 500 ppm. Histopathological examination of organs in F_{1b} parents and F_{2b} pups at 0 and 500 ppm revealed no treatment-related changes. Organ weight analysis in F_{1b} parents showed statistically significantly increased relative weights of the testes and ovaries at the highest dietary concentration.

The NOAEL for parental and reproductive toxicity was 100 ppm, equal to 8.6 mg/kg bw per day in F_0 parents, on the basis of reduced body-weight gains, effects on the weights of testes and ovaries, and reduced insemination indices at 500 ppm (Loeser & Eiben, 1984).

(b) Developmental toxicity

Rats

Groups of 20–22 mated female FB 30 rats (Long Evans) were given triadimenol (purity, 93.7%; A : B = 60 : 40) at a dose of 0, 10, 30 or 100 mg/kg bw per day by gavage from day 6 to day 15 of gestation. On day 20 of gestation, the fetuses were removed by cesarean section. This is a pre-GLP study and no statement of QA was provided.

At 100 mg/kg bw per day, the body-weight gain was slightly reduced and 20 out of 22 inseminated animals (90.9%) were fertilized; in all other groups, all females were fertilized. The study authors considered that the slight reduction at the highest dose was not treatment related. At the highest dose, increased fetal and placental weights were observed.

The NOAEL for maternal and offspring toxicity was 30 mg/kg bw per day on the basis of minor fertility and developmental effects, and placental weight effects at 100 mg/kg bw per day (Machemer, 1977a).

Groups of 25 mated female Wistar/HAN rats were given triadimenol (purity, 97%; A : B = 80 : 20) at a dose of 0, 30, 60 or 120 mg/kg bw per day by gavage from day 6 to day 15 of gestation. On day 21 of gestation, the fetuses were removed by cesarean section. This study complied with OECD requirements for GLP. At 60 and 120 mg/kg bw per day, body-weight gain and the food consumption were slightly reduced (statistically significantly). Additionally, at the highest dose, an increase in postimplantation loss was observed.

The NOAEL for maternal toxicity was 30 mg/kg bw per day on the basis of body-weight effects at 60 mg/kg bw per day.

The NOAEL for offspring toxicity was 60 mg/kg bw per day on the basis of postimplantation losses at 120 mg/kg bw per day (Becker et al., 1987a).

Groups of 25 mated female Long Evans BAY : FB30 rats were given triadimenol (purity, 95.2%; A : B = 80 : 20) at a dose of 0, 10 or 30 mg/kg bw per day by gavage from day 6 to day 15 of gestation. On day 20 of gestation, the fetuses were removed by cesarean section. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

At the highest dose, maternal body-weight gain was reduced and placenta weights were increased. The study author stated that this was ". . . a familiar result of treatment with azoles".

The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of body-weight effects at 30 mg/kg bw per day. The NOAEL for offspring toxicity was 10 mg/kg bw per day on the basis of increased placental weights at 30 mg/kg bw per day (Renhof, 1984).

Groups of 28 mated female Charles River Crl : CDBR rats were given triadimenol (purity, 95%) at a dose of 0, 5, 15, 25 or 60 mg/kg bw per day by gavage from day 6 to day 15 of gestation. On day 20 of gestation, the fetuses were removed by cesarean section. This study complied with OECD requirements for GLP.

At doses of ≥15 mg/kg bw per day, body-weight gain was reduced (about 10%) from day 6 to day 16 with a concomitant reduction in feed consumption. Placenta weights were increased at 60 mg/kg bw per day (Table 8). At doses of ≥25 mg/kg bw per day, an increase in the incidence of supernumerary lumbar ribs was observed.

The NOAEL for maternal toxicity was 25 mg/kg bw per day on the basis of body-weight effects at 15 mg/kg bw per day. The NOAEL for offspring toxicity was 15 mg/kg bw per day on the basis of increased supernumerary lumbar ribs at 25 mg/kg bw per day (Clemens et al., 1990b).

Rabbits

Groups of 20 artificially inseminated female New Zealand White rabbits were given triadimenol (purity, 96%) at a dose of 0, 5, 25 or 125 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 29 of gestation, the fetuses were removed by cesarean section. This study complied with OECD requirements for GLP.

At the highest dose, body-weight gain was reduced, as was food consumption. In the groups receiving the intermediate or the highest dose, there was a statistically significant decrease in median litter size (5.2 and 5.6 versus 7.6), implantations per litter (5.7 and 6.3 versus 8.4) and an increase in pre-implantation losses (23.4% and 14.7% versus 10.7%) (Table 9). This corresponded to reduced numbers in corpora lutea graviditatis (7.0 and 7.0 versus 9.0). After comparing these values with the data for historical controls, the Meeting therefore judged that these minimal reproductive effects were not treatment-related. A statistically significant increase in placental weight was observed only at the intermediate dose.

Table 8. Developmental parameters in a study of developmental toxicity in rats given triademenol by gavage

	Dose (mg/kg bw per day)				
	0	5	15	25	60
Body-weight gain days 6–16 (g)	52.5	51.7	45.8*	45.9**	39.9**
Placental weights (g)	0.52	0.52	0.51	0.55	0.63**
Extra ribs	1	6	6	13**	42**

From Clemens et al. (1990b)
* $p = 0.05$
** $p = 0.01$

Table 9. Reproductive efficiency in a study of developmental toxicity in rabbits given triademenol by gavage

Parameter		Dose (mg/kg bw per day)				Range for six historical control groups
		0	5	25	125	
Litter size	Mean	7.6	7.3	5.2	5.6	5.6–8.5
	Median	8.0	7.5	5.0*	6.0*	6–8
Implantations per litter	Mean	8.4	7.5	5.7	6.5	5.6–8.5
	Median	9.0	8.0	6.0**	6.0*	6–9
Pre-implantation losses (%)	Mean	10.7	12.0	23.4	14.7	5.1–23.6
	Median	9.1	0.0	25	6.3	0–11.1

From Clemens et al. (1992)
* $p = 0.05$ using Dunn test
** $p = 0.01$ using Dunn test

The NOAEL for maternal toxicity was 125 mg/kg bw per day on the basis of body-weight effects at 125 mg/kg bw per day. The NOAEL for offspring toxicity was 125 mg/kg bw per day, the highest dose tested (Clemens et al., 1992).

Groups of 16 mated female Chinchilla rabbits were given triadimenol (purity, 97%; A : B = 80 : 20) at a dose of 0, 8, 40 or 200 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 28 of gestation, the fetuses were removed by cesarean section. This study complied with GLP (unknown authority) requirements.

Animals at the highest dose showed excited behaviour and hair loss on paws and chest, probably caused by gnawing and scratching. At ≥40 mg/kg bw per day, the body-weight gain was reduced (<10%), concomitantly with reduced food intake at the highest dose. At the highest dose, a slight increase of postimplantation losses was observed. No other treatment-related effects on reproduction and development were observed.

The NOAEL for maternal toxicity was 40 mg/kg bw per day on the basis of reduced body-weight gain at ≥40 mg/kg bw per day. The NOAEL for offspring toxicity was 40 mg/kg bw per day on the basis of slightly increased postimplantation losses at 200 mg/kg bw per day (Becker et al., 1987b).

2.6 Special studies: neurotoxicity

In a study of neurotoxicity, male mice (Bor : CFW1) and male rats (Bor : WISW) were given single doses of triadimenol (purity, 98%) by gavage. In mice, effects on hexobarbital sleeping time, spontaneous motility, behaviour, open field behaviour and reserpine-induced ptosis were examined, while in rats, behaviour in a "novel box" test was investigated.

In mice, doses of 3.75 to 60 mg/kg bw per day stimulated the spontaneous motility, increased the irritability escape response and certain reflexes; doses of 15 and 60 mg/kg bw per day intensified the effect of amphetamine; and doses of 12–48 mg/kg bw per day antagonized that of reserpine. The test substance prolonged hexobarbital sleeping time at 15 and 60 mg/kg bw per day.

In rats, triademenol at 48 mg/kg bw per day had an excitatory effect and therefore reduced the sleeping time.

A comparison with caffeine showed that behavioural effects of 2.5 mg of caffeine/kg bw corresponded approximately to that of 12–15 mg of triadimenol/kg, in the amphetamine potentiation test 2.5 mg of caffeine/kg bw compared with 4 mg of triadimenol/kg and in the antagonism of ptosis, 10 mg of caffeine/kg bw and 12 mg of triadimenol/kg had similar potency (Polacek, 1983a). Owing to small group sizes, uncertain significance of the end-points and the lack of standardized study protocols, the Meeting concluded that this study would not be considered for evaluation of a reference dose.

In a comparative study to reveal structure–activity relationships with respect to neurological effects, groups of 8–12 male Long Evans rats were given a single dose of one of 14 different triazole fungicides or structurally related compounds by gavage. Dose ranges for the individual compounds were selected mainly according to their acute toxicity. The animals were subjected to the figure-eight maze test. Behavioural changes expressed as increased activity were restricted to triadimenol and triadimefon, reaching statistical

significance at 100 mg/kg bw per day. In both cases, a tendency to increased activity was observed at the lowest dose tested, 50 mg/kg bw per day (Crofton, 1996).

In studies in vitro, it was shown that triadimenol and triadimefon had a significant dopamine transporter-binding capacity, with no dopamine-releasing function in the striatum but with a dopamine uptake-inhibiting effect in striatal synaptosomal preparations (Walker & Mailman, 1996; Ikaiddi et al., 1997).

3. Observations in humans

In a medical survey spanning more than 10 years, no substance-related effects were observed in persons producing and formulating triadimenol. However, it was stated that no evidence of exposure had been found (Kehrig, 1999).

TRIADIMEFON

Evaluation for acceptable daily intake: triadimefon

4. Biochemical aspects

4.1 Absorption, distribution, excretion and metabolism

Five male and five female Wistar rats were given [phenyl-UL-^{14}C]triadimefon (specific activity, 15.78 mCi [583.9 MBq]/mmol) at a dose of 5 mg/kg bw or 50 mg/kg bw by gavage. In a study of multiple doses, 10 animals of each sex were treated with unlabelled triadimefon at a dose of 5 mg/kg bw per day for 14 days and on day 15 with [phenyl-UL-^{14}C]triadimefon at a dose of 5 mg/kg bw. In all these studies, animals were terminated 96 h after treatment with the radiolabelled substance. Expired air, faeces and urine were collected for analyses of radioactivity and metabolite. This study complied with the requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) for GLP.

The pattern of excretion and metabolism of the radiolabel was not significantly affected at either dose, or by pretreatment with unlabelled triadimefon. In males, 24–28% and in females, 57–67% of the administered dose was excreted in the urine, and 63–66% and 32–41% was excreted in the faeces, respectively. Less than 1% of the administered dose was expired. After 96 h, 2% of the radiolabel remained in the bodies of females and 9% in males, with the highest residue concentrations in the liver and kidneys.

The metabolism of triadimefon starts either by direct oxidation of the *t*-butyl methyl group to the hydroxy or the carboxy compound, with subsequent glucuronidation, or these steps are preceded by reduction of the keto group to the putative intermediate triadimenol. Therefore many of the metabolites found in studies of the metabolism of triadimenol (Puhl & Hurley, 1978; Justus, 2002b) are also found with triadimefon. Nevertheless, the metabolism of triadimefon in rats provides a pathway of demethylation of the *t*-butyl group that is not seen, at least not to any significant degree, with triadimenol (Chopade, 1992).

A single lactating goat aged 2 years was treated orally with capsules containing [phenyl-UL-^{14}C]triadimefon at a dose of 2.59 mg/kg bw per day (specific activity, 38.5 mCi [1.4 GBq]/mmol) for 3 consecutive days. On the third day, the animal, milk, faeces, urine and organs were collected for radioactivity analyses. This study complied with FIFRA requirements for GLP.

Within 24 h, 83% of the radioactivity was excreted in the urine. Highest residue concentrations were found in the kidneys (3.5 mg/kg) and in the liver (1.6 mg/kg). The residue concentration found in muscle was 0.068 mg/kg. Residue concentrations found in the milk at 0–24 h and at 24–48 h were similar (0.029 mg/kg) (Hall & Hartz, 1993).

Qualitatively, the metabolic pattern of triadimefon in the goat closely resembled that in rats (Chopade, 1992).

Ten laying hens were treated orally with capsules containing [phenyl-UL-^{14}C]triadimefon (specific activity, 38.5 mCi [1.4 GBq]/mmol) at a dose of 2.45 mg/kg bw per day for 3 consecutive days. On the third day, liver, fat and muscle were collected for radioactivity analyses. This study complied with FIFRA requirements for GLP.

Concentrations of radioactive residue in eggs increased from 0.007 (day 1) to 0.088 mg/kg (day 3). In the liver, fat and muscle, residue concentrations of 0.731, 0.171 and 0.123 mg/kg, respectively, were found (Duah & Smasal, 1993).

Qualitatively, the metabolic pattern of triadimefon in hens closely resembled that in rats (Chopade, 1992).

4.2 Effects on enzymes and other biochemical parameters

In a set of experiments in vitro and in vivo, the interaction of triadimefon with liver microsomal enzymes from mice and rats was investigated. No statement of compliance with GLP was provided.

Spectral analyses showed binding of triadimefon to cytochrome P450 in vitro and inhibition of monooxygenase activity was observed.

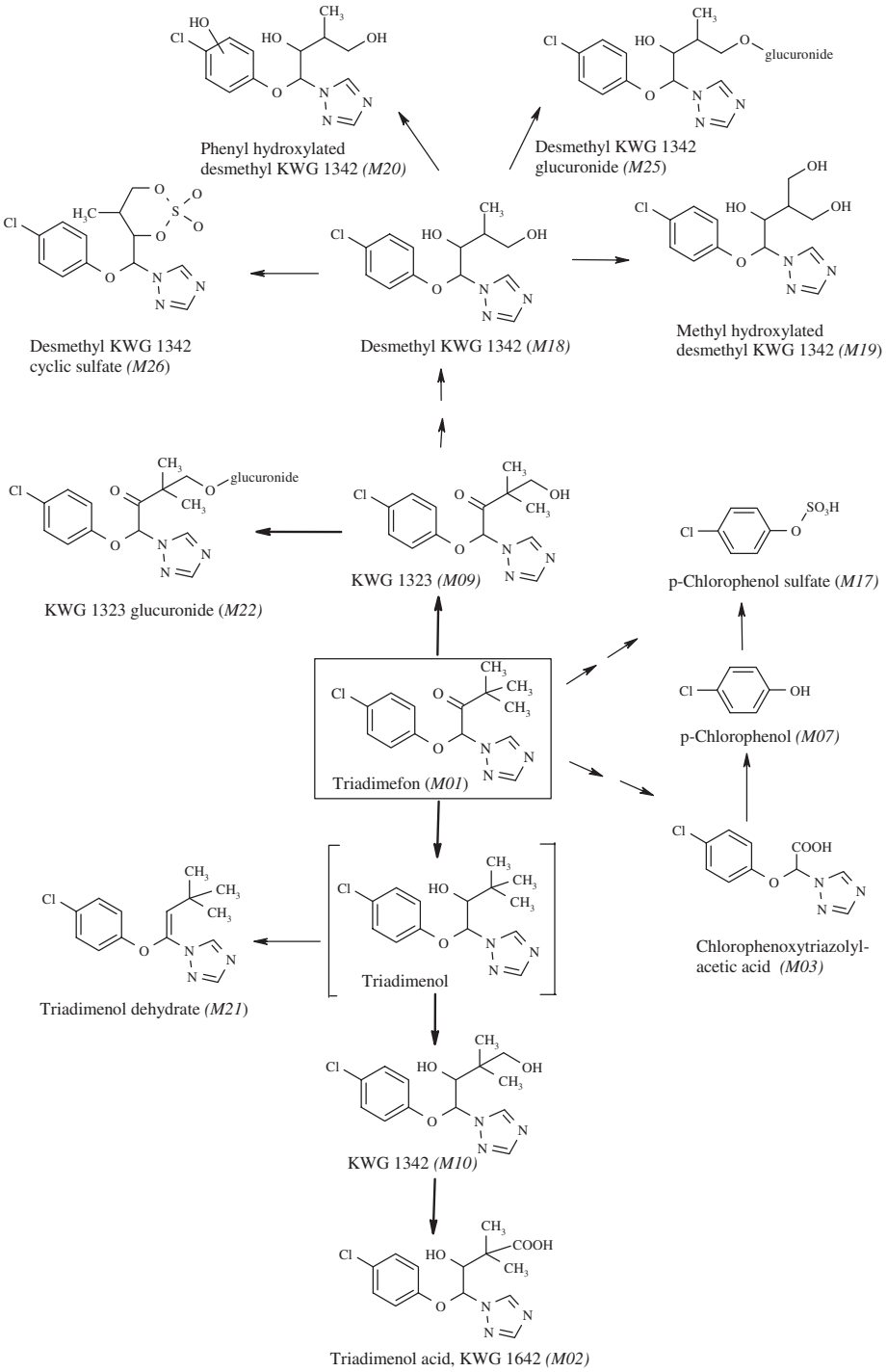
On exposure to triadimefon, induction of monooxygenase activity was observed, which was slight in rats and more pronounced in mice.

On the basis of the absence of any change in biphenyl 2-hydroxylase activity and the induction of aldrin epoxidation, it was concluded that enzyme induction by triadimefon more closely resembles that by phenobarbital than that by ligands of the aryl hydrocarbon (Ah) receptor (Schmidt, 1983). This is consistent with other azoles, e.g. propiconazole.

In a comparative assay for enzyme inhibition in vitro, the effects of triadimenol and triadimefon and other pesticides on aromatase activity were studied. Aromatase converts androgens to estrogens and therefore is important for achieving a balance in levels of sex steroids. Half maximal inhibition of human placental microsomal aromatase was observed for triadimenol and triadimefon at 21 µmol/l and 32 µmol/l, respectively. This was judged to be a weak inhibition when compared with that attributed to other azole compounds, such as prochloraz (Vinggaard et al., 2000).

Both compounds were found to be weak estrogen receptor agonists in MCF7 breast cancer cells (triadimefon and triadimenol at 10 µmol/l induced a 2.4-fold and 1.9-fold increase in cell proliferation respectively), but not in estrogen receptor alpha-transfected yeast cells. Other azole compounds such as prochloraz and imazalil either gave negative results in these assays or had very low activity (Vinggaard et al., 1999).

Figure 2. Proposed metabolism[a] of triadimefon in rats



From Bayer CropScience AG (2003)

[a]The scheme was reproduced from the manufacturer's evaluation

5. Toxicological studies

5.1 Acute toxicity

The acute toxicity of tridimefon is summarized in Table 10.

(a) Dermal and ocular irritation

In New Zealand White rabbits, slight treatment-related dermal and ocular irritation reactions were observed in a few cases (Sheets, 1990b; Sheets, 1990c).

(b) Dermal sensitization

Triadimefon (purity, 94.6%) was sensitizing to the skin of guinea-pigs in the Buehler topical test (Sheets, 1990a).

In the Magnusson-Kligman maximization test, technical-grade triadimefon (purity 94.6%) was used for intradermal and topical induction. Challenge with technical-grade triadimefon clearly resulted in sensitization, while challenge with purified triadimefon (purity, 99.6%) gave negative results (Diesing, 1991).

Table 10. Acute toxicity of triadimefon

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Reference
Mouse	NMRI	Male	Oral (fasted)	92.6	732	—	Mihail (1980)
Mouse	NMRI	Female	Oral (fasted)		1158	—	
Mouse	NMRI	Male	Oral	93.4	989	—	Thyssen & Kimmerle (1974)
Mouse	NMRI	Female	Oral		1071	—	
Mouse	NMRI	Male	Inhalation		—	>0.516	
Rat	Wistar	Male	Oral (fasted)	92.6	1855	—	Mihail (1980)
Rat	Wistar	Female	Oral (fasted)		1020	—	
Rat	Wistar	Male	Oral (fed)	93.4	568–1245	—	Thyssen & Kimmerle (1974); Mihail (1980)
Rat	Wistar	Female	Oral (fed)	92.6 and 93.4	363–793	—	Thyssen & Kimmerle (1974); Mihail (1980)
Rat	Wistar	Males and females	Dermal	92.6	>5000	—	Mihail (1980)
Rat	Wistar	Males and females	Intraperitoneal	92.6 and 93.4	213–321	—	Thyssen & Kimmerle (1974); Mihail (1980)
Rat	Wistar	Male	Dermal	93.4	>1000	—	Thyssen & Kimmerle (1974)
Rat	Wistar	Males and females	Inhalation		—	>0.455	
Rat	Sprague-Dawley	Male/Female	Inhalation	95.0	—	>3.27	Warren (1990)
Hamster		Male	Inhalation	93.4	—	>0.516	Thyssen & Kimmerle (1974)
Rabbit	NZW	Female	Oral		500	—	Thyssen & Kimmerle (1974)
Rabbit	NZW	Male	Oral (fasted)	92.6	250–500	—	Mihail (1980)
Hen		Female	Oral	93.4	500	—	Thyssen & Kimmerle (1974)
Quail		Female	Oral		1750–2500	—	Thyssen & Kimmerle (1974)

NZW, New Zealand White

5.2 Short-term studies of toxicity

Rats

Groups of 15 male and 15 female Wistar rats were given triadimefon (purity, "technical grade") at a dose of 0, 3.0, 10.0 or 30.0 mg/kg bw per day by gavage for 30 days. This is a pre-GLP study and no statement of QA was provided.

There were no treatment-related findings on behaviour, body-weight development, haematology, clinical chemistry, urine analysis or histopathology. The only treatment-related findings were increased relative and absolute weights of the liver in males at dosesof ≥10 mg/kg bw per day and in females at 30 mg/kg bw per day. There were no histopathological or clinical chemistry findings indicative of liver damage.

The NOAEL was 30.0 mg/kg bw per day, the highest dose tested (Thyssen et al., 1974).

Groups of 20 male and 20 female Wistar rats were given triadimefon (purity, 97%) at a dose of 0, 1, 5 or 25 mg/kg bw per day by gavage for 4 weeks, followed by a 4-week recovery period. This is a pre-GLP study and no statement of QA was provided.

At doses of ≥5 mg/kg bw per day, mild induction of microsomal enzymes was observed. This effect was reversible in the recovery period. No other parameters (appearance, behaviour, body weight, haematology, clinical chemistry, gross pathology, liver weight or histopathology) were affected.

The NOAEL was 25 mg/kg bw per day, the highest dose tested (Mihail & Kaliner, 1979).

Groups of 15 male and 15 female Sprague-Dawley rats were fed diets containing triadimefon (purity not reported) at a concentration of 0, 50, 200, 800 or 2000 ppm for 12 weeks. This is a pre-GLP study and no statement of QA was provided.

There were no treatment-related changes in appearance, behaviour, body weight, haematology, clinical chemistry, urine analysis, gross pathology or histopathology.

The NOAEL was 2000 ppm, equal to 150 mg/kg bw per day, the highest dose tested (Mohr, 1976).

Groups of five male and five female Sprague-Dawley rats were treated dermally with triadimefon (purity, 95.9%) at a dose of 0, 100, 300 or 1000 mg/kg bw per day for 6 h per day, 5 days per week, over a period of 3 weeks. This study complied with OECD requirements for GLP.

Females at the highest dose showed increased activity and reactivity as well as an increased incidence of diffuse acanthosis at the application site. No other parameters, including appearance, behaviour, body and organ weights, haematology, clinical chemistry, gross pathology, urine analysis or histopathology, were affected.

The NOAEL was 300 mg/kg bw per day on the basis of behavioural effects at 1000 mg/kg bw per day (Sheets & Phillips, 1992).

In the first of two studies of exposure by inhalation, groups of 10 male and 10 female Wistar rats were given triadimefon at a concentration of 0.454 mg/l by daily inhalation for 4 h on 5 consecutive days. The post-exposure period was 14 days (Thyssen et al., 1974).

In the second study, groups of 10 male and 10 female Wistar rats were given triadimefon at a concentration of 0.079 or 0.307 mg/l by daily inhalation for 6 h on 15 days (3×5 consecutive days over 3 weeks). After the last exposure, the animals were terminated. This is a pre-GLP study and no statement of QA was provided.

The only treatment-related finding was reduced body-weight gain in males at the higher dose in the second study and increased relative liver weights in males and females at the higher dose in the same study.

The NOAEC was 0.079 mg/l (Thyssen et al., 1974).

Rabbits

Groups of 15 mated female American Dutch rabbits were given triadimefon (purity, 94.7%) at a dose of 0, 20, 50 or 120 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 19 of gestation, the dams were terminated. No examinations on reproductive and developmental effects were performed. This study complied with OECD requirements for GLP.

At the highest dose, reduced food consumption, a loss in body weight and increased spleen and adrenal weights were observed. In the spleens, increased incidences of reticuloendothelial cell hyperplasia and macrophages with cell debris were found.

The NOAEL was 50 mg/kg bw per day on the basis of body weight and organ weight changes with histopathological correlates at 120 mg/kg bw per day (Clemens et al., 1990a).

Groups of three male and three female rabbits were treated dermally (intact or abraded skin) with triadimefon (purity, "pure technical grade") at a dose of 0, 50 or 250 mg/kg bw per day on 5 days per week for 4 weeks. This is a pre-GLP study and no statement of QA was provided.

The only treatment-related finding was slight erythema in all dosed animals (intact and abraded skin). No other parameters including appearance, behaviour, body and organ weights, haematology, clinical chemistry, gross pathology, urine analysis or histopathology were affected.

The NOAEL was 250 mg/kg bw per day, the highest dose tested (Thyssen & Weischer, 1976).

Dogs

Groups of four male and four female beagle dogs were fed diets containing triadimefon (purity, 99.6%) at a concentration of 0, 150, 600 or 2400 ppm for 13 weeks. This is a pre-GLP study and no statement of QA was provided. The average daily intakes of triadimefon were 0, 4.4, 17.3 and 65.8 mg/kg bw per day (calculated from the daily intakes per animal of 0, 43.5, 173 and 658 mg and a default body weight of 10 kg).

Table 11. Findings in dogs fed with triademefon for 2 years

	Dietary concentration (ppm)			
	0	100	300	1000–2000
Mean body weight at the end of study (kg)	10.86	11.74	10.76	10.05
N-Demethylase (nmoles/g per min)	35.8	40.5	43.4	99.6
AST (U/l)	20.4	20.8	23.7	19.0
aLT (U/l)	27.8	25.8	26.5	26.6
ALP (U/l)	113.6	114.5	133.0	565.63

From Hoffmann & Groening (1978)
ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase

Clinical inspections, haematology, clinical chemistry, urine analysis, gross and histological pathology were performed. Body-weight gain, food intake, relative weights of the liver and general appearance of animals at the highest dose were impaired. In this group, erythrocyte volume fraction, erythrocyte counts and haemoglobin were reduced. At doses of ≥600 ppm, animals showed an increased aminopyrine-*N*-demethylating activity, and at 2400 ppm, increased plasma ALP and ALT activity.

The NOAEL was 600 ppm, equal to 17.3 mg/kg bw per day, on the basis of a number of effects at 2400 ppm (Hoffmann & Luckhaus, 1974).

Groups of four male and four female beagle dogs were fed diets containing triadimefon (purity, 88.9%) at a concentration of 0, 100, 330 and 1000 ppm for 2 years. The average daily intakes of triademefon were 0, 3.26, 11.7 and 48.8 mg/kg bw per day. This study did not comply with GLP requirements, but was supervised by an internal QA unit. The dose of the group at 1000 ppm was increased to 2000 ppm from week 55 to 104.

At the highest dose, a slight significant decrease in body-weight gain and mild induction of hepatic microsomal enzymes was observed (Table 11). There were no other treatment-related findings.

The NOAEL was 2000 ppm, equal to 48.8 mg/kg bw per day, the highest dose tested (Hoffmann & Groening, 1978).

5.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 60 male and 60 female NMRI mice were fed diets containing triadimefon (purity not reported) at a concentration of 0, 50, 300 or 1800 ppm for 21 months. The average daily intakes were 0, 13.5, 76 and 550 mg/kg bw per day for males and 0, 19.6, 119 and 765 mg/kg bw per day for females. Ten animals per group were terminated at 12 months. This study complied with FIFRA requirements for GLP.

Males at the highest dose showed reduced body-weight gain (approximately 20%). In all treated females, reduced erythrocyte volume fraction and increased mean corpuscular haemoglobin concentration were found without any clear dose dependence. Increased activity of ALT and AST was observed at the highest dose at the end of the study in both sexes, and also in females at the intermediate dose at the interim kill. ALP activity was increased in males at the highest dose. In males at doses of ≥300 ppm, the number of animals with

nodular changes in the liver was increased and in both sexes at 1800 ppm, nodular changes, fatty droplets, discolouration and enlargement of the liver were found. These findings were accompanied by increases in absolute and relative weights of the liver. All dosed females and males at ≥300 ppm showed hepatocellular hypertrophy and an increase in single cell necrosis. Females at ≥300 ppm and males at 1800 ppm showed increased Kupffer cell proliferation, multifocal round cell infiltrations and lipofuscin deposits in macrophages. In males and females at the highest dose, an increase in hepatocellular adenomas was observed relative to that in controls (11 versus 3, and 9 versus 2) (Table 12).

The NOAEL was 50 ppm, equal to 13.5 mg/kg bw per day, on the basis of signs of liver damage at ≥300 ppm (Bomhard & Hahnemann, 1986).

Groups of 50 male and 50 female CF_1/W 74 mice were fed diets containing triadimefon (purity, 97%) at a concentration of 0, 50, 300 and 1800 ppm for 24 months. The average daily intakes of triademefon were 0, 9.29, 60.5 and 421 mg/kg bw per day for males and 0, 12.0, 75.6 and 471 mg/kg bw per day for females. This is a pre-GLP study and no statement of QA was provided.

Animals of both sexes at the highest dose showed reduced body-weight gain (<10%) and increased liver weights; the livers appeared swollen, and hardened or brittle (Table 13). Males in this group also had reduced kidney weights. All dosed males showed reduced mean corpuscular haemoglobin concentrations. At the end of the study, both sexes in the group receiving the highest dose had increased erythrocyte counts and females had increased thrombocyte counts and increased haemoglobin, erythrocyte volume fraction and mean corpuscular haemoglobin concentrations. At the highest dose, animals of both sexes had increased activity of ALP, AST and ALT.

Table 12. Incidence of liver adenomas in mice fed with triademefon for 21 months

	Dietary concentration (ppm)			
	0	50	300	1800
No. of animals of each sex examined	50	50	50	50
Males	3	3	4	11
Females	2	1	0	9

From Bomhard & Hahnemann (1986)

Table 13. Findings at the end of a 24-month study in mice fed diets containing triademefon

Finding	Dietary concentration (ppm)			
	0	50	300	1800
No. of animals examined of each sex	50	50	50	50
Liver weight in males (mg)	2241	2233	2605	4176**
Liver weight in females (mg)	1833	2021	1950	3819**
Kidney weight in males (mg)	853	750*	831	686**
ALP in males (U/l)	151	113	221	1845
ALP in females (U/l)	330	322	291	1135

From Bomhard & Loeser (1980)
ALP, alkaline phosphatase
*$p = 0.05$
**$p = 0.01$

An increase of hyperplastic liver nodules was reported at the highest dose compared with controls (males, 15 versus 7; and females, 15 versus 4). Re-examination of the relevant slides of liver sections 10 years later resulted in reclassification of most of these nodules as liver adenomas and carcinomas. Since not all slides could be re-examined, no final conclusions could be made by the re-examination group regarding putatively increased incidences of neoplastic lesions and their classification.

The NOAEL was 300 ppm, equal to 60.5 mg/kg bw per day, on the basis of body-weight effects and changes in the blood profile (Bomhard & Loeser, 1980).

Rats

Groups of 60 male and 60 female Wistar (Bor : WISW) rats were fed diets containing triadimefon (purity, 94.4%) at a concentration of 0, 50, 300 or 1800 ppm for 105 weeks. Ten animals per group were terminated at 52 weeks. Average daily intakes of triademefon were 0, 2.7, 16.4 and 114 mg/kg bw per day for males and 0, 3.6, 22.5 and 199 mg/kg bw per day for females. This study complied with OECD requirements for GLP.

In spite of an increased food intake, body-weight gain was reduced in both sexes at the highest dose (5–10%) and females showed reduced haemoglobin, mean corpuscular haemoglobin concentration, erythrocyte volume fraction, and reduced erythrocyte counts. Decreased leukocyte counts were observed at different time-points, but there was no apparent dose dependence. In females receiving triademefon at doses of ≥300 ppm and in males at 1800 ppm, absolute and relative weights of the liver were increased. An increase (<50%) in the ALT activity was found in males at 1800 ppm and a decreased AST activity in all dosed females (twofold at the highest dose). In both sexes, a tendency to lower plasma bilirubin values at the intermediate and the highest doses and decreased creatinine values at the highest doses were observed. The males at the highest dose excreted significantly less protein in the urine. In all dosed groups, the incidence of fatty deposits in hepatocyte cytoplasm increased with dose. A marginally-increased incidence of thyroid cystic hyperplasia was found in both sexes at the highest dose, predominantly in females, and concomitantly, a minor increase in the incidence of thyroid follicular adenomas was found compared with controls (males, 3 versus 0; and females, 2 versus 0) (Table 14). A marked decrease in the incidence of several tumours (adrenals in males and mammary glands in females)was observed at the highest dose. In an addendum to the study, the incidences of thyroid adenomas were compared with those for historical controls of this rat strain; they fell well within the ranges for historical controls.

Table 14. Histopathological findings in the thyroid of rats fed diets containing triademefon for 105 weeks

Finding		Dietary concentration (ppm)				Range for historical controls
		0	50	300	1800	
Follicular cell adenomas	Males	0	0	1	3*	0–5.2%
	Females	0	1	0	2	0–4.0%
Cystic hyperplasia	Males	2	3	1	3	—
	Females	2	0	1	4	—

From Bomhard & Schilde (1991)
*$p = 0.05$ in the Peto et al. trend test

The NOAEL was 300 ppm, equal to 16.4 mg/kg bw per day, primarily on the basis of non-neoplastic changes in the thyroid at 1800 ppm (Bomhard & Schilde, 1991).

Groups of 50 male and 50 female Wistar rats were fed diets containing triadimefon (purity, "technical grade") at a concentration of 0 (100 animals of each sex), 50, 500 or 5000 ppm for 24 months. The average daily intakes of triademefon were 0, 2.38 and 24.3 mg/kg bw per day for males and 0, 3.19 and 33.3 mg/kg bw per day for females. No average intake was given for animals in the group at 5000 ppm, since no animals survived the first year of exposure. This is a pre-GLP study and no statement of QA was provided.

Starting on week 23, animals at the highest dose showed violent motor activity, feed refusal and many animals died. After severe body-weight loss, the surviving animals started to feed again but in the following weeks they showed the same symptoms again. On week 39, the last animals at the highest dose were terminated in a moribund state. None of the animals in the groups receiving triademefon at the lowest and the intermediate doses showed any effects on appearance or behaviour. Females at 500 ppm showed slightly reduced body-weight gain. The liver weights of males at ≥50 ppm and the liver and ovary weights of females at 500 ppm were increased (Table 15). There were no histopathological changes in the liver and no clinical chemistry effects indicating liver damage. Adrenal weights of both sexes were decreased at 500 ppm. Animals at the highest dose that died or were killed in extremis showed haemorrhagic lesions in the stomach mucosa, blood-filled and dilated alveolar vessels, degenerative processes in proximal kidney tubules of females, atrophied spleens with signs of decreased haematopoesis, some giant spermatids in testes, and decreased haematopoesis in the bone marrow of males. At doses of ≥500 ppm, females showed statistically significantly reduced and males statistically significantly increased erythrocyte counts (both about 10%). Additionally, males receiving triademefon at doses of ≥500 ppm had statistically significantly (about 70% of that of controls) reduced leukocyte counts. There were no significant enzyme changes indicative of liver damage. In the first 6 months, females at the highest dose showed increased cholesterol concentrations. There were no apparent changes in tumour incidences in any group.

The NOAEL was 50 ppm, equal to 2.38 mg/kg bw per day, on the basis of minimal organ weight changes and changed erythrocyte counts in both sexes at 500 ppm (Bomhard & Loeser, 1978).

Interpolating these two studies, the overall NOAEL was 16.4 mg/kg bw per day.

Table 15. Absolute organ weights (mg) of mice fed with triademefon for 24 months

Organ	Sex	Dietary concentration (ppm)		
		0	50	500
Liver	Males	13 209	15 279**	15 373**
	Females	9 237	9 328	10 143**
Adrenals	Males	46	45	42**
	Females	68	66	60*
Ovaries	Females	129	122	155**

From Bomhard & Loeser (1978)
*p = 0.05 in the Wilcoxon-Mann-Whitney U-test
**p = 0.01 in the Wilcoxon-Mann-Whitney U-test

Table 16. Studies of genotoxicity with triadimefon

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98	100–3333 µg/plate ±S9, in DMSO	93.1–94.2	Negative	San & Springfield (1990)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA100, TA98	0.1–1000 µg/plate ±S9, in DMSO	97.0	Negative	Inukai & Iyatomi (1977)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98, TA1950	0.1–1000 µg/plate ± mouse microsomes, in DMSO	97.0	Negative	van Dijck (1976)
Reverse mutation[b]	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98, TA1950 *E. coli* WP2 hcr	10–5000 µg/plate in ±S9, DMSO	97.0	Negative	Shirasu et al. (1978); Shirasu et al. (1979)
Reverse mutation	*Saccharomyces* S138 and S211c	0.01–10 µg/plate ±S9, in DMSO	Not reported	Negative	Jagannath (1980)
DNA damage	*E. coli* (K12)p 3478 (pol A_1^-) and W3110 (pol A^+)	625–10 000 µg/plate ±S9	86.0	Negative	Herbold (1984)
Rec assay	*B. subtilis* NIG17 (rec^+) and NIG45 (rec^-)	3–300 µg/disk	97.0	Negative	Inukai & Iyatomi (1977)
Rec assay	*B. subtilis* NIG17 (rec^+) and NIG45 (rec^-)	20–2000 µg/disk	97.0	Negative	Shirasu et al. (1978); Shirasu et al. (1979)
Cytogenetic changes[a]	Primary human lymphocytes	50–200 µg/ml ±S9	93.0	Negative	Herbold (1986)
CHO/HGPRT mutation[a]	Chinese hamster ovary K1 cells (CHO)	105–256 µg/ml ±S9	93.1	Negative	Harbell (1989)
In vivo					
Micronucleus formation	Bone marrow erythroblasts of male and female NMRI mice	Two oral doses at 200 mg/kg bw per day	Not reported	Negative	Machemer (1977b)
Dominant lethal mutation	Male mice	Single oral dose at 200 mg/kg bw	95.9	Negative	Machemer (1976c)
Unscheduled DNA synthesis	Primary rat hepatocytes from male Sprague-Dawley rats	5–160 µg/ml	96.4	Negative	Brendler (1991)

DMSO, dimethylsulfoxide; S9, 9000 × *g* rat liver supernatant

[a] Dose-related cytotoxicity observed at all doses

[b] Shirasu et al. (1978) used only the strains TA98 and TA100.

5.4 Genotoxicity

The results of studies of genotoxicity with triademefon are summarized in Table 16.

5.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a three-generation study, groups of 10 male and 20 female Wistar rats were fed diets containing triadimefon (purity, "technical grade") at a concentration of 0, 50, 300 or 1800 ppm. The pretreatment period before the first mating was 70 days. In each generation, the pups of the second of two matings (F_{1b}, F_{2b}, F_{3b}) were used to produce the next generation. All females were kept for longer than one estrus cycle consecutively with each of three males. This is a pre-GLP study and no statement of QA was provided.

Female and pup body-weight gain was reduced at ≥300 ppm in all generations, reaching statistical significance only at 1800 ppm. At 1800 ppm, fewer (85%) animals became pregnant in the second mating of the F_0. In the first mating of the $F_{1b,}$ only one female became pregnant, while in the second mating none of the females became pregnant. The

sizes of delivered litters at the highest dose were decreased and pup weight survival rate in the lactating period and body-weight gain were impaired. Histopathological examination of the F_{3b} pups revealed no treatment-related effects and at all doses no treatment-related malformations were seen.

The NOAEL for maternal toxicity was 300 ppm, equivalent to 22.8 mg/kg bw per day, on the basis of reduced body-weight gain at 300 ppm. The NOAEL for reproductive toxicity was 300 ppm, equivalent to 22.8 mg/kg bw per day, on the basis of reduced pup weight gain at 1800 ppm (Loeser, 1979).

The above study was supplemented by another study of reproductive toxicity in Bor:WISW rats. Groups of 10 males and 20 females were fed diets containing triadimefon (purity, 92.6%) at a concentration of 0, 50 or 1800 ppm. The pretreatment period before mating was 100 days. In each generation, only one mating was conducted to produce the next generation. All female animals were kept consecutively with each of three males for a week. There was no report on compliance of this study with any GLP standards and no statement on quality assurance was given.

The fertility of the F_0 animals at 1800 ppm was not affected, but the viability (79% versus 93 in controls) and birth weights of the F_1 pups were reduced. The fertility of F_1 animals at 1800 ppm was 35% versus 85% in the control group and the insemination index was 50% versus 100%. Therefore, the ratio of pregnant:inseminated females was 70% at 1800 ppm and 85% in the control group. The litter size, viability and birth weights of the F_2 pups were reduced and the male:female ratio was 38:62, compared with 50:50 in the control group. In a cross mating test, F_1 males at 1800 ppm were mated with F_1 control females (test 1) and F_1 control males were mated with F_1 females of the group at 1800 ppm (test 2). In test 1, the fertility index was 47.4% and in test 2 it was 80%; the respective insemination indices were 63% and 100%, respectively. Therefore, the difference in fertility index probably arose owing to reduced mating willingness in the males at the highest dose. However, the litter size in test 2 was reduced compared to that in test 1 and the male:female ratio was 62:38 (i.e. inverted when compared with the sex ratio observed in the F_2 generation).

In males at the highest dose, the testosterone concentration was double that in control males and testes weights were increased. No correlation between individual testosterone concentrations and spermiograms and mating willingness was observable. Reduced mating willingness appeared to correlate with reduced body weight. In agreement with the study authors, the Meeting concluded that prenatal, but not postnatal exposure of males affects mating willingness.

The NOAEL for reproductive toxicity was 50 ppm, equivalent to 3.75 mg/kg bw per day, on the basis of impaired reproductive performance at 1800 ppm (Eiben, 1984).

(b) Developmental toxicity

Rats

Groups of 26 mated female CD-SD rats were given triadimefon (purity, 93.2%) at a dose of 0, 10, 30 or 90 mg/kg bw per day by gavage from day 6 to day 15 of gestation. On day 20 of gestation, the fetuses were removed by caesarian section. This study complied with FIFRA requirements for GLP.

Table 17. Supernumerary ribs in a study of developmental toxicity in rats treated with triademefon by gavage

Dose (mg/kg bw per day)	Litters affected/total litters	Fetuses affected/total fetuses
0	1/20	1/48
10	10/17	16/52
30	10/19	13/54
90	19/22	57/84

From Unger et al. (1982)

At the highest dose, the body-weight gain of the dams was statistically significantly reduced (29 g compared with 38 g in the controls, only significant from day 6 to day 15 of gestation), and in fetuses, an increase in supernumerary ribs was found (Table 17). No other signs of developmental toxicity were observed. The NOAEL for parental and offspring toxicity was 30 mg/kg bw per day, on the basis of supernumerary ribs in fetuses and body weight depression in dams at 90 mg/kg bw per day (Unger et al., 1982).

Groups of 22–24 mated female FB 30 (Long Evans) rats were given triadimefon (purity not reported) at a dose of 0, 10, 30 or 100 mg/kg bw per day by gavage in test 1 and at a dose of 0, 50, 75 or 100 mg/kg bw per day in test 2, from day 6 to day 15 of gestation. On day 20 of gestation, the fetuses were removed by caesarian section. This is a pre-GLP study and no statement of QA was provided. At doses of ≥30 mg/kg bw per day, the body-weight gain of dams was reduced by ≤50% at 100 mg/kg bw per day. At the highest dose in test 2, there was a slight increase in placental weights.

At 75 mg/kg bw per day and in the two groups at 100 mg/kg bw per day, 2 out of 220 pups and 5 out of 394 pups, respectively, had cleft palates, while there were none in any of the other groups. In 143 historical control groups with 2975 litters (dated 1971 to 1984), 7 out of 32 354 pups showed this specific malformation.

The NOAEL for maternal toxicity in these two tests was 10 mg/kg bw per day on the basis of reductions in body-weight gain at 30 mg/kg bw per day. The NOAEL for offspring toxicity was 50 mg/kg bw per day on the basis of cleft palates at 75 mg/kg bw per day (Machemer, 1976b).

Groups of 20–22 mated female FB30 (Long Evans) rats were exposed to triadimefon (purity not reported) at a concentration of 0, 0.014, 0.033 or 0.114 mg/l for 6 h per day on 10 consecutive days (day 6 to day 15 of gestation) by inhalation. On day 20 of gestation, the fetuses were removed by caesarian section. This is a pre-GLP study and no statement of QA was provided. At concentrations of ≥0.033 mg/l, the body-weight gain of the dams was reduced. There was no other evidence of effects on maternal, embryonic, or developmental toxicity. The NOAEC was 0.014 mg/l on the basis of body-weight effects in dams at 0.033 mg/l (Machemer & Kimmerle, 1976).

Rabbits

Groups of 10–13 mated female Himalayan rabbits were given triadimefon (purity not reported) at a dose of 0, 5, 15 or 50 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 29 of gestation, the fetuses were removed by caesarian section. This is a pre-GLP study and no statement of QA was provided. There was no evidence of maternal

or developmental toxicity. The NOAEL was 50 mg/kg bw per day, the highest dose tested (Machemer, 1976a).

Groups of 12 mated female Himalayan rabbits were given triadimefon (purity, 93.5%) at a dose of 0, 10, 30 or 100 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 29 of gestation, the fetuses were removed by caesarian section. There was no report on compliance of this study with any GLP standards and no statement of QA was provided. At 100 mg/kg bw per day, dams had changed faeces consistency, diarrhoea, and swollen and inflammated external vaginas. At doses of ≥30 mg/kg bw per day, the animals showed severely reduced body-weight gain, resulting in body-weight loss at the highest dose (Table 18).

At the highest dose, three animals showed complete resorption of their litters, with one in the control group and none in any other dosed group. One of 53 pups at the highest dose showed multiple malformations, although a relation to treatment is unlikely.

The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of body-weight changes of dams at 30 mg/kg bw per day. The NOAEL for offspring toxicity was 30 mg/kg bw per day on the basis of increased resorptions at 100 mg/kg bw per day (Roetz, 1982).

Groups of 20 American Dutch rabbits were given triadimefon (purity, 94.3%) at a dose of 0, 20, 50 or 120 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 28 of gestation, the fetuses were removed by caesarian section. This study complied with OECD guidelines for GLP.

At the highest dose, reduced feed consumption and a loss in body weight was found (Table 19). Fetuses in the group receiving the highest dose showed delayed ossification in

Table 18. Mean maternal body-weight gain in a study of developmental toxicity in Himalayan rabbits given triademefon by gavage

Dose (mg/kg bw per day)	Maternal body-weight gain (g)
0	39.1
10	47.5
30	4.6
100	−64.3

From Roetz (1982)

Table 19. Mean maternal body-weight gain in a study of developmental toxicity in American Dutch rabbits given triademefon by gavage

Dose (mg/kg bw per day)	Mean maternal body-weight gain (g)		
	Days 6–10	Days 6–18	Days 0–28
0	20	120	220
20	10	70	210
50	0	110	280
120	−80	50	270

From Clemens & Hartnagel (1990)

Table 20. Mean maternal body-weight gain in a study of developmental toxicity in American Dutch rabbits given triademefon by gavage

Dose (mg/kg bw per day)	Mean maternal body-weight gain (g)	
	Days 6–19	Days 0–28
0	170	310
40	70	260
60	80	290
80	70	290

From Clemens et al. (1991)

skeletal elements, malformations of caudal vertebrae and of spinous elements of the scapula (59 out of 118 fetuses, 50%) and cleft palates (2 out of 118 fetuses, 1.7%). The incidence of irregular spinous process of the scapula was also significantly increased (32 out of 121 fetuses, 26.4%) in the group receiving triademefon at a dose of 50 mg/kg bw per day. In 21 historical control groups with 335 litters (dated 1982 to 1988), only 2 out of 2034 pups had cleft palates and 18 out of 602 pups had irregularly formed scapulae. The NOAEL for offspring toxicity in this study was 20 mg/kg bw per day on the basis of scapula malformations at 50 mg/kg bw per day (Clemens & Hartnagel, 1990).

Groups of 20 American Dutch rabbits were given triadimefon (purity, 92.9%) at a dose of 0, 40, 60 or 80 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 28 of gestation, the fetuses were removed by caesarian section. This study complied with OECD requirements for GLP.

All dosed animals had decreased body-weight gain (Table 20) and a slight increase in the incidence of reticulo-endothelial hyperplasia in the spleen was found. Scapula malformations were found in fetuses of all dosed groups (13.4%, 19.3% and 26.7% in the groups receiving the lowest, intermediate and highest doses respectively; for data on historical control groups, see above) and delayed ossification in skeletal elements at doses of ≥60 mg/kg bw per day. The uncommon finding of umbilical hernia was observed in one out of 119 fetuses at 60 mg/kg bw per day and in two out of 105 fetuses at 80 mg/kg bw per day. In this study, no NOAEL for maternal and developmental toxicity could be established (Clemens et al., 1991).

5.6 Special studies

(a) Neurotoxicity

Groups of 12 male and 12 female Wistar rats were given a single dose of triadimefon (purity, 95.8%) at 0, 2, 35 or 600 (males) or 400 (females) mg/kg bw by gavage and then observed for 14 days. This study complied with OECD requirements for GLP.

One male and four females in the group receiving the highest dose died within 2 days after dosing and the body-weight gain in this group was decreased. Male animals at the intermediate and the highest dose and females at the highest dose showed stereotypic behaviour, self mutilation and other signs of general toxicity. In the functional observational battery (FOB), animals at the intermediate or the highest dose showed effects shortly after dosing (most pronounced at 40 min), including affected posture and gait, increased activity, searching and cleaning gestures and increased rearing incidence. Overall, the effects on

FOB were reversible within 14 days. Although attenuated, the increased open-field rearing remained in males. The NOAEL was 2 mg/kg bw per day on the basis of signs of neurotoxicity at 35 mg/kg bw per day (Dreist & Popp, 1996a).

Groups of 18 male and 18 female Wistar rats were fed diets containing triadimefon (purity, 95.8%) at a concentration of 0, 50, 800 or 2200 ppm for 13 weeks. At the end of this period, six animals of each sex per group were terminated for neuropathology examination and the remaining animals were fed basal diet and observed for reversibility of any effects for another 4 weeks (males) or 10 weeks (females). This study complied with OECD requirements for GLP. The average intake of triadimefon was 0, 3.4, 54.6 and 150 mg/kg bw per day in males and 0, 4.3, 68.7 and 190 mg/kg bw per day in females. Intake was calculated according to food intake of the control animals, since a prominent increase in food consumption was observed in males at the highest dose (28% increased cumulative food intake, on the basis of grams/kg bw) and intermediate dose, and females at the highest dose (cumulative food intake was increased by 26% and 102%, respectively) which probably resulted from increased activity of the animals.

Body-weight gain was reduced in males at the intermediate (−5%) and highest doses (−13%) and in females at the highest dose (−15%). Males in the group receiving the highest dose and females in the groups receiving the intermediate and highest doses showed increased motility lasting for several weeks during the recovery period. Hyperactivity, indicated by effects on posture, increased rearing in the open field and pacing, was observed in animals of both sexes at the intermediate and highest doses. Most of the effects were reversible or attenuated in the recovery period.

The NOAEL was 50 ppm, equivalent to 3.4 mg/kg bw per day, on the basis of signs of neurotoxicity at 800 ppm (Dreist & Popp, 1996b).

There are several publications reporting on neurotoxicity induced by triadimefon in rats, as indicated by increased locomotor activity and stereotypical behaviour changes. These include studies in which triadimefon was administered orally, as well those in which it was administered by intraperitoneal application, from a dose of tens to a few hundred milligrams per kg bw per day. The magnitude of the effects and the time to recovery were related to dose (Crofton et al., 1988; Moser & MacPhail, 1989; Walker et al., 1990).

In a comparative study of neurotoxicity to reveal structure–activity relationships, male Long Evans rats were treated with one of 14 triazole fungicides or structurally related compounds. Eight to twelve animals per group received triadimefon or triadimenol at a dose of 0, 50, 100, 200 or 400 mg/kg bw. Signs of neurotoxicity were restricted to triadimefon and triadimenol and were reported as hyperactivity, which was statistically significant at the intermediate and the highest doses (Crofton, 1996).

Several studies suggest that the mechanism by which triadimefon causes neurotoxicity is via its potentiation of dopaminergic activity. In vitro, it was shown that triadimefon and triadimenol have a significant dopamine transporter-binding capacity, no dopamine-releasing function in the striatum, but a dopamine uptake-inhibiting effect in striatal synaptosomal preparations (Walker & Mailman, 1996; Ikaiddi et al., 1997).

In another study in rats, it was shown that the animals developed tolerance to triadimefon-induced enhanced locomotor and stereotypy behavioural patterns, since a new

challenge posed 14 days after the first of 12 consecutive exposures did not generate a response. This was also true in a similarly designed test for cross-sensitization with cocaine. These findings were accompanied by significant changes in dopaminergic biochemistry, which were interpreted by the study authors as adaptive responses to both single and repetitive exposures to triadimefon (Hill et al., 2000).

In a subsequent study on neurotoxicity in mice treated with triadimefon, animals were pretreated with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), *N*-methyl-D-aspartate (NMDA) and dopamine D1 and D2 receptor antagonists before they were exposed to triadimefon. Pretreatment with AMPA, NMDA or dopamine D1 receptor antagonists prevented the animals from developing behavioural changes, but the dopamine D2 receptor antagonist was inactive in this respect. The authors concluded that in addition to effects on dopamine re-uptake, AMPA, NMDA and dopamine D1 receptors are also involved in the development of triadimefon-mediated neurotoxicity (Reeves et al., 2004).

Male Bor:CFW1 mice and male Bor:WISW rats were tested for several pharmacological reactions after single oral exposures to triadimefon (purity, 92.6%) at a dose of 0, 0.3, 1.0 or 3.0 mg/kg bw.

Mice were examined for effects on hexobarbital anaesthesia, central coordination capability, analgesia, convulsion, anti-convulsion, traction capability, catalepsy, locomotor inhibition and spontaneous motility, and rats were tested for catalepsy, lingomandibular reflex and neuromuscular transmission. The only finding was a slight increase in spontaneous motility in mice of all dosed groups, which was not related to dose (Polacek, 1983b).

Although there is evidence from studies of acute toxicity and from longer-term studies specifically designed to identify such end-points that triadimefon has effects on the central nervous system, the majority of studies with repeated doses did not report such effects. Because of the age of the studies and the lack of information about monitoring of the animals, it is not clear whether such signs may have been present but were not noted or reported.

(b) Metabolites of triadimenol and triadimefon in rats

Owing to the close structural relationship between triadimenol and triadimefon, KWG 1342, KWG 1640 and KWG 1323 were identified as metabolites of triadimenol as well as of triadimefon in rats. To a very minor extent, free triazole is mentioned as a minor metabolite of triadimenol in rats. There was no such mention for triadimefon. The toxicity of triazole is discussed in section 7 (metabolites in plants).

In studies of acute oral toxicity, the LD_{50} for hydroxytriadimenol (KWG 1342) was >1000 mg/kg bw in fed female rats and >5000 mg/kg bw in fasted male rats (Heimann, 1985b), while the LD_{50} for carboxytriadimenol (KWG 1640) was >1000 mg/kg bw in male and female fasted rats (Heimann, 1985c) and hydroxytriadimefon (KWG 1323) had an LD_{50} of >5000 mg/kg bw in male and female fasted rats (Heimann, 1985a).

6. Observations in humans

Persons working with pure triadimefon showed no effects attributable to possible exposure (Kehrig & Steffens, 2003).

7. Metabolites of triazole fungicides in plants

In this section, the toxicity of the plant metabolites triazole, triazolyl alanine and triazole acetic acid is evaluated. Triazole was also identified in rats as a minor metabolite of triadimenol. Triazolyl alanine and triazole acetic acid are also produced from propiconazole by plants, but not by mammals (see propiconazole, p •• this volume).

A complete degradation of the chemical structure of triadimenol and triadimefon and formation of 4-chlorophenol and 1,2,4-triazole can occur in the soil. If 1,2,4-triazole is then taken up by the plant, it is conjugated by an enzymatic reaction with serine to form triazole alanine, which can be further transformed into triazole hydroxyl propanoic acid and triazole acetic acid. This chain of reactions can also occur in the soil.

7.1 Triazole

(a) Biochemical aspects

(i) Absorption, distribution, excretion and metabolism

Groups of two male and two female Sprague-Dawley rats were given ^{14}C-labelled 1,2,4-triazole as a single dose at 0.08, 9.8 or 173 mg/animal by gavage. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

Irrespective of the dose, about 90% of the administered dose was excreted in the urine and 10% in the faeces. Up to 98% of the dose was excreted in the first 48 h and at the final termination after 7 days the limits of quantification (0.002, 0.2 and 4 ppm, respectively, for the three doses, in increasing order) in tissues were exceeded only in fat (4.46 ppm in one female), testes (4.79 mg/kg in one male) and erythrocytes (8.57 ppm in one male) (Lai & Simoneaux, 1986c).

Groups of five male Sprague-Dawley rats were given ^{14}C-labelled 1,2,4-triazole as single doses at 0.1 and 100 mg/kg bw administered intravenously, 1 mg/kg bw administered orally, or 1 mg/kg bw administered intraduodenally. This is a pre-GLP study and no statement of QA was provided.

After oral administration, absorption of the substance was nearly 100%. After intravenous dosing, 50%, 1.5% and 0.3% was recovered in the body after 8 h, 3 days and 6 days, respectively. After oral or intravenous administration, only 0.1% of the administered dose was found in the exhaled air. Irrespective of the route of administration, 92–94% of the dose was excreted in the urine and 3–5% in the faeces. Studies with bile-duct fistulated rats showed 12% excretion in the bile, suggesting that the substance is reabsorbed. On day 6 after dosing, tissue concentrations were all near the limit of detection (Weber et al., 1978).

Ten male Sprague-Dawley rats were given ^{14}C-labelled 1,2,4-triazole as a single oral dose at 10 mg/kg bw per day and urine was analysed for metabolites. This is a pre-GLP study and no statement of QA was provided.

Of the excreted radiolabel, 90% was unchanged ^{14}C-labelled 1,2,4-triazole; other metabolites were not identified (Ecker, 1980).

Table 21. Acute toxicity of triazole

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	LC_{50} (mg/l of air)	Reference
Rat	Crl:CD	Male	Oral	92.8	500–5000	—	Procopio & Hamilton (1981)
Rat	Wistar	Males and females	Oral	NR	1649	—	Thyssen & Kimmerle (1976a)
Rat	Wistar	Male	Dermal	NR	4200	—	Thyssen & Kimmerle (1976a)
Rat	Wistar	Female	Dermal	NR	3192	—	Thyssen & Kimmerle (1976a)
Rat[a]	Wistar	Male	Inhalation	NR	—	NA	Thyssen & Kimmerle (1976a)
Mouse[a]	NMRI	Male	Inhalation	NR	—	NA	Thyssen & Kimmerle (1976a)
Rabbit	NZW	Male	Dermal	92.8	200–2000	—	Procopio & Hamilton (1981)

NA, not applicable; NR, not reported; NZW, New Zealand White
[a]No effects observed; mice were exposed for 6 h and rats for 4 h; concentrations of triazole in air not given

(b) Toxicological studies

(i) Acute toxicity

The acute toxicity of triazole is summarized in Table 21.

(ii) Dermal and ocular irritation

In a study in New Zealand White rabbits, the triazole proved to be slightly irritating to the skin and the eyes (Procopio & Hamilton, 1981). In another study in New Zealand White rabbits, no effect on skin but strong irritation of the eyes was reported (Thyssen & Kimmerle, 1976a). Additionally, no effect on skin of humans was observed in this study.

(iii) Dermal sensitization

Triazole was not sensitizing to the skin of guinea-pigs in the Magnusson-Kligman maximization test. This study complied with the OECD requirements for GLP. Ten animals of each sex were dosed intradermally with 0.1 ml of a 10% formulation of triazole and then 1 week later topically with a 75% formulation of triazole. The challenge with a 75% formulation was performed 2 weeks after the dermal application (Frosch, 1998).

(iv) Short-term studies of toxicity

Rats

Groups of 15 male and 15 female Wistar rats were fed diets containing triazole (purity, 99.6%) at a concentration of 0, 100, 500 and 2500 ppm for 3 months. The average daily intakes of triazole were 0, 7.8, 37.9 and 212 mg/kg bw per day for males and 0, 10.2, 54.2 and 267 mg/kg bw per day for females. This is a pre-GLP study and no statement of QA was provided.

At 2500 ppm, body-weight gain was reduced in both sexes as was (temporarily) the food intake. In males at the highest dose, increased accumulation of fat in the liver and significantly lower haemoglobin concentration, erythrocyte volume fraction, mean corpuscular volume and mean corpuscular haemoglobin were observed.

The NOAEL was 500 ppm, equal to 37.9 mg/kg bw per day, on the basis of effects on body < weight, liver and blood at 2500 ppm (Bomhard et al., 1979).

Table 22. Studies of genotoxicity with triazole

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation[a]	*S. typhimurium* TA1535, TA1537, TA100, TA98	10–5000 µg/plate ±S9, in DMSO	99.7	Negative	Poth (1989)
Reverse mutation[b]	*S. typhimurium* TA1535, TA1537, TA100, TA98	100–7500 µg/plate ±S9, in DMSO	92.8	Negative	Melly & Lohse (1982)

DMSO, dimethylsulfoxide
[a] Cytotoxicity was observed at ≥1000 µg/plate
[b] Cytotoxicity was observed at ≥2000 µg/plate

(v) Genotoxicity

The results of studies of genotoxicity with triazole are summarized in Table 22.

(vi) Reproductive toxicity: developmental toxicity

Rats

In a comparative study of teratology in vitro, rat embryos were exposed to the antifungals flusilazole and fluconazole and to 1,2,4-triazole. This study did not comply with GLP. Unlike flusilazole and fluconazole, 1,2,4-triazole did not induce malformations in the branchial apparatus (Menegola et al., 2001).

In a comparative study of reproductive toxicity, Wistar rats were exposed to a set of substances with known effects on reproduction and development. 1,2,4-Triazole served as one of the negative controls in this study. This study did not comply with GLP requirements. As was expected, 1,2,4-triazole showed no effects on either reproduction or on development (Wickramaratne, 1987).

Groups of 25 mated female Wistar (Bor:WISW) rats were given 1,2,4-triazole (purity, 94%) at a dose of 0, 100 or 200 mg/kg bw per day by gavage from day 6 to day 15 of gestation. On day 20 of gestation, the fetuses were removed by cesarean section. This study complied with OECD requirements for GLP. This study supplemented a previous study of the same design that did not show any effects on fetuses at doses of 10, 30 and 100 mg/kg bw per day (Renhof, 1988b).

At the highest dose, the body-weight gain of dams was reduced. In the fetuses at ≥100 mg/kg bw per day, reduced body weights and higher incidences of undescended testicles (controls, 0.8%; 100 mg/kg bw, 4.9%; and 200 mg/kg bw, 4.3%) were reported, while at 200 mg/kg bw per day, increased implantation losses, reduced viability of the fetuses and increased malformations of the hind legs (2.9% versus 0% in the two other groups) and cleft palates (2.9% versus 0% in the two other groups) were observed. In historical controls of this strain, only two out of 13 892 fetuses (0.01%) had cleft palates and 75 (0.54%) had limb malformations (limb and type of malformation not further specified).

A NOAEL could not be identified in this study. Therefore, the NOAEL for developmental effects can be considered to be 30 mg/kg bw per day, from the first study (Renhof, 1988a).

In vitro

In a study on the kinetics of aromatase enzymes in granulosa cells in vitro, substituted triazole derivatives were found to be potent inhibitors of aromatase. 1,2,4-Triazole was found to give essentially negative results in this assay for inhibitors (Wickings et al., 1987).

7.2 Triazolyl alanine

The toxicity of triazolyl alanine was evaluated by the Meeting in 1989 (Annex 1, reference 58).

(a) Biochemical aspects

(i) Absorption, distribution, excretion and metabolism

In a balance study, groups of four male and four female Tif:RAIf rats were given ^{14}C-labelled triazolyl alanine (purity, >99%) at a dose of 0.5 or 50 mg/kg bw by gavage. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

At both doses, 95–105% of the administered dose was excreted within 24 h. Seven days after administration, 2–12% of the administered dose was excreted in the faeces, 88–108% in the urine and <1% in the exhaled air. At the lower dose, no radiolabel was detectable in tissues after 168 h, while at the higher dose radiolabel was detected at a concentration of <0.02 ppm. Electrophoretic characterization revealed that 86% of the administered dose was excreted unchanged (Hamboeck, 1983a).

In another study of balance and metabolism, groups of two male and two female Sprague-Dawley rats were given ^{14}C-labelled triazolyl alanine (purity, >99%) at a dose of 0.56, 54.4 or 994 mg/kg bw by gavage. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

At all doses, the renal excretion was 83%, while at increasing doses 16.1%, 6.2% and 7.7%, respectively, of the radiolabel was excreted in the faeces. Within the first 48 h, 97.4%, 87.3% and 88.2%; respectively, of the radiolabel was excreted at increasing doses. At final termination, with a few exceptions the tissue residue levels were below the limit of quantification at all doses.

In thin-layer chromatography performed on samples of urine, only two radioactive zones were identified. On comigration analyses it was estimated that 82–93% of the administered dose was excreted as unchanged triazolyl alanine and 13–30% as *N*-acetyltriazolyl alanine (Lai & Simoneaux, 1986b; Lai & Simoneaux, 1986e).

The findings of the above study (Lai & Simoneaux, 1986b; Lai & Simoneaux, 1986e) were generally confirmed in a similar study using nuclear magnetic resonance (NMR) and mass spectrometry (MS) for analyses. Additionally, both triazolyl alanine and *N*-acetyltriazolyl alanine were found at a level of approximately 1% in the faeces of rats (Hamboeck, 1983b).

Table 23. Acute toxicity of triazolyl alanine

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Purity (%)	References
Mouse	NMRI	Males and females	Oral, fasted	>5000	"Pure"	Mihail (1986)
Rat	Wistar	Males and females	Oral, fasted and fed	>5000	"Pure"	Mihail (1986)
Rat	Not reported	Males and females	Oral	>2000	Not reported	Henderson & Parkinson (1981)
Rat	Wistar	Males and females	Intraperitoneal	>5000	"Pure"	Mihail (1986)

(b) Toxicological studies

(i) Acute toxicity

The acute toxicity of triazolyl alanine is summarized in Table 23.

(ii) Short-term studies of toxicity

Rats

Groups of 10 male Wistar rats were given drinking-water containing triazolyl alanine (purity, approximately 100%) at a concentration of 0, 3000 or 10 000 mg/l for 2 weeks. The average daily intakes of triazolyl alanine were 0, 448 and 1490 mg/kg bw per day. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

There were no treatment-related findings related to appearance, behaviour, body-weight gain or gross examination of organs.

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

Groups of 20 male and 20 female Wistar rats were given triazolyl alanine (purity, described as "pure") at a dose of 0, 25, 100 and 400 mg/kg bw per day by gavage for 4 weeks followed by a 4-week recovery period. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

There were no treatment-related findings after haematological, clinical-chemical, gravimetric, macroscopic and histopathological examinations.

The NOAEL was 400 mg/kg bw per day, the highest dose tested (Mihail & Vogel, 1983).

Groups of 20 male and 20 female Wistar (Bor:WISW) rats were fed diets containing triazolyl alanine (purity, 97.5%) at a concentration of 0, 1250, 5000 or 20 000 ppm for 3 months. The average daily intakes of triazolyl alanine were 0, 90, 370 and 1510 mg/kg bw per day for males, and 0, 100, 400 and 1680 mg/kg bw per day for females. This study did not comply with GLP requirements, but was supervised by the internal QA unit.

At the highest dose, the body-weight gain in males was slightly reduced relative to that in controls. There were no other treatment-related findings.

The NOAEL was 5000 ppm, equal to 370 mg/kg bw per day, on the basis of impairment of body-weight gain in males at 20 000 ppm (Maruhn & Bomhard, 1984).

Dogs

Groups of four male and four female beagle dogs were fed diets containing triazolyl alanine (purity, 97.5%) at a concentration of 0, 3200, 8000 or 20 000 ppm for 13 weeks. The average daily intakes of triazolyl alanine were 0, 119, 291 and 690 mg/kg bw per day (calculated from the daily intakes per animal of 0, 1185, 2914 and 6900 mg and a default body weight of 10 kg). This study did not comply with GLP requirements, but was supervised by the internal QA unit.

At the highest dose, the body-weight gain and food consumption of females was reduced. There were no other treatment-related findings.

The NOAEL was 3200 ppm, equivalent to 139 mg/kg bw per day, on the basis of impairment of body-weight gain at 8000 ppm (Keutz & Groening, 1984).

(iii) Genotoxicity

The results of studies of genotoxicity with triazolyl alanine are summarized in Table 24.

Table 24. Studies of genotoxicity with triazolyl alanine

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation[a]	*S. typhimurium* TA1535, TA1537, TA100, TA98, TA102	20–5000 µg/plate ±S9, in DMSO	97.4	Negative	Deparade (1986)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98	20–12 500 µg/plate ±S9, in DMSO	NR	Negative	Herbold (1983b)
Reverse mutation	*E. coli* WP2 *uvra* and *S. typhimurium* TA1535, TA1537, TA100, TA98	312.5–5000 µg/plate ±S9, in DMSO	>96	Negative	Hertner (1993)
CHO/HGPRT mutation assay	Chinese hamster ovary cells	500–10 000 µg/ml ±S9	97.4	Negative	Dollenmeier (1986)
DNA damage	*E. coli* polA1+ and polA1−	62.5–1000 µg/plate ±S9, in DMSO	NR	Negative	Herbold (1983a)
Rec assay	*Bacillus subtilis* H17 (rec^+) and M45 (rec^-)	20–1000 µg/disk ±S9	>96	Negative	Watanabe (1993)
Unscheduled DNA synthesis	Primary rat hepatocytes from a male rat	0.25–10 000 µg/ml	97.5	Negative	Puri (1986)
Transformation	BALB/3T3	62.5–1000 µg/ml ±S9	97.4	Negative	Beilstein (1984)
Transformation	BHK 21C13	500–8000 µg/ml −S9 1000–16 000 µg/ml +S9	"No impurities identified"	Positive Positive	Richold et al. (1981)
In vivo					
Micronucleus formation	Bone marrow erythroblasts of male and female Chinese hamsters	5000 mg/kg bw, orally	97.4	Negative	Strasser (1986)
Micronucleus formation	Bone marrow erythroblasts of male CBC F1 mice	2500 and 5000 mg/kg bw, intraperitoneally	NR	Negative	Watkins (1982)
Micronucleus formation	Bone-marrow erythroblasts of male and female Bor:NMRI mice	8000 mg/kg bw, orally	NR	Negative	Herbold (1982)

DMSO, dimethyl sulfoxide; NR, not reported; S9, 9000 × *g* supernatant of rodent liver

[a] Precipitations at concentrations >78 µg/plate

(iv) Reproductive toxicity

Multigeneration studies

In a two-generation study in AP rats, groups of 6 males and 12 females were fed diets containing triazolyl alanine (purity, 48%; at the beginning of the study a purity of >90% was assumed) at a concentration of 0, 150, 625, 2500 or 10 000 ppm. There was no report on compliance of this study with any GLP standards and no statement of QA was provided. The pretreatment period before the first mating was 42 days.

In the group receiving the highest dose, the mean litter body weight on postnatal day 1 was slightly reduced, but returned to normal on postnatal day 5. In the parent females in the group receiving the highest dose, a tendency to prolonged intervals in the estrus cycle was observed.

The NOAEL was 2500 ppm on the basis of pup birth weight effects and possible effects on the estrus cycle at 10 000 ppm (Birtley, 1983).

In a two-generation study in Alpk:AP rats, groups of 15 males and 30 females were fed diets containing triazolyl alanine (purity, 97.8%) at a concentration of 0, 500, 2000 or 10 000 ppm. The pretreatment period before the first mating was 84 days. This study complied with the FIFRA requirements for GLP.

There was a slight reduction in birth weights of pups in the F_{1b} and F_{2a} generations at 10 000 ppm.

The NOAEL was 2000 ppm (Milburn et al., 1986).

Developmental toxicity

Groups of 24 mated female Alpk:AP rats were given triazolyl alanine (purity, 94.8%) at a dose of 0, 100, 300 or 1000 mg/kg bw per day by gavage from day 7 to day 16 of gestation. On day 22 of gestation, the fetuses were removed by cesarean section. This study did not comply with GLP requirements but was supervised by the internal QA unit.

A slight increase in non-ossification of odontoid processes was observed at 300 mg/kg bw per day, while an increase in retarded ossification of different bones was observed at 1000 mg/kg bw per day.

The NOAEL was 100 mg/kg bw per day on the basis of slight effects on skeletal development at 300 mg/kg bw per day (Clapp et al., 1983).

7.3 Triazole acetic acid

(a) Biochemical aspects

(i) Absorption, distribution, excretion and metabolism

In a balance study, groups of two male and two female Sprague-Dawley rats were given ^{14}C-labelled triazole acetic acid (purity, >99%) at a dose of 0.58, 58.6 or 1030 mg/kg bw by gavage. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

Irrespective of sex and dose, 90.6–102.5% of the administered substance was excreted in the urine and 3.1–4.3% in the faeces. Within the first 48 h, excretion was nearly complete and at 7 days after dosing, residues in tissues (plasma and testes) were found to be only incidental above the level of quantification (Lai & Simoneaux, 1986a).

In a study of metabolism, groups of two male and two female Sprague-Dawley rats were given ^{14}C-labelled triazole acetic acid (purity, >99%) at a dose of 0.58, 58.6 or 1030 mg/kg bw by gavage. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

In the urine, unchanged triazole acetic acid was the only radioactive substance found (Lai & Simoneaux, 1986d).

(b) Toxicological studies

(i) Acute toxicity

The acute toxicity of triazole acetic acid is summarized in Table 25.

(ii) Short-term studies of toxicity

Rats

Groups of five male and five female RAIf rats were fed diets containing triazole acetic acid at a concentration of 0, 100, 1000 or 8000 ppm for 14 days. The average daily intakes of triazole acetic acid were 0, 11, 103 and 788 mg/kg bw per day for males and 0, 10, 97 and 704 mg/kg bw per day for females. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

There were no treatment related findings.

The NOAEL was 704 mg/kg bw per day, the highest dose tested (Thevenaz, 1986).

(iii) Genotoxicity

The results of studies of genotoxicity with triazole acetic acid are summarized in Table 26.

Table 25. Acute toxicity of triazole acetic acid

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Purity (%)	Reference
Rat	Tif: RAIf	Males and females	Oral	>5000	>99	Thevenaz (1994)

Table 26. Studies of genotoxicity with triazole acetic acid

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA100, TA98	20–5120 µg/plate ±S9	>99	Negative	Deparade (1984)

Comments

Triadimenol

In rats, radiolabelled triadimenol is rapidly absorbed from the gastrointestinal tract, with radioactivity reaching peak concentrations in most tissues between 1 h and 4 h after dosing. Up to 90% of the administered dose was excreted, with an elimination half-life for the radiolabel of between 6 h and 15 h. Excretion was essentially complete within 96 h. After 5–6 days, the amount of radioactivity in most organs was below the limits of quantification.

Renal excretion accounted for ≤21% of the orally administered dose in males and ≤48% in females. The remainder was found in the faeces. In bile-duct cannulated males 93% of the administered dose was recovered in the bile and only 6% in the urine. Thus a substantial amount of the administered dose undergoes enterohepatic recycling. Radioactivity in expired air was negligible.

Triadimenol was extensively metabolized, predominantly by oxidation of one of the *t*-butyl methyl groups to give hydroxy or carboxy derivatives. The putative intermediate triadimefon has not been isolated. Cleavage of the chloro-phenyl and the triazole group was of minor significance. In the urine and faeces most of the metabolites were not conjugated, but in bile the metabolites were found to be extensively glucuronidated.

Triadimenol has low to moderate acute toxicity. The acute oral LD_{50} both in mice and rats was in the range of 700 to 1500 mg/kg bw, with increasing toxicity for increasing isomer ratios A (1RS,2SR):B (1RS,2RS). This finding was supported by an oral LD_{50} of 579 mg/kg bw for isomer A and 5000 mg/kg bw for isomer B tested separately. In rats, the dermal LD_{50} was >5000 mg/kg bw and the LC_{50} upon inhalation was >0.954 mg/l of air (after an exposure of 4 h).

Triadimenol is not an ocular or dermal irritant in rabbits and is not a sensitizer in the maximization test in guinea-pigs.

In short-term studies in mice, rats and dogs, the main effect of triadimenol was on the liver.

In a study comparing the 80:20 and 60:40 isomer mixtures, rats were treated for 28 days by gavage. Both isomer compositions slightly increased motor activity at ≥45 mg/kg bw per day, and induced mixed function oxidase activity and reversibly increased liver weight at 100 mg/kg bw per day. In mice fed diets containing triadimenol at a concentration of 160 to 4500 ppm for 13 weeks, one out of ten males at 4500 ppm died. In both sexes at ≥1500 ppm, there were increased liver weights accompanied by increased alanine aminotransferase and AST activities. Reduced erythrocyte volume fraction and increased mean corpuscular haemoglobin concentration were observed in females at the highest dose. The NOAEL was 500 ppm, equal to 76.8 mg/kg bw per day.

In two 3-month feeding studies in rats, liver weights were increased at ≥600 ppm (<10% at 600 ppm), with cellular hypertrophy at 3000 ppm. Liver enzyme activities in serum were not increased. In one study, kidney and ovary weights were also increased at the highest dose of 2400 mg/kg. At the highest doses in both studies, there were slight changes in some haematology parameters. The lowest NOAEL after oral administration in the short-term

studies in rats was 600 ppm, equal to 39.6 mg/kg bw per day. In a 3-week study in rats treated by inhalation, no effects were observed at up to the highest dose of 2.2 mg/l of air.

In a 3-week study in rabbits, dermal application of triadimenol did not cause any dermal or systemic reactions at the highest dose tested, 250 mg/kg bw per day.

In a 3-month, a 6-month and a 2-year study, dogs were given diets containing triadimenol at concentrations of ≤2400 ppm. The only significant findings were decreased body-weight gain at 2400 ppm, liver and kidney weight increases at the highest doses and increased cytochrome P450 levels. The overall NOAEL was 600 ppm, equal to 21.1 mg/kg bw per day.

In two long-term studies, mice were given diets containing triadimenol at a concentration of ≤2000 ppm. In one study, Crl:CD-1(ICR)BR mice were kept for 80 weeks, and in the other study CF_1/WF 74 mice were kept for 2 years. At 2000 ppm, reduced body-weight gains were recorded and liver weights were increased, as were testes weights in one study. Additionally, liver enzyme activity was higher. In one study, histopathological examination of the liver showed more basophilic foci at ≥80 ppm, predominantly in males, but there was a poor dose–response relationship and similar values have been reported in control groups in other studies. Hepatocellular hypertrophy and single cell necrosis were found at ≥400 ppm. At 2000 ppm, additional histopathological changes to the liver were reported. At the intermediate dose, 400 ppm, but not at the highest dose, males had slightly more liver adenomas and carcinomas. There was no clear dose–response relationship, and values were within the historical control range of 6–17%. In females at the highest dose, two out of 50 animals had luteomas; this was within the range for historical controls of 0.9–10%. In the other study, females at the intermediate and highest dose had more liver adenomas and in both sexes at the highest dose, the incidences of liver hyperplastic nodules and thyroid cystic alterations were increased. The increase in liver adenomas is a common finding in mice, which is considered to be of questionable relevance for humans. The overall NOAEL was 500 ppm, equal to 140 mg/kg bw per day.

In a long-term feeding study in rats, reduced body-weight gain was found in both sexes at the highest concentration of 2000 mg/kg, as were changes in the weights of a number of organs, including spleen, lung and testes. However, there was a poor relationship with dose. In females, kidney, liver and ovarian weights were higher at the highest dose. In both sexes at 2000 ppm, the activities of liver enzymes (ALT and AST in both sexes and glutamate dehydrogenase in males) were slightly increased. At the highest dose, minor changes in haematology parameters were at the borderline of the physiological range at some time-points. There was no histopathological evidence for any non-neoplastic or neoplastic changes. The NOAEL was 500 ppm, equal to 25 mg/kg bw per day.

In a series of studies of genotoxicity in vitro and in vivo, triadimenol consistently gave negative results. The Meeting concluded that triadimenol is unlikely to be genotoxic.

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

To study reproductive performance during exposure to triadimenol, two- and three-generation feeding studies were performed in rats given diets containing triadimenol at

concentrations of ≤500 ppm and ≤2000 ppm, respectively. In the study in which the higher doses were administered, matings in all three generations consistently showed reduced fertility at ≥500 ppm; in F_0 matings, this finding was observed at 125 ppm. Reduced viability was observed in F_1 pups of both matings at 2000 ppm, F_2 pups from the first mating at ≥500 ppm, and F_2 pups of the second mating at 2000 ppm. All F_3 pups from the first mating died at ≥500 ppm, but not those from the second mating. At 500 ppm, increased testicular and ovarian weights were observed in F_{1b} parents in the study in which lower doses were administered, and increased testicular weights in the F_{2b} parents at 2000 ppm. The lowest NOAEL in these studies was 100 ppm, equal to 8.6 mg/kg bw per day.

Several studies of developmental toxicity were performed in rats, over a dose range of 5 to 120 mg/kg bw per day. In one study, an increase in supernumerary lumbar ribs was found at ≥25 mg/kg bw per day, and in another study there was an increase in postimplantation losses at 120 mg/kg bw per day. In three out of the four studies, increased placental weights were noted at doses of 30 to 100 mg/kg bw per day. Such effects have been reported with other azoles. Triadimenol did not induce malformations in studies of developmental toxicity and clear NOAELs for developmental toxicity could be established; the lowest NOAEL was 15 mg/kg bw per day.

The NOAEL for offspring toxicity in rabbits was 4 mg/kg bw per day on the basis of slightly increased postimplantation losses at the maternally toxic dose of 200 mg/kg bw per day.

Clinical signs (general restlessness, alternating phases of increased and reduced motility, aggressivity) observed during tests for acute toxicity suggested possible effects on the central nervous system.

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

A medical survey of personnel working in the production of triadimenol gave no indication of any substance-related effects.

Toxicological evaluation

Although a series of tests for acute neurotoxicity in mice were available, a NOAEL for triadimenol for neurotoxicity could not be identified because of technical shortcomings in these studies. As triadimenol is closely related to triadimefon in terms of chemical structure and toxicological effects, and in the view of the lack of sound studies of neurotoxicity with triadimenol, the Meeting concluded that studies of neurotoxicity performed with triadimefon could serve as a basis for derivating an ADI and an ARfD for triadimenol. This was supported by evidence for similar neurotoxic potential in a published study of acute toxicity with triadimenol and triadimefon.

The Meeting established an ADI of 0–0.03 ppm based on the NOAEL of 3.4 mg/kg bw per day for hyperactivity in a study of neurotoxicity with triadimefon in a 13-week feeding study in rats, and with a safety factor of 100.

The Meeting established an ARfD of 0.08 mg/kg bw on the basis of the NOAEL of 2 mg/kg bw for hyperactivity in a study of acute neurotoxicity in rats treated with triadimefon by gavage. A safety factor of 25 was applied because the effects were C_{max}-dependent and reversible (see comments on triadimefon).

Triadimefon

In a study on the absorption, distribution, metabolism and excretion of triadimefon in rats, the dose given and pretreatment with non-labelled triadimefon did not significantly affect excretion and metabolism patterns. In males about one third and in females about two thirds of the administered dose was excreted in the urine, and vice versa in the faeces. After 96 h, 2% of the radioactivity remained in females and 9% in males, with the highest residue concentrations found in liver and kidneys.

The metabolism of triadimefon starts either by direct oxidation of a *t*-butyl methyl group to the hydroxy or the carboxy compound with subsequent glucuronidation, or these steps are preceded by reduction of the keto group of triadimefon to the putative intermediate, triadimenol. Therefore, many of the metabolites found in triadimenol metabolism studies are also found with triadimefon. Nevertheless, the metabolism of triadimefon in rats provides a pathway for demethylation of the *t*-butyl group, which is not seen with triadimenol. This might be owing to very low biotransformation of triadimenol via triadimefon as intermediate.

The acute oral LD_{50} in mice and rats was in the range of 363 to 1855 mg/kg bw. The dermal LD_{50} was >5000 mg/kg bw and the LC_{50} on inhalation was >3.27 mg/l of air.

In rabbits, a few treatment-related effects including skin and eye irritation were recorded, but the irritation potential of triadimefon was very low. In guinea-pigs, technical-grade triadimefon of low purity was a sensitizer in the Büehler test for skin sensitization. However, purified triadimefon did not have any sensitizing potential in guinea-pigs in the Magnusson & Kligman maximization test, even after induction with technical-grade triadimefon of low purity.

In short-term studies in rats and dogs, the main effects of triadimefon were on the liver.

In three short-term studies in rats (treated by gavage at doses of ≤30 mg/kg bw per day for 30 days, by gavage at doses of ≤25 mg/kg bw per day for 4 weeks, and given diets containing triadimefon at concentrations of ≤2000 ppm for 12 weeks) the overall NOAEL was 150 mg/kg bw per day, the highest dose tested.

In two studies in dogs fed with diets containing triadimefon for 13 weeks and 2 years, the highest concentrations administered were 2400 ppm and 2000 ppm, respectively. Bodyweight decreases, relative liver weight increases and liver enzyme induction were observed predominantly in the group receiving the highest dose, and, in the short-term study only, there were also effects on haematology parameters. The overall NOAEL in these studies was 600 ppm, equal to 17.3 mg/kg bw per day, in the 2-year study.

The dermal application of triadimefon at 1000 mg/kg bw per day to rats for 3 weeks (6 h per day for 5 days per week) caused diffuse acanthosis at the application site and

increased activity and reactivity. The NOAEL was 300 mg/kg bw per day. The dermal application of triadimefon at 50 and 250 mg/kg bw per day to rabbits for 4 weeks (5 days per week) caused mild erythema at the application sites. Rats exposed by inhalation to triadimefon at 0.3 mg/l of air had reduced body-weight gain and increased liver weights.

In two 2-year feeding studies in mice, severely decreased body-weight gains, changes in several haematology parameters and increased liver weights and increased enzyme activity were observed at the highest dietary concentration of 1800 ppm. Starting at 300 ppm, histopathological changes, including nodular changes, hypertrophy and single cell necrosis, were found in the liver. These effects were more pronounced at the highest dose, and in one study an increase in hepatocellular adenomas was also reported. In the other study, a re-examination of histopathology slides led to re-classification of findings for adenomas and carcinomas. Owing to incomplete re-examination, a final conclusion on whether the incidences were increased or not was not possible. However, liver adenomas in the presence of liver toxicity in mice are generally not believed to be of toxicological concern for humans.

The lowest NOAEL was 50 ppm, equal to 13.5 mg/kg bw per day, on the basis of nodular changes and single cell necrosis in the liver at 300 ppm.

With the exception of behavioural changes and severe histopathological lesions in several organs observed in one study at the highest dose of 5000 ppm, the toxicological profile in two 2-year feeding studies in rats was very similar to that of the studies in mice. After 23 weeks of exposure to the highest dose at 5000 ppm, animals showed violent activity and refused the feed and became moribund. The surviving animals in this group were terminated at week 39. They showed haemorrhagic lesions in the stomach mucosa, blood-filled and dilated alveolar vessels, degenerative processes in proximal kidney tubules of females, atrophied spleens with signs of decreased haematopoesis, some giant spermatids in testes, and decreased haematopoesis in the bone marrow of males. At the lower dietary concentrations of 1800 and 500 ppm, reduced body-weight gains, increased liver weights and mildly increased liver enzyme activities were recorded. In one study, ovary weights were higher and adrenal weights lower. Mild effects on haematology were found in both studies. In one study at the highest dietary concentration of 1800 ppm, a marginal increase in thyroid cystic hyperplasias and more thyroid follicular adenomas (five versus zero for both sexes taken together) were found. When compared with historical controls, this effect was not significant. The overall NOAEL was 300 ppm, equal to 16.4 mg/kg bw per day.

In a series of studies of genotoxicity in vitro and in vivo, all results were consistently negative. The Meeting concluded that triadimefon is unlikely to be genotoxic.

In view of the lack of genotoxicity and the finding only of liver adenomas in mice and equivocal changes in thyroid follicular adenomas in rats at concentrations at which organ toxicity was observed, the Meeting concluded that triadimefon is not likely to pose a carcinogenic risk to humans.

In two related multigeneration studies, rats received diets containing triadimefon at concentrations of ≤1800 ppm. Maternal and pup weight development was reduced at doses of ≥300 ppm and, in the first generation at the highest dose, the viability of the pups was reduced. At the highest dose, two matings of the F_1 animals to give F_2 generation pups resulted in one female becoming pregnant in the first mating and none in the second. In the second study, again at 1800 ppm, the fertility of the F_0 generation was not affected, but that

of the F_1 generation was, albeit not to the same extent as in the first study. Viability and pup weights were reduced. In a cross mating in which only one sex was exposed to triadimefon, only the matings with exposed males gave significantly reduced fertility, correlating with reduced insemination indices. Therefore, reduced fertility seemed to have resulted mainly from impaired mounting willingness of exposed males. In males at the highest dose, the concentration of testosterone was double that in control males, and testes weights were increased. However, no correlation between individual testosterone concentrations and spermiograms and mating willingness was observed, although reduced mating willingness did appear to correlate with reduced body weight. It appears that prenatal, but not postnatal, exposure of males affected mating willingness. The lowest NOAEL was 50 ppm, equivalent to 3.75 mg/kg bw per day, based on a LOAEL of 1800 ppm for reproductive effects.

In studies of developmental toxicity in rats treated by inhalation (one study) and by gavage (two studies), inhalation exposure at air concentrations of ≤0.114 mg/l of air on day 6 to day 15 of gestation did not result in any findings indicative of developmental toxicity. In the studies of rats treated by gavage, however, supernumerary ribs in one study at 90 mg/kg bw per day, increased placental weights at 100 mg/kg bw per day, and cleft palates at doses of ≥75 mg/kg bw per day were found. These doses also reduced the body-weight gains of dams by ≤50% over the exposure period, but not when averaged over the whole gestation period. In four studies in rabbits, body-weight loss in dams was observed at a dose of ≥30 mg/kg bw per day. Over the dose range of 60 to 120 mg/kg bw per day, increased litter losses, and caudal vertebrae malformations and cleft palates were found either in one or the other study and delayed ossification and scapula malformations were observed in both studies. Additionally, in one study, the uncommon finding of umbilical hernia was recorded in pups at 60 and 80 mg/kg bw per day. Scapula deformations were also found at 40 mg/kg bw per day, the lowest dose tested in the study. Overall, the lowest NOAEL for offspring toxicity was 20 mg/kg bw on the basis of scapula deformations at 40 mg/kg bw in rabbits.

Several studies provide evidence that triadimefon has neurotoxic potential. In a study in which single doses of triadimefon were administered by gavage and in a 13-week feeding study, several signs of hyperactivity, increased motility and stereotypic behaviour were found. The NOAEL in the former study was 2 mg/kg bw on the basis of reversible neurotoxic effects at 35 mg/kg bw. These were considered to be C_{max}-dependent effects in view of the fact that a dose of 54.6 mg/kg bw per day in the short-term feeding study caused similar effects only after several days. The NOAEL for this study was 50 ppm, equivalent to 3.4 mg/kg bw. In a comparative study of acute neurotoxicity in Long Evans rats treated by gavage with a group of 14 triazoles or structurally related compounds, hyperactivity at 100 mg/kg bw, but not at 50 mg/kg bw, was recorded for both triadimenol and triadimefon. In this study, the dose–response curves for triadimenol and triadimefon were very similar, suggesting a common mechanism of neurotoxicity.

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

A medical survey of the personnel working in the production of triadimefon gave no indication of any substance-related effects.

Toxicological evaluation

The Meeting established an ADI of 0–0.03 mg/kg bw on the basis of the NOAEL of 3.4 mg/kg bw per day for hyperactivity in a study of neurotoxicity in rats fed with triadimefon and a safety factor of 100.

The Meeting established an ARfD of 0.08 mg/kg bw based on the NOAEL of 2 mg/kg bw for hyperactivity in a study of acute neurotoxicity in rats given triadimefon by gavage. A safety factor of 25 was used since the effects were C_{max}-dependent and reversible.

Plant metabolites of triadimefon, triadimenol and other triazole fungicides

Triazole, triazolyl alanine and triazole acetic acid are plant metabolites of several triazole fungicides, including triadimenol and triadimefon.

After oral administration of triazole, triazolyl alanine and triazole acetic acid to rats, these compounds are rapidly and completely absorbed. Urinary excretion is the main excretion pathway for ≥90% of the administered dose, and only a few percent are found in the faeces. Except for triazolyl alanine, which is metabolized to a minor extent to *N*-acetyltriazolyl alanine, these compounds are virtually not metabolized and are excreted unchanged. Owing to rapid and complete excretion, there is no potential for accumulation in the body for any of these plant metabolites.

The acute oral toxicity of all three compounds is low, with LD_{50}s of >5000 mg/kg bw, except for triazole, with an LD_{50} of 1649 mg/kg bw.

Only a few tests for genotoxicity have been performed on triazole and triazole acetic acid and all gave negative results. Triazolyl alanine was more extensively tested; only one test for cell transformation in vitro gave a positive result, while the results of another similar test and all other tests were negative.

In a 3-month feeding study in rats, triazole induced fat deposition in the liver and changes in haematological parameters at the highest dose of 2500 ppm. In 3-month feeding studies in rats and, the only effect of triazolyl alanine was to reduce body-weight gain at the highest dose of 20 000 ppm. No effects were recorded in a 2-week study in rats fed with triazole acetic acid at the highest dose of 8000 ppm.

In a study of developmental toxicity with triazole in rats, at ≥100 mg/kg bw per day fetuses showed increased incidence of undescended testicles and at 200 mg/kg bw per day malformations of the hind legs were found. In studies of reproductive and developmental toxicity with triazolyl alanine in rats, only very minor effects on pups, indicative of general toxicity, such as reduced birth weights and retarded ossification processes were found at high doses. There were no studies of reproductive and developmental toxicity with triazole acetic acid.

Since triazolyl alanine and triazole acetic acid were of low systemic toxicity and developmental effects with triazole occur at doses of ≥100 mg/kg bw per day, these metabolites were judged not to pose an additional risk to humans.

Triadimenol

Levels relevant to risk assessment of triadimenol#

Species	Study	Effect	NOAEL	LOAEL
Mouse	80-week study of toxicity and carcinogenicitya	Toxicity	500 ppm, equal to 140 mg/kg bw per day	2000 ppm, equal to 620 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 140 mg/kg bw per day	2000 ppm, equal to 620 mg/kg bw per day
Rat	2-year study of toxicity and carcinogenicity[a]	Toxicity	500 ppm, equal to 25 mg/kg bw per day	2000 ppm, equal to 105 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 105 mg/kg bw per dayc	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	100 ppm, equal to 8.6 mg/kg bw per day	500 ppm, equal to 43.0 mg/kg bw per day
		Pup toxicity	100 ppm, equal to 8.6 mg/kg bw per day	500 ppm, equal to 43.0 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	25 mg/kg bw per day	60 mg/kg bw per day
		Embryo- and fetotoxicity	15 mg/kg bw per day	25 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	40 mg/kg bw per day	200 mg/kg bw per day
		Embryo- and fetotoxicity	40 mg/kg bw per day	200 mg/kg bw per day
Dog	13-week study of toxicity[a]	Toxicity	600 ppm equal to 21.1 mg/kg bw per day	2400 ppm equal to 85.9 mg/kg bw per day

See comments on triadimefon
[a] Diet
[b] Gavage
[c] Highest dose tested

Estimate of acceptable daily intake for humans

0–0.03 mg/kg bw

Estimate of acute reference dose

0.08 mg/kg bw

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

Further observations in humans

Summary of critical end-points for triadimenol

Absorption, distribution, metabolism and excretion in animals	
Rate and extent of oral absorption	Rapid (peak within 1.5 h); >90%
Distribution	Widely distributed
Potential for accumulation	Low, half-lives of 6–15 h
Rate and extent of excretion	79–90% within 2 h
Metabolism	Very extensive; predominantly oxidation of *t*-butyl methyl group
Toxicologically significant compounds (animals, plants and the environment)	Triadimenol, triadimefon, triazole
Acute toxicity	
Rat, LD_{50}, oral	579–5000 mg/kg bw (varies with isomer composition)
Rat, LD_{50}, dermal	>5000 mg/kg bw
Rat, LC_{50}, inhalation	>0.95 mg/l
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Skin sensitization	Not sensitizing (Magnusson & Kligman maximization test)
Short-term studies of toxicity	
Critical effects	Liver toxicity (2-year study in dogs)
Lowest NOAEL	21.1 mg/kg bw
Genotoxicity	Negative results in vitro and in vivo
Long-term studies of toxicity and carcinogenicity	
Critical effects	Body and organ weight changes (2-year study in rats)
Lowest NOAEL	25 mg/kg bw
Carcinogenicity	Liver adenomas in female mice; unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Critical effects	Increased ovary and testes weights (rat)
Lowest reproductive NOAEL	8.6 mg/kg bw
Critical effects	Increased supernumerary lumbar ribs; not teratogenic (rat)
Lowest developmental NOAEL	15 mg/kg bw
Neurotoxicity/delayed neurotoxicity	
Critical effects at LOAEL	See triadimefon
Lowest NOAEL	See triadimefon
Other toxicological studies	Metabolites are of no greater toxicological concern than the parent
Medical data	No effects on health in manufacturing personnel

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.03 mg/kg bw	Rat, short-term study of neurotoxicity with triadimefon (see triadimefon)	100
ARfD	0.08 mg/kg bw	Rat, study of acute neurotoxicity with triadimefon (see triadimefon)	25

Triadimefon

Levels relevant to risk assessment of triadimefon

Species	Study	Effect	NOAEL	LOAEL
Mouse	21-month study of toxicity and carcinogenicity[a]	Toxicity	50 ppm, equal to 13.5 mg/kg bw per day	300 ppm, equal to 76 mg/kg bw per day
		Carcinogenicity	300 ppm, equal to 76 mg/kg bw per day	1800 ppm, equal to 550 mg/kg bw per day
Rat	105-week study of toxicity and carcinogenicity[a]	Toxicity	300 ppm, equal to 16.4 mg/kg bw per day	1800 ppm, equal to 114 mg/kg bw per day
		Carcinogenicity	1800 ppm, equal to 114 mg/kg bw per day[c]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	300 ppm, equal to 22.8 mg/kg bw per day	1800 ppm, equal to 136.8 mg/kg bw per day
		Pup toxicity	300 ppm, equal to 22.8 mg/kg bw per day	1800 ppm, equal to 136.8 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	30 mg/kg bw per day
		Embryo- and fetotoxicity	30 mg/kg bw per day	90 mg/kg bw per day
	Acute neurotoxicity[b]	Neurotoxicity	2 mg/kg bw	35 mg/kg bw
	13-week study of neurotoxicity[a]	Neurotoxicity	50 ppm, equivalent to 3.4 mg/kg bw per day	800 ppm, equivalent to 54.6 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	30 mg/kg bw per day
		Embryo- and fetotoxicity	20 mg/kg bw per day	50 mg/kg bw per day
Dog	2-year study of toxicity[a]	Toxicity	300 ppm equal to 11.7 mg/kg bw per day	200 ppm equal to 48.8 mg/kg bw per day

[a] Diet
[b] Gavage
[c] Highest dose tested

Estimate of acceptable daily intake for humans

0–0.03 mg/kg bw

Estimate of acute reference dose

0.08 mg/kg bw

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

Further observations in humans

Summary of critical end-points for triadimefon

Absorption, distribution, metabolism and excretion in animals	
Rate and extent of oral absorption	≥28% in females, ≥67% in males as urinary excretion
Distribution	Widely distributed in kidneys and liver
Potential for accumulation	Low
Rate and extent of excretion	90–98% excretion within 96 h
Metabolism	Very extensive; predominantly oxidation of t-butyl methyl group
Toxicologically significant compounds (plants, animals and the environment)	Triadimenol, triadimefon, triazole
Acute toxicity	
Rat, LD_{50}, oral	363–1855 mg/kg bw
Rat, LD_{50}, dermal	>5000 mg/kg bw
Rat, LC_{50}, inhalation	>3.27 mg/l
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Skin sensitization	Technical-grade triadimefon is sensitizing, purified triadimefon is not sensitizing (Büehler, and Magnusson & Kligman maximization tests)
Short-term studies of toxicity	
Critical effects	Liver effects (dog)
Lowest NOAEL	17.3 mg/kg bw
Genotoxicity	Negative in vitro and in vivo
Long-term studies of toxicity and carcinogenicity	
Critical effects	Liver nodular changes, hypertrophy and single cell necrosis
Lowest NOAEL	13.5 mg/kg bw per day
Carcinogenicity	Liver adenomas in mice; unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Critical effects	Impaired reproductive performance (rat)
Lowest reproductive NOAEL	22.8 mg/kg bw per day
Critical effects	Scapula malformations at maternal toxic doses (rabbit)
Lowest developmental NOAEL	20 mg/kg bw per day
Neurotoxicity/delayed neurotoxicity	
Critical effects	Increased activity in study of acute neurotoxicity after gavage administration (rat)
Lowest NOAEL	2 mg/kg bw
Critical effects	Increased activity in short-term feeding study (rat)
Lowest NOAEL	3.4 mg/kg bw
Other toxicological studies	Metabolites are of no greater toxicological concern than the parent
Medical data	No effects on health in manufacturing personnel

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.03 mg/kg	Rat, short-term study of neurotoxicity	100
ARfD	0.08 mg/kg	Rat, study of acute neurotoxicity	25

References

Bayer CropScience AG (2003) Triadimenol dossier according to directive 91/414/EEC. Annex II, 5. Toxicology and Metabolism. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Becker, H., Frei, D., Vogel, W. & Terrier, C. (1987a) Embryotoxicity (including teratogenicity) study with KWG 0519 in the rat. Unpublished report No. R3988 from RCC, Research and Consulting Company AG, Itingen, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Becker, H., Mueller, E., Vogel, W. & Terrier, C. (1987b) Embryotoxicity (including teratogenicity) study with KWG 0519 in the rabbit. Unpublished report No. R4145 from RCC, Research and Consulting Company AG, Itingen, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Beilstein, P. (1984) CGA 131013 tech.—Transformation/liver-microsome test. Unpublished report No. 840324 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Birtley, R.D.N. (1983) Triazole alanine—preliminary reproduction study in the rat. Unpublished report No. CTL/L/470 from Imperial Chemical Industries PLC, Cheshire, UK. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E. (1982) THS 2212—preliminary subacute toxicity study on male rats, administration in the drinking water. Unpublished report No. 11253 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E. & Hahnemann, S. (1986) MEB 6447 (common name: triadimefon, the active ingredient of Bayleton). Carcinogenicity study on NMRI mice (21-month administration in the feed). Unpublished report No. 16719 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E. & Loeser, E. (1978) MEB 6447—chronic toxicity study on rats (two-year feeding experiment). Unpublished report No. 7707 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E. & Loeser, E. (1980) MEB 6447—chronic toxicity study on mice (two-year feeding experiment). Unpublished report No. 9344 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E. & Loeser, E. (1982) KWG 0519 (triadimenol, Baytan active ingredient). Chronic toxicological study on mice (feeding experiment over two years). Unpublished report No. 10855 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E. & Schilde, B. (1991) MEB 6447—chronic toxicity and cancerogenicity studies on Wistar rats with administration in diet over a period of 105 week. Unpublished report No. 20774 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E., Loeser, E. & Schilde, B. (1979) 1,2,4-triazole—subchronic toxicological study with rats (feeding study over three months). Unpublished report No. 8667 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Brendler, S. (1991) MEB 6447—mutagenicity test on unscheduled DNA synthesis in rat liver primary cell cultures in vitro. Unpublished report No. 20027 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Chopade, H.M. (1992) Disposition and metabolism of (phenyl-UL-14C)triadimefon in rats. Unpublished report No. 102640 from Miles Inc. Agriculture Division, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Cifone, M.A. (1982) Mutagenicity evaluation of KWG 0519 in the mouse lymphoma forward mutation assay. Final report. Unpublished report No. R2204 from Litton Biocinetics, Inc., Kensington, MD, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Clapp, M.J.L., Killick, M.E., Hollis, K.J. & Godley, M.J. (1983) Triazole alanine—teratogenicity study in the rat. Unpublished report No. CTL/P/875 from Imperial Chemical Industries PLC, Cheshire, England. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Clemens, G.R. & Hartnagel, R.E. (1990) Teratology study in the rabbit with MEB 6447 (triadimefon). Unpublished report No. BC5096 (MTD0149) from Toxicology Department Miles Inc., Elkhart, IN, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Clemens, G.R., Jasty, V., Troup, C.M. & Hartnagel, Jr, R.E. (1990a) A toxicity study in the rabbit with MEB 6447 (triadimefon). Unpublished report No. BC5106 (MTD0150) from Bayer CropScience AG. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Clemens, G.R., Troup, C.M. & Hartnagel, R.E. (1990b) Developmental toxicity study in the rat with Baytan technical. Unpublished report No. BC5142 from Miles Laboratories Inc., Elkhart, IN, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Clemens, G.R., Jasty, V., Troup, C.M. & Hartnagel, R.E. (1991) Developmental toxicity study in the rabbit with MEB 6447 (triadimefon). Unpublished report No. R5318 from Miles Laboratories Inc., Elkhart, IN, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Clemens, G.R., Grosso, D.S., Anderson, E.J. & Hartnagel, R.E. (1992) A developmental toxicity study in rabbits with Baytan technical. Unpublished report No. BC6692 from Miles Laboratories Inc., Elkhart, IN, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Crofton, K.M. (1996) A structure–activity relationship for the neurotoxicity of triazole fungicides. *Toxicol. Lett.*, **84**, 155–159.

Crofton, K.M., Boncek, V.M. & Reiter, L.W. (1988) Hyperactivity induced by triadimefon, a triazole fungicide. *Fundam. Appl. Toxicol.*, **10**, 459–465.

Deparade, E. (1986) CGA 131013 tech.—Salmonella/mammalian microsome mutagenicity test. Unpublished report No. 860187 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Deparade, E. (1984) CGA 142856—Salmonella/mammalian-microsome mutagenicity test. Unpublished report No. 840864 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Diesing, L. (1991) MEB 6447 (common name: triadimefon)—studies on skin sensitizing effect in guinea pigs (maximization test according to Magnusson and Kligman). Unpublished report No. 20867 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Dollenmeier, P. (1986) CGA 131013—point mutation test with Chinese hamster cells V79 (OECD conform). Unpublished report No. 860258 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Dreist, M. & Popp, A. (1996a) MEB 6447 (common name: triadimefon)—acute oral neurotoxicity screening study in Wistar rats. Unpublished report No. 24636 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Dreist, M. & Popp, A. (1996b) MEB 6447 (common name: triadimefon)—subchronic neurotoxicity screening study in Wistar rats (thirteen-week administration in the diet with a four-week recovery period in males and a ten-week recovery period in females). Unpublished report No. 25315 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Duah, F.K. & Smasal, T.M. (1993) Metabolism of triadimefon in laying hens. Unpublished report No. 105183 from Miles Inc. Agriculture Division, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Ecker, W. (1980) Biotransformation of 1,2,4-(3(5)-14C) triazole in rats. Unpublished report No. PF1471 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Eiben, R. (1984) MEB 6447 (triadimefon)—two-generation study with rats (supplementary study). Unpublished report No. 12712 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Flucke, W. (1979a) KWG 0519 form A—determination of acute toxicity (LD50): rat p.o. Unpublished report No. MO-00-010367 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Flucke, W. (1979b) KWG 0519 form B—determination of acute toxicity (LD50): rat. p.o. Unpublished report No. MO-00-010360 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Flucke, W. (1979c) KWG 0519 mixture 60 : 40—determination of acute toxicity (LD50). Unpublished report No. MO-00-010366 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Flucke, W. (1981) KWG 0519 (triadimenol)—study of sensitization effect on guinea pigs (maximization test of Magnusson & Kligman). Unpublished report No. 9894 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Frosch, I. (1998) Evaluation of skin sensitisation by 1,2,4-triazole with the guinea pig maximisation test. Unpublished report No. ToxLabs/1998/7050SEN from ToxLabs Prueflabor GmbH, Greppin, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hall, L.R. & Hartz, A.L. (1993) The metabolism of (14C) triadimefon in the lactating goat. Unpublished report No. 105161 from Miles Inc. Agriculture Division, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hamboeck, H. (1983a) Distribution, degradation and excretion of D,L-2-amino-3-(1-H-1,2,4-triazol-1-yl)propanoic acid (D,L-triazolylalanine) in the rat. Unpublished report No. 1/83 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hamboeck, H. (1983b) The metabolism of D,L-2-amino-3-(1H-1,2,4-triazol-1-yl)-propanoic acid (D,L-triazolylalanine) in the rat. Unpublished report No. 11/83 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Harbell, J.W. (1989) Bayleton technical—CHO/HGPRT mutation assay with confirmation. Unpublished report No. BC1143 from Microbiological Associates Inc., Rockville, MD, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Heimann, K.G. (1985a) KWG 1323 N (metabolite of KWG 0519)—study for acute oral toxicity to rats. Unpublished report No. 13618 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Heimann, K.G. (1985b) KWG 1342 N (metabolite of KWG 0519)—study for acute oral toxicity to rats. Unpublished report No. 13617 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Heimann, K.G. (1985c) KWG 1640 N (metabolite of KWG 0519)—study for acute oral toxicity to rats. Unpublished report No. 13616 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Heimann, K.G. & Schilde, B. (1984) KWG 0519 (suggested common name: triadimenol, the active ingredient of Baytan (TM))—subacute dermal toxicity study on rabbits. Unpublished report No. 12496 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Henderson, C. & Parkinson, G.R. (1981) R152056—acute oral toxicity to rats. Unpublished report No. CTL/P/600 from Imperial Chemical Industies Ltd, Cheshire, England. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1978a) KWG 0519—micronucleus test on mouse to evaluate KWG 0519 for potential mutagenic effects. Unpublished report No. 7588 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1978b) KWG 0519—dominant lethal study on male mouse to test for mutagenic effects (study No. 002). Unpublished report No. 7900 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1979a) KWG 0519—Salmonella/microsome test for detection of point-mutagenic effects (study No. KWG 0519/008). Unpublished report No. 8189 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1979b) KWG 0519—micronucleus test on mouse to evaluate KWG 0519 for potential mutagenic effects (study No. KWG 0519/009). Unpublished report No. 8584 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1981) KWG 0519 (triadimenol, the active ingredient of Baytan)—study of DNA damage using the *E. coli* Pol A1 test. Unpublished report No. 10265 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1982) THS 2212 triazolylanine—micronucleus test for mutagenic effect on mice. Unpublished report No. 11054 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1983a) THS 2212 triazolylalanine—Pol A1 test on *E. coli* during testing for effects harmful to DNA. Unpublished report No. 11390 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1983b) THS 2212 triazolylalanine—Salmonella/microsome test for point mutagenic effect. Unpublished report No. 11388 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1984) MEB 6447 (common name: triadimefon)—POL-test on *E. coli* to evaluate for potential DNA damage. Unpublished report No. 12780 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1986) MEB 6447 (common name: triadimefon)—cytogenetic study with human lymphocyte cultures in vitro to evaluate for harmful effect on chromosomes. Unpublished report No. 14982 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hertner, T. (1993) CGA 131013 technical—Salmonella and Escherichia/liver microsome test. Unpublished report No. 933002 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hill, D., Ikaiddi, M., Mazzio, E. & Soliman, K.F. (2000) The neurochemical basis for the behavioral effects of triadimefon. *Ann. NY Acad. Sci.*, **914**, 336–353.

Hoffmann, K. (1984) KWG 0519 (common name: triadimefon)—second chronic study of toxicity to dogs on oral administration (six-month feeding study). Unpublished report No. 12971 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hoffmann, K. & Groening, P. (1978) MEB 6447—long-term toxicity on dogs (two-year feeding study). Unpublished report No. 7882 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hoffmann, K. & Kaliner, G. (1977) KWG 0519—subchronic toxicity study on dogs (thirteen-week feeding experiment). Unpublished report No. 6633 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hoffmann, K. & Luckhaus, G. (1974) MEB 6447—subchronic toxicity study on dogs (thirteen-week feeding experiment). Unpublished report No. 5071 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hoffmann, K. & Vogel, O. (1984) KWG 0519 (common name: triadimefon)—first chronic study of toxicity to dogs on oral administration (two-year feeding study). Unpublished report No. 12970 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Ikaiddi, M.U., Akunne, H.C. & Soliman, K.F. (1997) Behavioral and neurochemical effects of acute and repeated administration of triadimefon in the male rat. *Neurotoxicology*, **18**, 771–780.

Inukai, H. & Iyatomi, A. (1977) MEB 6447—mutagenicity test on bacterial systems. Unpublished report No. 68 from Nitokuno, Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Jagannath, D.R. (1980) Mutagenicity evaluation of MEB 6447 in the reverse mutation induction assay. Unpublished report No. R1675 from Litton Bionetics Inc., Kensington, Maryland, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Justus, K. (2002a) [Phenyl-UL-14C]triadimenol: distribution of the total radioactivity in male rats determined by quantitative whole body autoradiography. Unpublished report No. MR-091/02 from Bayer AG, Bayer CropScience, Monheim, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Justus, K. (2002b) [Phenyl-UL-14C]triadimenol: investigation of the biokinetic behaviour and the metabolism (ADME) in the rat. Unpublished report No. MR-088/02 from Bayer AG, Bayer CropScience, Monheim, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Kehrig, B. (1999) Occupational medical experiences with triadimenol. Unpublished report No. MO-99-017584 from Bayer AG, Dormagen, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Kehrig, B. & Steffens, W. (2003) Occupational medical experiences with triadimefon/Bayleton. Unpublished report No. MO-03-004770 from Bayer AG, Dormagen, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Keutz, E.V. & Groening, P. (1984) THS 2212 (triazolylalanine)—subchronic toxicity to dogs on oral administration (13-week feeding study). Unpublished report No. 12562 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Kimmerle, G. (1976) KWG 0519—subacute inhalation toxicity study on rats. Unpublished report No. 6321 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Kroetlinger, F. (1993) KWG 0519—study for skin and eye irritation/corrosion in rabbits. Unpublished report No. 22036 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Kroetlinger, F., Loeser, E., Schilde, B. & Diesing, L. (1982) KWG 0519 (triadimenol, the active ingredient of Baytan)—chronic toxicity study on rats (2-year feeding experiment). Unpublished report No. 11009 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Lai, K. & Simoneaux, B. (1986a) Balance study of ^{14}C-triazole acetic acid in orally dosed rats. Unpublished report No. ABR-86022 from Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Lai, K. & Simoneaux, B. (1986b) Balance study of ^{14}C-triazole alanine in orally dosed rats. Unpublished report No. ABR-86023 from Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Lai, K. & Simoneaux, B. (1986c) Balance study of ^{14}C-triazole in orally dosed rats. Unpublished report No. ABR-86021 from Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Lai, K. & Simoneaux, B. (1986d) The metabolism of ^{14}C-triazole acetic acid in the rat. Unpublished report No. ABR-86028 from Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Lai, K. & Simoneaux, B. (1986e) The metabolism of triazole alanine in the rat. Unpublished report No. ABR-86041 from Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Leeling, J.L., Helms, R.J. & Polomack, S.C. (1988) Dermal absorption of triadimenol-^{14}C in rats. Unpublished report No. 1055 from Miles Inc., Elkhart, Indiana, USA. Submitted to WHO for JMPR 1989 but not for the present Meeting.

Loeser, E. (1979) MEB 6447—multigeneration reproduction study on rats. Unpublished report No. 8297 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Loeser, E. & Eiben, R. (1982) KWG 0519—multigeneration study on rat. Unpublished report No. 11035 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Loeser, E. & Eiben, R. (1984) KWG 0519 (proposed common name: triadimenol)—generation study on rats. Unpublished report No. 12390 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Loeser, E. & Kaliner, G. (1977) KWG 0519—subchronic toxicity study on rats (three-month feeding experiment). Unpublished report No. 6811 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Machemer, L. (1976a) MEB 6447 (triadimefon)—evaluation for embryotoxic and teratogenic effects on rabbits following oral administration. Unpublished report No. 6297 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Machemer, L. (1976b) MEB 6447 (triadimefon)—evaluation for embryotoxic and teratogenic effects on rats following oral administration. Unpublished report No. 6294 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Machemer, L. (1976c) MEB 6447—dominant lethal study on male mice to test for mutagenic effects. Unpublished report No. 5837 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Machemer, L. (1977a) KWG 0519—evaluation for embryotoxic and teratogenic effects on orally dosed rats. Unpublished report No. 7038 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Machemer, L. (1977b) MEB 6447 (triadimefon, Bayleton active ingredient)—micronucleus test on mice to evaluate MEB 6447 for mutagenic effects. Unpublished report No. 6622 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Machemer, L. & Kimmerle, G. (1976) Evaluation of MEB 6447 (triadimefon) for embryotoxic and teratogenic effects on rats following inhalation in dynamic flow apparatus. Unpublished report No. 6298 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Maruhn, D. & Bomhard, E. (1984) Triazolylalanine (THS 2212)—study for subchronic toxicity to rats (three-month feeding study). Unpublished report No. 12397 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Melly, J.G. & Lohse, K. (1982) 1,2,4-Triazole—microbial mutagen test. Unpublished report No. 81R-252 from Rohm and Haas Company, Spring House, PA, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Menegola, E., Broccia, M.L., Di Renzo, F. & Giavini, E. (2001) Antifungal triazoles induce malformations in vitro. *Reprod. Toxicol*., **15**, 421–427.

Mihail, F. (1980) MEB 6447—acute toxicity studies. Unpublished report No. 9277 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Mihail, F. (1986) Triazolylanine (THS 2212). Unpublished report No. 11229A from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Mihail, F. & Kaliner, G. (1979) MEB 6447—subacute oral cumulative toxicity study on rats. Unpublished report No. 8195 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Mihail, F. & Thyssen, J. (1980) KWG 0519 (triadimenol)—acute toxicity studies (isomer ratio 80 : 20). Unpublished report No. 9451 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Mihail, F. & Vogel, O. (1981) KWG 0519 (triadimenol)—study of comparative toxicity to rats after 4-week treatment using two test compound samples. Unpublished report No. 9949 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Mihail, F. & Vogel, O. (1983) Triazolylanine (THS 2212)—subacute oral toxicity study on rats. Unpublished report No. 11491 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Milburn, G.M., Birtley, R.D.N., Pate, I., Hollis, K. & Moreland, S. (1986) Triazole alanine—two-generation reproduction study in the rat. Unpublished report No. CTL/P/1168 from Imperial Chemical Industries PLC, Cheshire, England. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Mohr, U. (1976) MEB 6447—subchronic toxicity study on rats (twelve-week feeding experiment). Unpublished report No. R840a from Medizinische Hochschule Hannover, Hannover, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Moser, V.C. & MacPhail, R.C. (1989) Neurobehavioral effect of triadimefon, a triazole fungicide, in male and female rats. *Neurotoxicol. Teratol*., **11**, 285–293.

Myhr, B.C. (1982) Evaluation of KWG 0519 in the primary rat hepatocyte unscheduled DNA synthesis assay—final report. Unpublished report No. R2212 from Litton Biocinetics, Inc., Kensington, MD, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Nagane, M., Hatanaka, J. & Iyatomi, A. (1982) KWG 0519—mutagenicity test on bacterial system. Unpublished report No. JAP220 from Nihon Tokusho Noyaku Seizo K.K., Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Nagashima, Y. (1982a) Primary eye irritation study of KWG 0519 in rabbits. Unpublished report No. BOZO/B-402 from Bozo Research Center Inc., Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Nagashima, Y. (1982b) Primary skin irritation study of KWG 0519 in rabbits. Unpublished report No. BOZO/B-401 from Bozo Research Center Inc., Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Nishimura, N. (1983) Subacute toxicity study of KWG0519 in dietary administration to rats for 13 weeks. Unpublished report No. BOZO/B-398 from Bozo Research Center Inc., Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Polacek, I. (1983a) Central nervous effects of KWG 0519 (pilot study). Unpublished report No. R2695 from Toxicological Institute Regensburg, Regensburg, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Polacek, I. (1983b) Safety pharmacology study with MEB 6447 on oral administration. Unpublished report No. R 2428 from Toxicological Institute, Regensburg, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Poth, A. (1989) *Salmonella typhimurium* reverse mutation assay with 1H-1,2,4-triazole. Unpublished report No. 158400 from CCR—Cytotest Cell Research GmbH & Co. KG, Rossdorf, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Procopio, K.R. & Hamilton, J.D. (1981) 1,2,4-Triazole—acute toxicity range-finding study. Unpublished report No. 81R-057A from Rohm and Haas Company, Spring House, PA, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Puhl, R.J. & Hurley, J.B. (1978) The metabolism and excretion of Baytan TM by rats. Unpublished report No. 66489 from Mobay Chemical Corporation, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Puri, E. (1986) CGA 131013 technical—autoradiographic DNA repair test on rat hepatocytes. Unpublished report No. 860184 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Putman, D.L. (1987) Baytan—sister chromatid exchange assay in chinese hamster ovary (CHO) cells. Unpublished report No. BC1022 from Microbiological Associates Inc., Rockville, MD, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Reeves, R., Thiruchelvam, M. & Cory-Slechta, D.A. (2004) Development of behavioral sensitization to the cocaine-like fungicide triadimefon is prevented by AMPA, NMDA, DA D1 but not DA D2 receptor antagonists. *Toxicol. Sci.*, **79**, 123–136.

Renhof, M. (1984) KWG 0519 (common name: triadimenol)—study for embryotoxic effects on rats after oral administration. Unpublished report No. 12687 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Renhof, M. (1988a) 1,2,4-triazole—investigations into embryotoxic effects on rats after oral administration—supplement to study No. T5019339. Unpublished report No. 17402 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Renhof, M. (1988b) 1,2,4-triazole—investigations into embryotoxic effects on rats after oral administration. Unpublished report No. 17401 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Richold, M., Allen, J.A., Williams, A. & Ransome, S.J. (1981) Cell transformation test for potential carcinogenicity of R152056. Unpublished report No. CTL/C/1085 from Huntingdon Research Centre Ltd., Huntingdon, England. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Roetz, R. (1982) MEB 6447—evaluation for embryotoxic effects on the rabbit after oral administration. Unpublished report No. 10831 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

San, R.H.C. & Springfield, K.A. (1990) Bayleton technical—Salmonella/mammalian microsome plate incorporation mutagenicity assay (Ames test). Unpublished report No. 5262 from Microbiological Associates, Inc., Rockville, MD, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Schladt, L. (1998) KWG 0519—oncogenicity study on CD-1 mice. Dietary administration over 18 months. Unpublished report No. 28299 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Schladt, L. & Sander, E. (1998) KWG 0519—subchronic toxicity study in CD-1 mice (administration in the feed over 13 weeks). Unpublished report No. 27070 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Schmidt, U. (1983) Triadimefon—interaction of triadimefon with liver microsomes—studies on rat and mouse in vivo and in vitro. Unpublished report No. 11812 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Sheets, L.P. (1990a) Dermal sensitization study with technical grade triadimefon (Bayleton) in guinea pigs. Unpublished report No. 5154 from Mobay Chemical Corporation, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Sheets, L.P. (1990b) Primary dermal irritation study with technical grade triadimefon (Bayleton) in rabbits. Unpublished report No. 5164 from Mobay Chemical Corporation, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Sheets, L.P. (1990c) Primary eye irritation study with technical grade Triadimefon (Bayleton) in rabbits. Unpublished report No. 5163 from Mobay Chemical Corporation, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Sheets, L.P. & Phillips, S.D. (1992) A repeated dose 21-day dermal toxicity study with technical grade triadimefon (Bayleton) in rats. Unpublished report No. BC6649 from Miles Inc. Agriculture Division, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Shirasu, Y., Moriya, M. & Miyazawa, T. (1978) Triadimefon—mutagenicity test on bacterial systems. Unpublished report No. 66747 (MO-02-005595) from Institute of Environmental Toxicology, Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Shirasu, Y., Moriya, M. & Miyazawa, T. (1979) Triadimefon—mutagenicity test on bacterial systems. Unpublished report No. 89028 (MO-02-004915) from Institute of Environmental Toxicology, Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Strasser, F. (1986) CGA 131013 technical—micronucleus test (Chinese hamster). Unpublished report No. 860185 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Tanahashi, N. & Moriya, M. (1982) Triadimenol—microbial mutagenicity study. Unpublished report No. MO-00-014878 from Institute of Environmental Toxicology, Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thevenaz, P. (1986) CGA 142856 technical—14-day subacute toxicity study in rats (dietary administration). Unpublished report No. 841140 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thevenaz, P. (1994) CGA 142856—acute oral LD50 in the rat. Unpublished report No. 840887 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thyssen, J. & Kaliner, G. (1976) KWG 0519—subacute oral cumulative toxicity study on rats (four-week treatment). Unpublished report No. 6481 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thyssen, J. & Kimmerle, G. (1974) MEB 6447—acute toxicity studies. Unpublished report No. 4416 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thyssen, J. & Kimmerle, G. (1976a) 1,2,4-Triazole—occupational toxicology study. Unpublished report No. 5926 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thyssen, J. & Kimmerle, G. (1976b) KWG 0519—acute toxicity studies. Unpublished report No. 6090 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thyssen, J. & Weischer, C.H. (1976) MEB 6447—subacute dermal cumulative toxicity study on rabbits. Unpublished report No. 6352 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thyssen, J., Kimmerle, G. & Luckhaus, G. (1974) MEB 6447—subacute toxicity studies. Unpublished report No. 4464 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Unger, T.M., Goethem, Dv. & Shellenberger, T.E. (1982) A teratological evaluation of Bayleton in mated female rats. Unpublished report No. BC324 from Midwest Research Institute, Kansas City, MI, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

van Dijck, P. (1976) Evaluation of Bayleton (MEB 6447, triadimefon) for mutagenetic potential by the Ames test with histidine-auxotrophic *Salmonella typhimurium* strains. Unpublished report No. 53107 (MO-02-005632) from Laboratorium voor Hygiene, Katholieke Universiteit Leuven, Leuven, Belgium. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Vinggaard, A.M., Breinholt, V. & Larsen, J.C. (1999) Screening of selected pesticides for oestrogen receptor activation in vitro. *Food Addit. Contam.*, **16**, 533–542.

Vinggaard, A.M., Hnida, C., Breinholt, V. & Larsen, J.C. (2000) Screening of selected pesticides for inhibition of CYP19 aromatase activity in vitro. *Toxicol. In Vitro*, **14**, 227–234.

Walker, Q.D. & Mailman, R.B. (1996) Triadimefon and triadimenol: effects on monoamine uptake and release. *Toxicol. Appl. Pharmacol*., **139**, 227–233.

Walker, Q.D., Lewis, M.H., Crofton, K.M. & Mailman, R.B. (1990) Triadimefon, a triazole fungicide, induces stereotyped behavior and alters monoamine metabolism in rats. *Toxicol. Appl. Pharmacol*., **102**, 474–485.

Warren, D.L. (1990) Acute four-hour inhalation toxicity study with Bayleton technical in rats. Unpublished report No. 5267 from Mobay Chemical Corporation, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Watanabe, M. (1993) CGA-131013—DNA repair test (rec assay)—final report. Unpublished report No. IET 93-0010 from Kodaira, Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Watkins, P.A. (1982) R152056: 3-(1,2,4-triazol-l-yl) alanine (ICI 156,342)—micronucleus test in CBC mice—TQM/4. Unpublished report No. CTL/C/1164 from Imperial Chemical Industries PLC, Cheshire, England. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Weber, H., Patzschke, K. & Wegner, L.A. (1978) 1,2,4-triazole-14C biokinetic studies on rats. Unpublished report No. PH7920 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Wickings, E.J., Middleton, M.C. & Hillier, S.G. (1987) Non-steroidal inhibition of granulosa cell aromatase activity in vitro. *J. Steroid Biochem*., **26**, 641–646.

Wickramaratne, G.A.D. (1987) The Chernoff-Kavlock Assay: Its validation and application in rats. *Teratog. Carcinog. Mutagen*., **7**, 73–83.

Zarn, J.A., Bruschweiler, B.J. & Schlatter, J.R. (2003) Azole fungicides affect mammalian steroidogenesis by inhibiting sterol 14 alpha-demethylase and aromatase. *Environ. Health Perspect*., **111**, 255–261.