PROPICONAZOLE

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Explanation

Propiconazole is the International Organization for Standardization (ISO) approved name for 1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl-methyl]-1*H*-1,2,4-triazole, a systemic fungicide that acts by inhibition of ergosterol biosynthesis. Propiconazole was evaluated toxicologically by the JMPR in 1987, when an acceptable daily intake (ADI) of 0–0.04 mg/kg bw was established on the basis of the no-observed-adverse-effect level (NOAEL) of 4 mg/kg bw per day for effects on body weight, clinical chemistry and haematology in a 2-year study in rats, and this was supported by the NOAEL of 7 mg/kg bw per day in a 1-year study in dogs. Propiconazole was considered by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues. The Meeting reviewed new data on propiconazole that had not been reviewed previously and relevant data from the previous evaluation.

Evaluation for acceptable daily intake

Most of the studies of toxicity with propiconazole were performed between 1979 and 1988 and used batches with a purity of 89–93%, which is considered to be representative of commercial propiconazole. Studies did not comply with good laboratory practice (GLP) unless specifically stated in the text.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

(a) Oral route

Mice

Groups of five male and five female CD1 mice were fed diets containing propiconazole at a concentration of 5, 100 or 2500 ppm for 21 days, followed by a single oral dose of [U-^{14}C]-phenyl propiconazole (specific activity, 2.1 MBq/mg; radiochemical purity, >97%) at a corresponding dose (0.8/1.0, 16.8/21.5 and 434/475 mg/kg bw for males/females). Additional groups of two female mice and two male RAIf rats received control diet and radiolabelled propiconazole as a single dose at 600 mg/kg bw or 9 mg/kg bw respectively. The radiolabelled propiconazole was administered by gavage in a vehicle of ethanol/polyethylene glycol/water (7 : 9 : 4). At the higher dose, unlabelled propiconazole (purity, 91.1%) was added to the radiolabelled preparation. Urine and faeces were collected at 24-h intervals. All animals were sacrificed 4 days after administration of the final dose and tissue concentration of radioactivity was determined by liquid scintillation counting (LSC) after appropriate processing of the sample.

Two mice in the control group died after receiving a dose of 600 mg/kg bw by gavage. Total recoveries of radioactivity were >85% of the administered dose. Urinary excretion occurred predominantly in the first 24 h and tended to be higher in males than in females (Table 1). Residual radioactivity was approximately proportional to the dose administered. Residues in female mice were higher than in male mice, except in the kidneys where values were higher or equal in males. Independent of the dose and sex of the animals, the highest concentrations of residues (up to 3.0 µg/g) were found in the liver (Bissig, 1986).

Table 1. Excretion patterns (% of administered dose) in mice pretreated with diets containing unlabelled propiconazole for 21 days before administration of a single dose of radiolabelled propiconazole by gavage

Sample	Dietary concentration (ppm)/^{14}C-labelled dose (mg/kg bw)					
	5/0.8	100/17	2500/434	5/1	100/22	2500/475
	Males			Females		
Faeces						
0–24 h	35	33	26	35	15	20
0–96 h	38	35	32	43	22	31
Urine						
0–24 h	47	58	57	39	71	44
0–96 h	54	60	67	45	81	52
Total excretion	91	95	99	87	102	83

From Bissig (1986)

Rats

Two male and two female rats were given single doses of triazole-labelled [3,5-^{14}C]propiconazole (radiochemical purity, >98%) at 0.5 or 25 mg/kg bw by gavage in ethanol/polyethylene glycol 200/water (3 : 2 : 5). Within 24 h, 74–84% of the administered radioactivity had been excreted. After 6 days, 0.04–0.15%, 28–46% and 53–67% of the radioactivity had been recovered from expired air, faeces and urine respectively. Total recoveries of radioactivity were >95%. The patterns of excretion were essentially independent of sex or dose, although there was an indication that females excreted more propiconazole at the lower dose via the urine. Less than 1% of the administered dose remained in the tissues. Highest tissue concentrations of residues at day 6 were found in the liver, blood and kidneys, all <1 µg equivalent/g. No unchanged propiconazole was excreted in the urine according to thin-layer chromatography (TLC) analyses (Hambock, 1979).

A single oral dose of triazole-labelled [3,5-^{14}C]propiconazole (specific activity, 1.44 MBq/mg; radiochemical purity, >98%) at about 32 mg/kg bw or of phenyl-[U-^{14}C]-labelled propiconazole (specific activity, 2.22 MBq/mg; radiochemical purity, >98%) was administered to groups of male Tif:RAIf rats (20 rats were given triazole-labelled propiconazole; three rats were given for phenyl-labelled propiconazole) via gavage. More than 80% of the administered radioactivity was excreted within 24 h, with an approximately even distribution between the urine and faeces (Table 2). There was no marked difference in excretion of propiconazole labelled on either of the two positions (Mücke, 1979).

The most extensive investigation of the toxicokinetics of propiconazole was performed in a GLP-compliant study (Cresswell & Hopkins, 1989). Groups of five male and five female Crl:CD(SD)BR rats received [U-^{14}C]phenyl propiconazole (specific activity, 1.44 MBq/mg; radiochemical purity, >99%) as a single dose at 0.5 or 50 mg/kg bw by gavage after a 16-h fast, or as a single dose at 0.5 mg/kg bw by intravenous administration. The lower dose was given either with no pretreatment or after 14 unlabelled doses. Vehicles were physiological saline for intravenous dosing and ethanol/polyethylene glycol 200/water (1 : 2 : 2) for gavage. All animals were sacrificed after 168 h, except for animals in the pretreated group, which were sacrificed after 120 h). Samples of urine and faeces were collected over nine intervals of up to 168 h. Exhaled carbon dioxide was collected during the first 24 h. At termination, samples from a range of tissue and blood were removed. Samples of excreta and tissues were assayed by LSC after appropriate processing.

Some animals lost weight during the study, but there were no clinical signs indicating toxicity. Adequate stability and content of the dosing solutions was demonstrated. No carbon dioxide was detected in samples of trapped air. Patterns of excretion were similar

Table 2. Excretion of radioactivity (% of administered dose) in male rats given a single dose of phenyl- or triazole-labelled propiconazole

	Triazole-[3,5-^{14}C]-labelled propiconazole at 31.4 mg/kg bw	Phenyl-[U-^{14}C]-labelled propiconazole at 32.5 mg/kg bw
Urine		
0–24 h	44.5	48.5
0–96 h	52.3	51.4
Faeces		
0–24 h	36.2	44.2
0–96 h	43.3	48.6

From Mücke (1979)

Table 3. Radioactivity (mean values) in samples of excreta and tissues from rats given [U-^{14}C]phenyl propiconazole by intravenous or oral administration

	Dose (mg/kg bw)							
	0.5	0.5	0.5 (14 + 1)[a]	50	0.5	0.5	0.5 (14 + 1)[a]	50
	IV	Gavage	Gavage	Gavage	IV	Gavage	Gavage	Gavage
	Males				Females			
Faeces								
0–24 h (%)	35	37	34	31	30	32	31	27
0–168 h (%)	42	50	48[b]	48	39	38	40[b]	37
Urine								
0–24 h (%)	40	36	36	34	44	43	43	46
0–168 h (%)	43	39	41[b]	39	46	44	46[b]	49
Liver (ppm)	0.021	0.012	0.022	0.94	0.01	0.007	0.018	0.78
Kidney (ppm)	0.006	0.004	0.006	0.35	0.005	0.004	0.007	0.37
Lung (ppm)	0.003	ND	0.001	0.08	ND	0.001	0.003	0.08
Adrenal (ppm)	0.003	ND	0.002	0.56	0.006	ND	0.01	0.26
Blood (ppm)	0.001	ND	ND	0.07	0.004	ND	0.002	0.16
Bone marrow (ppm)	0.05	ND	ND	ND	0.008	0.006	0.04	0.14
Total recovery (%)	86–92	92–102	95–98	88–99	80–108	90–98	93–102	89–97

From Cresswell & Hopkins (1989)
IV, intravenous; ND, not detected
[a] This dose was administered after 14 unlabelled doses.
[b] 120 h

in all groups irrespective of dosing regime or route of administration, with most of the administered dose being excreted within 24 h and an approximately equal distribution between faeces and urine (Table 3). Females tended to excrete a greater proportion of an oral dose in the urine than did males (Table 3). Intragroup variation in patterns of excretion was low. Concentrations of radioactivity remaining at the end of the study were low (≤1% of the administered dose), most tissue concentrations were <0.1 ppm (µg equivalents/g) with highest concentrations typically in the liver and kidney (Table 3). Radioactivity in bone marrow showed a high degree of intra- and intergroup variation. The similarity in urinary and faecal excretion patterns between oral and intravenous dosing indicated significant biliary excretion and that most of an oral dose is absorbed (Cresswell & Hopkins, 1989).

The absorption, distribution and excretion of [U-^{14}C]phenyl propiconazole (specific activity, 1.48 MBq/mg; radiochemical purity, ≥ 98%) was investigated in intact and bile-duct cannulated Tif:RAIf male rats. A single dose at 0.5 mg/kg bw was administered by gavage in ethanol/polyethylene glycol 200/water (1 : 2 : 2). Blood samples were collected, at regular intervals up to 48 h, from the retro-orbital plexus of three rats. Groups of six rats were sacrificed at 1, 8, 14 or 24 h for investigation of radioactivity in a range of tissues. Samples of urine, faeces (0–24 and 24–48 h) and bile (eight intervals during 48 h) were collected from five bile-duct cannulated rats.

Peak plasma concentrations of radioactivity were detected 1 h after dosing. Highest tissue concentrations were found in the liver and kidney (Table 4). Most of the administered dose was excreted within 24 h in the bile (58%), with smaller amounts in the urine (15%) and faeces (4%); total excretion during 48 h was >90%. When compared with the results of studies in intact animals in which about 50% of the administered dose was recovered in the urine, these findings indicate extensive enterohepatic recirculation. Taking into account both biliary and urinary components, these results indicate that >80% of an oral dose of propiconazole is absorbed (Bissig, 1992).

Table 4. Tissue concentrations of radioactivity (μg equivalents/g) in male rats given [U-^{14}C]phenyl propiconazole as a single dose at 0.5 mg/kg bw

Tissue	Time after dosing (h)			
	1	8	14	20
Liver	0.68	0.58	0.15	0.14
Kidney	0.25	0.23	0.07	0.08
Lung	0.11	0.10	0.05	0.03
Adrenal gland	0.14	0.11	0.03	0.03
Plasma	0.08	0.07	0.02	0.02

From Bissig (1992)

Table 5. Total radioactivity and levels of main metabolites in samples from Leghorn hens given 10 mg of [U-^{14}C]phenyl propiconazole per day for 8 days

Sample	Mean total radioactivity (ppm)	Metabolite (mean % of radioactivity in sample)		
		Propiconazole	CGA 118244	CGA91305
Liver	3.9	2	4	81
Kidney	4.2	2	2	47
Egg white	0.7	27	51	18
Egg yolk	1.7	15	11	62
Thigh muscle	0.6	7	2	80
Skin/fat	0.6	40	3	30

From Doweyko (1990a)
CGA 118244, (propyl) β-hydroxy-propiconazole
CGA 91305, hydroxy-de-dioxolanated propiconazole

Hens

Two Leghorn hens were given daily doses of gelatin capsules containing either 5.9 mg of [^{14}C]phenyl propiconazole or 5.5 mg of [^{14}C]triazole propiconazole, each day for 16 days. These doses equated to a dietary concentration of approximately 50 ppm. The hens were sacrificed 24 h after the last dose. Concentrations of residues in eggs, excreta and tissues were determined. More than 94% of the administered doses were found in excreta, with <1% remaining in eggs/tissues. The concentrations of residue in the egg white of the hen receiving phenyl-labelled propiconazole were unusual, declining to day 10 and then increasing markedly. Residues of the triazole label in eggs reached a plateau after 11 doses. Peak concentrations in eggs were 0.9 to 1.2 ppm, highest tissue concentrations were approximately 2 ppm in the liver and kidney. Concentrations of radioactivity were generally higher in eggs and tissues (especially muscle) from the animal receiving the triazole-labelled propiconazole, indicating cleavage of the link between the triazole and phenyl rings (Seim & Brown, 1984; Szolics & Simoneaux, 1985).

Four Leghorn hens received daily doses of 10 mg of [U-^{14}C]phenyl propiconazole (specific activity, 1.77 MBq/mg; radiochemical purity, 98.6%) in gelatin capsules for 8 days, before sacrifice 6 h after the final dose. Steady-state excretion accounted for approximately 80–85% of the administered dose. Concentration of radioactivity in tissues and eggs was determined by LSC after appropriate processing. Highest tissue concentrations were found in the liver and kidney (Table 5). Concentrations of radioactivity in eggs reached a plateau by days 5–7 (Table 5) (Doweyko, 1990a).

Goats

One lactating goat received 10 daily doses of 5 mg of triazole-labelled propiconazole (specific activity, 0.92 MBq/mg) equivalent to a dietary concentration of 4.5 ppm. Samples of milk, urine and faeces were taken during the study. The animal was sacrificed 27 h after the final dose and a range of tissues were removed. Total radioactivity was determined by LSC after sample processing. Most of the administered doses were excreted in the urine (70%), with 20% in the faeces and approximately 0.2% in the milk. Concentrations in milk reached a plateau at approximately 0.01–0.02 ppm after six doses, with most radioactivity being found in the whey (80%) and casein (17%), and approximately 1% in the fat. Peak tissue concentrations were found in the liver (0.1 ppm) and kidney (0.03 ppm), with 0.01 ppm in muscle (Fisher & Cassidy, 1980)

Two lactating goats were given four daily doses of 125 mg of [U-^{14}C]phenyl propiconazole (specific activity, 1.77 MBq/mg; radiochemical purity, 98.6%) in gelatin capsules. The doses equated to dietary concentrations of 67–92 ppm. Samples of urine, faeces and milk were collected throughout this GLP-compliant study. The goats were sacrificed 6 h after the final dose and samples of a range of tissues were taken. Samples of excreta, milk and tissue were analysed by LSC for total radioactivity. Excretion was extensive in the urine (50% of the administered dose) and faeces (40%) but not in milk. Tissue concentrations were highest in the liver (4 ppm) and kidney (2.5 ppm), with lower concentrations in blood (0.3 ppm). The highest concentration in milk (0.2 ppm) was seen on day 4 and it is possible that a plateau had not been reached (Doweyko, 1990b).

(b) Dermal route

Rats

Groups of four male and four female Sprague-Dawley rats were given triazole-U-^{14}C-propiconazole as single doses at 1.0 or 10.0 mg/kg bw as a 40% solution in a formulation blank applied to the dorsal skin. The application site did not appear to be covered and oral ingestion cannot be discounted. Extensive absorption was indicated, with 20% of the applied dose being excreted within 24 h and >75% of the applied radioactivity being recovered during 72 h in excreta (urine, 30–40%; faeces, 20–30%) or the tissues and carcass other than at the application site. The amount of residual radioactivity on the skin averaged 20% of the applied dose. Highest concentrations in tissues were generally found at 24 h, although females at the lower dose and males at the higher dose appeared to have an initial peak at 4 h. The report states that the metabolites in excreta were similar to those formed in animals dosed orally (no data presented) (Simoneaux, 1983).

Comparable results were reported by Murphy et al. (1986) in a study in which groups of four male Sprague-Dawley rats were given triazole-^{14}C-labelled propiconazole (radiochemical purity, >95%) at a dose of 0.1, 1 or 10 mg per rat by dermal administration in a formulation blank. Animals were sacrificed after 2, 4 or 10 h. Total recoveries of radioactivity were >85%. The extent of absorption increased with time, but was inversely proportional to applied dose. After 10 h, total absorption (excluding skin residue) was approximately 30%, 10% and 5% for the lowest, intermediate and highest dose respectively. Approximately 25% of the intermediate and highest doses, and 13% of the lowest dose remained at the application site after washing (Murphy et al., 1986).

1.2 Biotransformation

Mice

Samples of urine from the study of Bissig (1986), in which [U-^{14}C]phenyl-propiconazole was used (described above), were investigated using TLC, high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) and mass spectrometry (MS). The presence of conjugates was examined using glucuronidase or sulfatase treatments. The pattern of urinary metabolites of propiconazole demonstrated a marked sex dependency. In male mice, 60% of the radioactivity in urine collected at 0–24 h was represented by one metabolite (U2), while this metabolite accounted for only 30% in the sample of urine collected at 0–24 h in female mice and aproximately 10% in rats. U2 was identified as the glucuronic acid conjugate of 1-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol—a metabolite lacking the dioxolane ring substituent. Treatment of U2 with glucuronidase resulted in a significant increase in levels of U16 in mice, but little change in rats, indicating that it might consist of more than one component. Other major metabolites (U1 and U12; not identified) were present in greater amounts in females than in males. The results indicate that the major metabolic pathway of propiconazole in mice involves dioxolane ring cleavage followed by glucuronide conjugation (Bissig, 1986).

Rats

Samples of urine and faeces from animals receiving triazole-labelled propiconazole in the study by Mücke (1979), described above, were investigated using glucuronidase/arylsulfatase digestion and two-dimensional TLC and electrophoresis. Faecal metabolites were less polar than those in the urine. Approximately 20 metabolites were found in the urine. Unchanged propiconazole was present at approximately 5% in the faecal samples, but was not detected in urine. Conjugates represented approximately 10% of the administered dose. The primary urinary metabolite (U8) represented 24% of the urinary radioactivity. The structure of propiconazole was reported to be well preserved, with little cleavage of the dioxolane ring (Mücke, 1979).

The metabolism of [3,5-^{14}C]-triazole propiconazole (specific activity 23.1 µCi/mg (0.85 MBq/mg); radiochemical purity >98%) was investigated in male TIF:RAIf rats (group size not specified). Rats were fasted overnight before being given a dose of approximately 31.4 mg/kg bw by gavage in an ethanol/polyethylene glycol 200/water (3 : 2 : 5) vehicle. Samples of urine and faeces were taken during the first 24 h after dosing; these represented 45% and 36%, respectively, of the administered radioactivity. Samples were extracted and investigated using a wide range of techniques, including fractionation, derivitization, enzymic hydrolysis, TLC, electrophoresis, MS, HPLC, liquid chromatography (LC) and NMR. Nine reference compounds were used for confirmation of identity. Propiconazole was not found in urine, but was present in faeces at approximately 3% of the administered dose. A wide range of metabolites were identified (representing more than 50% of the administered radioactivity), most being present at <2% of the administered dose. The major site of metabolism was the propyl side-chain, with approximately 15–20% of the radioactivity consisting of carboxy acid and or hydroxy derivatives from the propyl side-chain. Dioxolane ring cleavage was evident in the urinary metabolites, representing 10–15% of the administered dose. Hydroxylation of both chlorophenyl and triazole rings was seen together with glucuronidation and sulfation. There was limited evidence of cleavage between the chlorophenyl and triazole rings (Mücke, 1983).

Pooled samples of urine and pooled samples of faeces from the study by Cresswell & Hopkins (1989) (see above), were extracted and analysed for metabolites by two-dimensional TLC. There was no investigation of conjugation. Comparisons were made with eight analytical standards of predicted metabolites. Propiconazole was found to be extensively metabolized; however, many of the components did not correlate with the standards (Table 6). Twenty-four urinary and forty-seven faecal components were detected among the range of samples, with great variation between the patterns for the sexes and at different doses (Table 6). It was not possible to compare faecal and urinary patterns directly, as different first-solvent systems were used. Unchanged propiconazole was present in the faeces of orally dosed groups (6–18% of the administered radioactivity) and in the urine of animals treated by intravenous administration (approximately 30% of the administered radioactivity). With many metabolites remaining unidentified, the metabolic pathway of propiconazole could not be determined with any certainty. The levels of CGA 91304 in pretreated

Table 6. Urinary and faecal metabolites (% of radioactivity of fraction) in samples from rats given [U-^{14}C]phenyl propiconazole by oral or intravenous administration

	Dose (mg/kg bw)							
	0.5	0.5	0.5 (14 + 1)[a]	50	0.5	0.5	0.5 (14 + 1)[a]	50
	IV	Gavage	Gavage	Gavage	IV	Gavage	Gavage	Gavage
	Males				Females			
Urine								
Propiconazole	27	—	—	—	29	—	—	—
CGA 188245	62	19	—	—	2	14	—	49
CGA 217495	9	—	4	—	59	—	—	—
CGA 91304	2	12	29	—	—	15	—	—
CGA 118244	—	—	4	—	4	—	—	—
CGA 217496	—	—	—	—	—	—	—	—
Fraction 7	—	30	—	—	—	14	—	—
Fraction 8	—	40	—	—	—	57	—	18
Fraction 9	—	—	—	—	6	—	—	—
Fraction 10	—	—	53	—	—	—	—	2
Fraction 11	—	—	9	—	—	—	—	9
Fraction 12	—	—	2	1.5	—	—	—	—
Fraction 13	—	—	—	—	—	—	37	—
Fraction 14	—	—	—	—	—	—	32	—
Fraction 15	—	—	—	—	—	—	32	—
Fraction 16	—	—	—	34	—	—	—	—
Fraction 17	—	—	—	42	—	—	—	—
Fraction 18	—	—	—	4	—	—	—	—
Fraction 19	—	—	—	8	—	—	—	—
Fraction 20	—	—	—	2	—	—	—	—
Fraction 21	—	—	—	23	—	—	—	3
Fraction 22	—	—	—	3	—	—	—	—
Fraction 23	—	—	—	—	—	—	—	14
Fraction 24	—	—	—	—	—	—	—	4
Faeces								
Propiconazole	—	7	15	6	—	14	14	18
CGA 188245	10	8	6	—	9	—	—	11
CGA 91305	7	8	8	—	5	8	—	8

From Cresswell & Hopkins (1989)
—, not identified; IV, intravenous
[a] This dose was administered after 14 unlabelled doses
CGA 188245, (propyl) γ-hydroxy-propiconazole
CGA 217495, dioxalane 4-carboxy acid derivative of propiconazole
CGA 91304, keto-de-dioxolanated propiconazole
CGA 118244, (propyl) β-hydroxy-propiconazole
CGA 217496, dioxalane 4-methylcarboxy acid derivative of propiconazole
CGA 91305, hydroxy-de-dioxolanated propiconazole

males (29%) show a significantly higher rate of cleavage of the dioxolane ring than was found by Bissig (1986). Indications were that oxidation of the propyl side-chain was a significant reaction. Samples from pretreated animals had a different pattern to untreated animals, indcating induction of metabolism (Cresswell & Hopkins, 1989).

The pattern of results found by Cresswell & Hopkins (1989), with two or three components predominating in each group, differs from that found by Mücke (1983), where no component was present at >10%.

Samples from bile-duct cannulated male rats (Bissig, 1992; see above) were investigated for metabolites by TLC. Sixteen metabolite fractions were detected in the urine, the primary urine metabolite (U11; 5.5% of the administered dose) was identified as an α-hydroxy acid derivative of propiconazole. The primary radioactive component of faeces was unchanged propiconazole. There were approximately 16 metabolites/fractions found in samples of bile; the main biliary metabolites were not identified, but the neutral and non-polar components had retention factors (R_f) that were similar to those of some urinary metabolites (Bissig, 1992).

Hens

Samples from hens given 10 mg of [U-^{14}C]phenyl propiconazole for 8 days (Doweyko, 1990a; see above) were investigated by TLC, HPLC and MS after sub-sampling, solvent extraction and, in the case of kidney, incubation with glucuronidase and arylsulfatase. Three major metabolites (propiconazole, CGA118244 and CGA91305) represented >80% of the residue in most tissues, with the metabolite pattern differing between tissues. The metabolite profile in the kidney altered after incubation with arylsulfatase but not with glucuronidase. These results demonstrate that cleavage of the dioxolane ring occurs in hens, with retention of the cleavage products (Doweyko, 1990a).

Goats

Samples of urine, milk and liver from one lactating goat receiving 10 daily doses of 5 mg of triazole-labelled propiconazole (Fisher & Cassidy, 1980; see above) were extracted, fractionated and analysed by two-dimensional TLC, electrophoresis and ion-exchange chromatography either before or after Kjeldahl/sulfuric acid reactions; twelve analytical standards were available for comparison. Conjugation was investigated by incubation with glucuronidase or arylsulfatase. Most metabolites were not conclusively identified. The majority of metabolites in the urine (92%), but <20% of those in the milk and liver were considered to contain both triazole and chlorophenyl rings. TLC patterns for milk and liver were qualitatively similar (Madrid & Cassidy, 1981).

TLC comparison with urine samples from rats (Mücke, 1979) showed that the major urinary metabolites in rats and goats differ, but that the general pattern and extent of metabolism is similar. The primary reaction in both species was oxidation of the propyl side-chain of the dioxolane ring to carboxylic acids. Sulfate conjugation was more extensive in rats than in goats (Madrid & Cassidy, 1981).

Milk and tissue samples from goats given four daily doses of 125 mg of [U-^{14}C]phenyl propiconazole in gelatin capsules were analysed by LSC then extracted and investigated for metabolites by MS, TLC and HPLC (including comparison with eight analytical standards). Most radioactivity was extracted into acetonitrile. Three main metabolites (propiconazole,

Table 7. Levels of metabolites in tissues and milk from goats given four daily doses of 125 mg of [U-^{14}C]phenyl propiconazole

Sample	Concentration (ppm) or % of sample radioactivity	Metabolite						
		Propiconazole	CGA118244	CGA93105	D	E	F	G
Liver	(ppm)	0.63	0.95	0.72	0.67	ND	ND	ND
	(%)	14	21	16	15	ND	ND	ND
Kidney	(ppm)	0.12	0.25	0.47	0.83	ND	ND	ND
	(%)	5	9	18	31	ND	ND	ND
Tenderloin	(ppm)	<0.01	0.01	0.02	0.015	ND	ND	ND
	(%)	1.7	13	30	19	ND	ND	ND
Omental fat	(ppm)	0.01	0.02	0.02	ND	ND	ND	ND
	(%)	20	34	31	ND	ND	ND	ND
Milk	(ppm)	ND	0.05	0.05	0.01	0.01	0.01	0.02
	(%)	ND	23	24	6	5	6	11

From Doweyko (1990b)
ND, not detected

Table 8. Acute toxicity of propiconazole

Species	Strain	Sex	Route	Vehicle	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Purity (%)	Reference
Rat	Tif:RAIf	Male and female	Oral	CMC	1517	—	93	Bathe (1978)
Mouse	Tif:MAG	Male and female	Oral	CMC	1490	—	93	Bathe (1979a)
Rat	Tif:RAIf	Male and female	Dermal	None	>4000	—	93	Bathe (1979b)
Rabbit	NZW	Male and female	Dermal	None	>6000	—	93	Ullmann (1979a)
Rat	Tif:RAIf	Male and female	Inhalation (4-h, nose only, MMAD approximately 2.6 µm)	Ethanol	—	>5	91	Hartmann & Gfeller (1988)

CMC, carboxymethylcellulose; NZW, New Zealand White; MMAD, mass median aerodynamic diameter

CGA 118244 and CGA 91305) represented a significant proportion of the radioactivity in a particular tissue (Table 7), with an unidentified compound representing >30% of the radioactivity in kidney samples. Milk samples were unaltered by treatment with glucuronidase, but hydrolysis by arylsulfatase indicated the presence of sulfate conjugates. Tissue samples were not investigated for the presence of conjugates (Doweyko, 1990b).

2. Toxicological studies

2.1 Acute toxicity

(a) General toxicity

Most of the studies of acute toxicity with propiconazole were performed before the adoption of test guidelines and GLP, but the overall quality of these studies was adequate to determine the acute toxicity of propiconazole. The results are summarized in Table 8. Propiconazole is of moderate acute toxicity via the oral route and low acute toxicity via the dermal and inhalation routes. There were no specific signs of toxicity reported.

(b) Ocular irritation, dermal irritation and dermal sensitization

Propiconazole was moderately irritating to rabbit skin (Ullmann, 1978a) and produced only minimal irritation to rabbit eyes (Ullmann, 1978b). Weak reactions were seen in 3 out of 19 guinea-pigs in an "optimization" test for skin sensitization (Ullmann, 1979b). A pos-

itive result was reported in a test for skin sensitization in guinea-pigs that was performed to a protocol for the Magnusson & Kligman maximization test (Sommer, 1999).

2.2 Short-term studies of toxicity

Oral toxicity was investigated in 4-week and 90-day studies in rats; 13- and 17-week studies in mice; and 90-day and 1-year studies in dogs. Dermal toxicity has been investigated in a 21-day (15 exposures) study in rabbits. A 13-week (65 exposures) inhalation study was performed in rats.

(a) Oral administration

Mice

Groups of 40 male CD1 (ICR) BR mice received diets containing propiconazole (purity, 92%; batch, FL850083) at a concentration of 0, 20, 500, 850, 1450 or 2500 ppm for up to 13 weeks. At 4 weeks and 8 weeks, 10 mice per group were sacrificed, with the remainder being sacrificed at 13 weeks. Routine examinations included survival, clinical signs, body weight and food consumption. Ophthalmoscopy was performed before dosing and before termination. Blood for clinical chemistry investigations (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, sorbitol dehydrogenase and cholesterol) was taken before sacrifice. An extensive necropsy examination was performed, but only brain and liver weights were taken. Only livers were examined histopathologically, including staining with oil red O. Statements of compliance with GLP were provided, but the study does not comply with OECD guideline 408 owing to the limited range of examinations undertaken.

Homogeneity and achieved content of the diet were acceptable. Achieved intakes were 0, 2.8, 71, 121, 199 or 360 mg/kg bw per day. There were no deaths during the study, nor any clinical signs associated with exposure to propiconazole. Body-weight gain was reduced at 2500 ppm from the first week of dosing (0.8 g versus 2.2 g in the controls). Similar clinical chemistry results were seen at 4, 8 and 13 weeks (Table 9), with decreases in

Table 9. Findings in male mice given diets containing propiconazole for up to 13 weeks

	Dietary concentration (ppm)					
	0	20	500	850	1450	2500
4 weeks (n = 10):						
Liver weight (g)	1.3 ± 0.1	1.2 ± 0.2	1.5 ± 0.1	1.8 ± 0.2*	2.5 ± 0.3	2.8 ± 0.4
ALT (U/l)	24 ± 8	26 ± 8	29 ± 17	42 ± 17	56 ± 24*	86 ± 28*
SDH (U/l)	26 ± 5	30 ± 6	39 ± 22	45 ± 11*	58 ± 18*	86 ± 28*
Cholesterol (U/l)	129 ± 26	121 ± 32	122 ± 27	92 ± 18*	81 ± 22*	47 ± 18*
13 weeks (n = 20):						
Liver weight (g)	1.4 ± 0.1	1.4 ± 0.1	1.6 ± 0.2*	1.9 ± 0.2*	2.4 ± 0.4*	2.9 ± 0.3*
ALT (U/l)	22 ± 9	23 ± 6	25 ± 9	35 ± 17	53 ± 15*	79 ± 30*
SDH (U/l)	22 ± 4	25 ± 5	25 ± 5	31 ± 11*	45 ± 10*	58 ± 21*
Cholesterol	122 ± 22	113 ± 24	102 ± 22*	86 ± 24*	75 ± 20*	67 ± 19*
All animals combined (n = 40):						
Hepatocyte hypertrophy	0	0	10*	35*	40*	40*
Hepatocyte necrosis	1	0	4	9*	31*	34*
Hepatocyte vacuolation	0	1	2	5*	15*	33*

From Potrepka & Turnier (1991a)
ALT, alanine aminotransferase; AST, aspartate aminotransferase; SDH, sorbitol dehydrogenase
*$p < 0.05$

cholesterol at 500 ppm and greater and increases in sorbitol dehydrogenase and alanine aminotransferase activities at 850 ppm and greater. Gross histopathology findings identified a number of changes to the livers of animals receiving diets containing propiconazole at 850 ppm and greater; these findings were confirmed histopathologically. Liver weights were increased by >10% at dietary concentrations of 500 ppm and greater by week 4 (Table 9). Hepatocyte necrosis and vacuolation were significantly increased at 850 ppm and greater, with hypertrophy increased at dietary concentrations of 500 ppm and greater (Table 9). An increase in hepatocellular necrosis at 500 ppm is not statistically significant. Histopathological findings did not increase notably with duration of dosing (Potrepka & Turnier, 1991a).

The NOAEL was 500 ppm (equal to 71 mg/kg bw per day) on the basis of statistically significant increases in liver weight, hepatocyte necrosis and serum activities of alanine aminotransferase and sorbitol dehydrogenase at 850 ppm (equal to 121 mg/kg bw per day).

Groups of 20 male and female CD1 (ICR) BR mice received diets containing propiconazole (purity, 92%; batch, FL850083) at a concentration of 0, 20, 500, 850, 1450 or 2500 ppm for males and 0, 20, 500 or 2500 ppm for females, for 17 weeks. Routine examinations included survival, clinical signs, body weight and food consumption. Ophthalmoscopy was performed before dosing and before termination. Blood for clinical chemistry investigations (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and cholesterol) was taken at 13 weeks (non-fasted) and 17 weeks (fasted). An extensive necropsy examination was performed, but only brain and liver weights were taken. Only livers were examined histopathologically, including specific staining for fat vacuolation. Statements of compliance with GLP were provided, but the study does not comply with OECD guideline 408 owing to the limited range of examinations undertaken.

Homogeneity and achieved content of the diet were acceptable. Achieved intakes were 0, 2.7, 65, 112, 194 or 352 mg/kg bw per day in males and 0, 3.4, 85 or 434 mg/kg bw per day in females. Two males died during the study (one at 20 ppm and one at 850 ppm); these deaths were not considered to be treatment-related. There were no clinical signs associated with exposure to propiconazole. Body-weight gain was reduced by approximately 10% in both sexes at 2500 ppm; in males this was associated with increased food consumption. Similar clinical chemistry results were seen at both 13 and 17 weeks: serum cholesterol was decreased in males receiving propiconazole at dietary concentrations of 850 ppm and greater; alanine aminotransferase activities were increased in females at 2500 ppm and males at 1450 ppm and greater. Gross histopathology findings identified enlarged livers with discoloured foci in males at 1450 ppm and in both groups at the highest dose. Liver weights were increased dose relatedly by >10% in males at dietary concentrations of 500 ppm and greater, and in females at 2500 ppm (Table 10). Histopathological changes in the liver were seen at 850 ppm and greater in males and in females at the highest dose (Table 10). Increased vacuolation observed at 500 ppm was not reproduced at 850 or 1450 ppm and is not considered to be a biologically significant finding (Potrepka & Turnier, 1991b).

The NOAEL was 500 ppm (equal to 65 mg/kg bw per day) on the basis of changes in clinical chemistry, increases in liver weight and histopathology findings in males at 850 ppm and in females at 2500 ppm.

The findings at 500 ppm in these two studies did not show a consistent pattern and support the choice of this dose as the overall NOAEL.

Table 10. Findings in mice given diets containing propiconazole for 17 weeks

Parameter	Dietary concentration (ppm)					
	0	20	500	850	1450	2500
Males						
Liver weight (g)	1.4 ± 0.1	1.4 ± 0.2	1.7 ± 0.2*	1.8 ± 0.2*	2.5 ± 0.3	2.8 ± 0.4
Hepatocyte hypertrophy	0	0	4	14*	20*	20*
Hepatocyte necrosis	1	0	2	4	10*	18*
Vacuolation	0	0	6*	2	3	16*
ALT (U/l)	17 ± 3	33 ± 61	28 ± 24	29 ± 8	65 ± 23*	128 ± 70*
Cholesterol (U/l)	119 ± 20	104 ± 24	105 ± 24	91 ± 25*	66 ± 33*	67 ± 29*
Females						
Liver weight (g)	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	—	—	2.1 ± 0.3*
Hepatocyte hypertrophy	0	0	0	—	—	17*
Hepatocyte necrosis	0	0	0	—	—	6*
Hepatocyte vacuolation	0	0	0	—	—	3
ALT (U/l)	17 ± 4	19 ± 7	21 ± 11	—	—	61 ± 25*
Cholesterol (U/l)	83 ± 20	93 ± 69	76 ± 22	—	—	64 ± 25
AST	45 ± 9	47 ± 15	55 ± 18	—	—	68 ± 21*

From Potrepka & Turnier (1991b)
ALT, alanine aminotransferase; AST, aspartate aminotransferase
*$p < 0.05$

Rats

Groups of 10 male and 10 female RAIf rats were given propiconazole (purity, 91.9%; batch, P4-6) at a dose of 0, 50, 150 or 450 mg/kg bw per day via gavage in 2% carboxymethyl cellulose for 28 days. Animals were observed routinely for mortality, clinical signs, body weight and food consumption. Haematology examinations were performed on samples obtained from five animals of each sex per group before sacrifice; investigations of clinical chemistry (cholesterol not measured) and urine analysis were performed on samples from the remaining five animals of each sex per group. Ophthalmoscopic examinations and investigations of auditory response were performed before dosing and before termination. The study was performed before GLP and did not comply with the main requirements of OECD guideline 407 (1981) as only the liver was examined histopathologically.

Three females died during the study—two from the group at the highest dose and one from the group at the intermediate dose; one of the animals at the highest dose was in a poor condition before death and this death might have been treatment-related. Clinical signs (sedation, dyspnoea and ruffled coat) related to administration of propiconazole were seen in females at a dose of 450 mg/kg bw per day during the first week of the study only. Body-weight gain was reduced by approximately 20% in males at the highest dose; female body weights were similar in all groups. Food consumption was significantly reduced in females at the highest dose and there was a pattern of reduced food consumption in males at the highest dose. Reductions in erythrocyte volume fraction, haemoglobin and erythrocyte numbers were seen in females at the highest dose (Table 11). Increased concentrations of serum glucose were seen in both sexes at 450 mg/kg bw per day (Table 11) and reductions in concentrations of serum chloride were detected in females at the highest dose (Table 11). Variations in plasma proteins were within the physiological ranges and not considered to be adverse. Activities of serum enzymes of liver damage were similar in all groups. Liver weights were increased in all groups of females and males receiving propiconazole at a dose of 150 mg/kg bw per day or greater (Table 11), but were considered to be adaptive and not adverse. The results of urine analysis did not indicate any reduced renal performance. The increased liver weights at 150 mg/kg bw per day and greater were consistent with liver

Table 11. Findings in rats given propiconazole by gavage for 28 days

Parameter	Sex	Dose (mg/kg bw per day)			
		0	50	150	450
Haematology (n = 5)					
Erythrocytes (10^6/µl)	Male	7.3	7.6	7.6	7.6
	Female	7.3	7.3	7.0	6.6*
Erythrocyte volume fraction (%)	Male	41	43*	44	42
	Female	42	43	42	39*
Haemoglobin (mmol/l)	Male	8.4	8.9	8.9	8.7
	Female	8.6	8.7	8.7	7.7*
Clinical chemistry (n = 5)					
Glucose (mmol/l)	Male	6.9	6.5	7.1	7.4
	Female	6.2	6.9	6.8	8.2*
Chloride (mmol/l)	Male	98.5	97.5	97.8	97.0
	Female	101.3	100.4	100.9	96.8*
Liver (n = 10)					
Liver weight (g)	Male	13.8 ± 1.7	13.5 ± 2.3	18.2 ± 1.7*	18.9 ± 3.1*
	Female	8.6 ± 1.1	11.6 ± 0.4*	13.9 ± 0.9*	14.3 ± 0.8*
Liver hypertrophy	Male	0	0	4	10*
	Female	0	0	8*	10*
Liver necrosis	Male	0	0	1	0
	Female	0	0	0	3

From Basler & Gfeller (1980)
*$p < 0.05$

hypertrophy seen in the histopathological examination (Table 11). Three females from the group at 450 mg/kg bw per day had necrosis of the liver parenchyma (Basler & Gfeller, 1980).

The NOAEL was 150 mg/kg bw per day on the basis of clinical signs of toxicity, liver necrosis and reduced erythrocyte parameters in females at 450 mg/kg bw per day.

Groups of 20 male and 20 femlae Tif:RAIf rats received diets containing propiconazole (purity, 90%; batch, INA 35/1) at a concentration of 0, 240, 1200 or 6000 ppm for 13 weeks. Animals were observed routinely for mortality, clinical signs, body weight and food consumption. Haematology examinations were performed on samples obtained from 10 animals of each sex per group during weeks 4, 8 and 13; investigations of clinical chemistry (cholesterol not measured) and urine analysis were performed on samples from the remaining five animals of each sex per group. Ophthalmoscopic examinations were performed before dosing and before termination. At termination, all animals received a gross examination and a wide range of tissues were examined microscopically after normal and special staining. The study was performed before GLP, but complied with the main requirements of OECD guideline 408 (1981).

Analyses of diet samples confirmed acceptable levels of incorporation. Achieved intakes were 0, 16, 76 or 461 mg/kg bw per day in males and 0, 17, 78 or 481 mg/kg bw per day in females. Body-weight gain was reduced in groups receiving the highest dose from week 2 onwards and to a lesser extent (<10%) in females at 1200 ppm from week 9 onwards. Food consumption in all treated groups was lower than that in the control groups; in groups at the highest dose, the reduction was statistically significant for the first 2 months, but food consumption was higher than that of controls over the final 2 weeks. Food conversion efficiency was reduced at the highest dose only. Changes in erythrocyte parameters in groups at the highest dose were first noted at week 4 and persisted until the end of the study (Table

Table 12. Findings in rats given propiconazole by gavage for 13 weeks

Parameter	Sex	Dietary concentration (ppm)			
		0	240	1200	6000
Body weight (n = 20)					
Body weight (g) at week 13	Male	470 ± 42	463 ± 36	459 ± 46	372 ± 34*
	Female	293 ± 14	286 ± 22	269 ± 22*	235 ± 21*
Haematology (n = 10)					
Erythrocytes (10^6/µl)	Male	8.1	8.1	8.2	7.8*
	Female	7.7	7.9	7.7	7.2*
Erythrocyte volume fraction (%)	Male	44	43	45	43
	Female	44	44	44	41*
Haemoglobin (mmol/l)	Male	9.5	9.5	9.7	9.3
	Female	9.3	9.5	9.4	8.9*
Reticulocytes (%)	Male	5	4	4*	5
	Female	3	4*	4*	4*
Clinical chemistry (n = 10)					
Glucose (mmol/l)	Male	7.5	7.7	8.1*	7.8
	Female	6.4	6.3	7.4*	7.6*
Chloride (mmol/l)	Male	100.0	99.7	99.0	96.8*
	Female	99.7	99.3	98.2	97.4*
γ-GT (U/l)	Male	2.4	2.3	3.0	3.5*
	Female	2.0	2.4	2.4	4.7*
Liver (n = 20)					
Relative liver weight (%)	Male	3.6	3.5	3.7	4.3*
	Female	3.7	3.7	3.9	4.5*

From Sacchsse et al. (1979)
γ-GT, γ-glutamyl transferase
*$p < 0.05$

12). An apparent increase in reticulocytes in all groups of females appears to be associated with a low value for the controls (typical range, 4–5%) and is not considered to be biologically significant. A wide range of statistically significant clinical chemistry changes were seen in animals at the highest dose, including alterations in electrolytes, and increases in blood urea nitrogen, serum protein concentrations and albumin : globulin ratio. Increased serum glucose concentrations were seen in both sexes receiving propiconazole at a dietary concentration of 1200 ppm and greater; in males there was no clear dose–response relationship (Table 12) and the values were within physiological ranges. Reductions in serum concentrations of chloride were detected in the groups at the highest dose (Table 12). With the exception of γ-GT (γ-glutamyl transferase) (Table 12) activities of serum enzymes of liver damage showed no consistent pattern. The results of urine analysis did not indicate any reduced renal performance. Liver weights relative to body weight were increased by approximately 20% in animals receiving propiconazole at a dietary concentration of 6000 ppm (Table 12). Increases in relative brain, heart and adrenal weights appear secondary to reduced body weight. The only histological finding was an increased incidence and severity of splenic haemosiderosis in females at the highest dose (Sacchsse et al., 1979a).

The NOAEL was 1200 ppm (equal to 76 mg/kg bw per day) on the basis of reduced body-weight gain, increased liver weight and altered erythrocyte and clinical chemistry parameters at 6000 ppm.

Dogs

Groups of four male and four female beagle dogs were given diets containing propiconazole (purity, 93%; batch No. IN 35/5) at aconcentration of 0, 50, 250 or 1250 ppm

(equal to 0, 1.3, 6.9 or 35 mg/kg bw per day in males, and 0, 1.6, 7.6 or 36 mg/kg bw per day in females) for 13 weeks. Adequate homogeneity, stability and content of the diets were demonstrated. Dogs were observed routinely for mortality, signs of ill health, hearing ability, body weight and food consumption. Urine analysis, haematology and clinical chemistry investigations were conducted on all dogs during pre-test, and after 4, 8, and 13 weeks of treatment. Ophthalmology examinations were performed on all dogs during pre-test and after 13 weeks of treatment. At termination, all dogs were necropsied, major organs were weighed and an extensive range of tissues, from all animals, was examined histologically. The study complied with OECD test guideline 452 of 1981 and a statement of GLP compliance was provided.

There were no deaths or clinical signs of toxicity. Body-weight gain exhibited some reduction at the intermediate and highest dose in females, but this was considered to be related to the higher body weight at the start of the study (9.9 kg at 1250 ppm versus 7.8 kg in the controls). Food consumption was reduced by approximately 5% in females at the highest dose, but this was possibly a consequence of the reduced energy requirement of these larger animals. There were no adverse effects recorded during, haematology, clinical chemistry or urine analysis investigations. One female at the highest dose had grey streaks on the optic disc of the tapetum. Decreased plasma concentrations of glucose in females were present before dosing and was not considered to be treatment-related. Organ weights were similar in all groups of females when corrected for body-weight differences, but in males at the highest dose, absolute and relative weights of the liver were increased by approximately 10%. Three males at the highest dose and one female at the intermediate dose were reported to have "an increased amount" of lymphoid follicles in the mucous membrane of the pyloric area of the stomach. Possibly related alterations of the gastrointestinal tract were reported in the 1-year study in dogs (see below) and they are thus considered to be treatment-related. The gastrointestinal effects were likely to be cuased by a local reaction to propiconazole, which is irritating to the skin, and the relevance to exposures in humans could not be discounted. The NOAEL for local effects was 250 ppm (equal to 6.9 mg/kg bw per day) on the basis of lymphoid changes in the stomach at 1250 ppm. The NOAEL for systemic effects was 1250 ppm (equal to 35 mg/kg bw per day, the highest dose tested (Sachsse et al., 1980b).

Four groups of five male and five female beagle dogs (and groups of two males and two females for the recovery segment of the study) were given diets containing propiconazole (purity, 90.2%; batch No., FL831527) for 1 year. A recovery subgroup comprising two males and two females in the control group and in the group receiving the highest dose was maintained on untreated diet for 1 month after the end of the 1-year treatment phase. Dietary concentrations were 0, 5, 50 or 250 ppm (equal to 0, 0.17, 1.9 or 8.4 mg/kg bw per day in males, and 0, 0.19, 1.9 or 8.9 mg/kg bw per day in females). A nominal ration of 400 g of food was provided, but this was increased to 500 g for male dogs from week 18 onwards. Adequate homogeneity, stability and content of the diets were demonstrated. Dogs were observed routinely for mortality, signs of ill health, and measurement of body weight and food consumption. Urine analysis, haematology and clinical chemistry investigations were conducted on all dogs during pre-test, and after 3, 6, and 12 months of treatment. Ophthalmology examinations were performed on all dogs during pre-test and after 1 year of treatment. At termination (at 12 months for main groups or 13 months for recovery groups), all dogs were necropsied, major organs were weighed and an extensive range of tissues, from all animals, was examined histologically. The study complied with OECD test guideline 452 of 1981 and a statement of GLP compliance was provided.

Table 13. Incidence of gastrointestinal findings in dogs receiving propiconazole for 1 year

	Dietary concentration (ppm)			
	0 (control)	5	50	250
Males (n = *5*)				
Colon hyperaemia	0	0	0	1
Ileum hyperaemia	0	0	0	1
Jejunum hyperaemia	0	0	0	1
Caecum hyperaemia	0	0	0	1
Duodenum hyperaemia	0	0	1	2
Stomach hyperaemia	0	0	1	3
Females (n = *5*)				
Duodenum hyperaemia	0	2	0	2

From Johnson et al. (1985)

There were no deaths or clinical signs of toxicity. Body-weight gain exhibited transient effects with no clear pattern, but, in an attempt to resolve this, the food ration for males was increased. Food consumption was similar in all groups. There were no adverse effects recorded during ophthalmoscopy, or investigations of haematology, clinical chemistry or urine analysis. Occasional findings, e.g. increased plasma concentrations of glucose at 6 months in females showed a high degree of variability and were not reproduced at other time-points. Organ weights were similar across all groups. Gross pathological examination identified reddening of the gastrointestinal tract, which was confirmed as hyperaemia by microscopic examination (Table 13). The findings were seen predominantly in males in the group receiving the highest dose and were not evident after the recovery phase. The study report gave no indication of the extent or severity of the hyperaemia. Similar results were reported in the shorter-term study in dogs (see above), but there was no evidence of similar effects in the long-term studies in mice and rats. The gastrointestinal effects were likely to be caused by a local reaction to propiconazole, which is irritating to the skin, and the relevance to exposures in humans could not be discounted. The NOAEL for local effects was 50 ppm (equal to 1.9 mg/kg bw per day) on the basis of hyperaemia of the gastrointestinal tract at 250 ppm. The NOAEL for systemic effects was 250 ppm, equal to 8.4 mg/kg bw per day, the highest dose tested (Johnson et al., 1985)

(b) Dermal exposure

Rabbits

Groups of 10 male and 10 female KA 46 New Zealand White rabbits received undiluted propiconazole (purity, 91.9%; batch, P4-6) at a dose of 0, 200, 1000 or 5000 mg/kg bw per day applied to the shorn skin for 6 h per day, 5 days per week, for 3 weeks (total of 15 applications). Five rabbits per group had the application site abraded, for the remaining rabbits, the application site was intact. Routine observations were performed for survival, clinical signs, body weight and food consumption; blood samples for haematology and clinical chemistry were taken before dosing and at termination. Gross and microscopic examinations were performed. This study complies with the main requirements of OECD test guideline 410, but was performed before GLP.

There were no deaths. Local skin lesions, which might have lead to enhanced absoption, were present at intact and abraded sites in all treated groups at much increased incidence and severity relative to controls. Clinical signs (tremor, dyspnoea, ataxia) were seen

from day 4 onwards at 1000 mg/kg bw per day and greater. Body-weight gain was reduced in males at the highest dose. In females, the impact on body weight was compounded by the wide intergroup variation in initial weights, but an approximate 300 g reduction in body weight at 5000 mg/kg bw per day, between days −3 and +1, is considered to be treatment-related. A range of clinical chemistry changes were seen, but most were without any clear pattern. Findings likely to be related to treatment included: increases in blood urea nitrogen concentration, bilirubin and urea in males at the highest dose; erythrocyte count and haemoglobin concentration were decreased by approximately 10% in females at the highest dose, γ-GT activity was increased in both sexes at 5000 mg/kg bw per day, and serum concentration of sodium, which normally shows minimal variation, was increased in males, but decreased in females at the highest dose. Liver weights were increased by approximately 25% in the groups receiving the highest dose. There were no gross or microscopic changes indicative of systemic toxicity (Sachsse et al., 1980a).

This study failed to determine a NOAEL for local effects. The NOAEL for systemic effects was 200 mg/kg bw per day (5 days per week) on the basis of clinical signs at 1000 mg/kg bw per day.

(c) Inhalation exposure

Rats

Groups of 20 male and 20 female RAIf rats were exposed to aerosols of propiconazole (purity, 91.9%; batch, P4-6) as an 80% solution in acetone, for 6 h per day, 5 days per week, for 13 weeks. Concentrations in the atmosphere were determined gravimetrically as 21, 85 or 191 mg/m^3 (0.021, 0.085, 0.191 mg/l) and the particle size was determined to be in the respirable range (80% of particles had a diameter of <7 μm); an air control group and an acetone control group were also used. Animals were observed routinely for deaths, clinical signs, body weight and food consumption. Samples of blood for haematology and clinical chemistry (fasted samples) examinations was taken from 10 rats of each sex per group in weeks 6 and 13 (within a 1-h period). Ophthalmoscopy examinations were performed pre-test and before sacrifice. Gross and microscopic examinations were performed. The study complied with the main elements of OECD guideline 413, but was performed before GLP.

Two deaths (one male in the control group and one female at the lowest dose) were not considered to be treatment-related. There were no clinical signs of toxicity. Body-weight gain was reduced consistently (15–20%, $p < 0.01$) in females at the highest dose; reductions in body-weight gains in females at the lowest dose and in males at the intermediate dose were not part of a dose–response relationship and were not considered to be a direct effect of propiconazole. There were no consistent changes in haematology parameters. Changes in a number of clinical chemistry parameters were seen, but only reductions in serum concentrations of glucose in all groups of females exhibited any degree of consistency. However, the values were within the normal physiological range and are not considered to be adverse. Liver weight was increased by approximately 10% in females exposed at 191 mg/m^3 (0.191 mg/l). There were no ophthalmic, gross or histopathological changes associated with exposure to propiconazole (Sachsse et al., 1979b).

The no-observed-adverse-effect concentration (NOAEC) was 85 mg/m^3 (0.85 mg/l; approximately 20 mg/kg bw per day) on the basis of reduced body weights in females at 191 mg/m^3 (0.191 mg/l).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 52 male and 52 female CD1 mice received diets containing propiconazole (purity, 92%; batch, P4-6) at a concentration of 0, 100, 500 or 2500 ppm for 104 weeks. Satellite groups of 12 male and 12 female mice were fed diets containing propiconazole at the same dietary concentrations and sacrificed after 53 weeks of treatment. Routine observations included clinical signs, mortality, body weight, food consumption, food efficiency and water consumption. Haematology, clinical chemistry and urine analysis were performed at 1 year and before sacrifice at 102–104 weeks. A gross examination was performed, a range of organs were weighed and a comprehensive histopathological examination performed. Results were analysed using a range of appropriate statistical tests.

Mean intakes of propiconazole were 10, 49 or 344 mg/kg bw per day in males and 11, 56 or 340 mg/kg bw per day in females at 100, 500 or 2500 ppm respectively. There were no abnormal clinical signs or increases in palpable masses associated with propiconazole. During the first 26 weeks, mortality was significantly increased in males at 2500 ppm. Overall survival was adequate for a study of carcinogenicity in mice: >50% at 92 weeks in all groups except males at the highest dose, for which survival dropped below 50% after week 84. At 2500 ppm, body-weight gain was reduced significantly throughout the study in male and female mice. Body-weight gain was reduced by approximately 10% in males at 100 and 500 ppm during weeks 1–13, but as this was not seen subsequently, nor in females, it was not considered to be biologically relevant. Food consumption was increased in males at the highest dose during the entire study and during the first 5 months in females. Erythrocyte volume fraction and haemoglobin concentration were decreased at 2500 ppm in males at 1 and 2 years and in females at 2 years. Aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activity were significantly increased in males at the highest dose by approximately twofold at week 52 and approximately fourfold at week 104, and to a lesser extent in females at the highest dose at week 52. Cholesterol concentrations were reduced in a statistically significant manner in males at the highest dose at week 52 and in females at the highest dose at termination. Slight, non-statistically significant effects on alanine aminotransferase and cholesterol were present at 500 ppm in males. No evidence of renal toxicity was evident in the results of urine analysis.

Liver weight was significantly increased in males and females at the highest dose and in males at the intermediate dose (Table 14). The gross examination revealed a higher incidence of liver masses and/or enlarged livers in males and females receiving propiconazole at a dietary concentration of 2500 ppm. The gross findings were confirmed by microscopic examination. In the group receiving the highest dose at 12 months, there was an increase in liver weight, hepatocellular necrosis and inflammatory cell infiltration (Table 14). After 24 months at 2500 ppm, there was an increase in hepatocellular enlargement and vacuolation and eosinic foci in males and females and pigmented Kupffer cells in males (Table 14). At 500 ppm, there was an increase in hepatocyte vacuolation in females, with increases in hepatocellular enlargement and eosinophilic foci in males (Table 14). An increase in the incidence of benign and malignant liver cell tumours was seen in males at 2500 ppm in both interim and main study groups. In an addendum to the original report (Hardisty, 1991), liver findings were re-examined according to contemporary criteria. The overall conclusions were unchanged after the re-evaluation and the results are presented in Table 14. There were no treatment-related effects on organs other than the liver (Hunter et al., 1982a).

Table 14. Histopathology results in mice given diets containing propiconazole for up to 104 weeks

	Dietary concentration (ppm)			
	0	100	500	2500
Interim group, 53 weeks:				
Males				
No. of animals examined	11	11	11	9
Liver weight (g)	2.3 ± 0.4	2.5 ± 0.4	2.8 ± 0.6*	4.4 ± 1.4*
Hepatocyte enlargement, mild/moderate	2	2	5	9*
Hepatocyte necrosis	0	0	0	4
Inflammatory cell infiltration	1	0	2	6
Hepatocellular adenoma only (No. of mice)	1	0	4	1
Hepatocellular carcinoma (No. of mice)	0	0	0	3
Females				
No. of animals examined	12	11	11	12
Liver weight (g)	1.7 ± 0.4	1.7 ± 0.2	1.7 ± 0.3	2.4 ± 0.5
Hepatocyte enlargement	0	0	0	7
Main group, 102 to 104 weeks:				
Males				
No. of animals examined	53	53	51	55
Liver weight (g)	3.2 ± 1.8	2.9 ± 1.2	3.4 ± 1.1	7.4 ± 2.8*
Hepatocyte enlargement, mild/moderate	8	3	16*	44*
Hepatocyte necrosis	5	2	4	3
Inflammatory cell infiltration	30	26	26	38
Basophilic foci	5	5	7	1
Eosinophilic foci	1	1	5*	6*
Pigmented Kupffer cells	7	8	8	37*
Hepatocyte vacuolation	7	5	7	19*
Hepatocellular adenoma only (No. of mice)	11	7	9	22*
Hepatocellular carcinoma (No. of mice)	16	9	13	22
Hepatocellualr adenoma & carcinoma combined	27	16	22	44**
Females				
No. of animals examined	52	53	53	52
Liver weight (g)	2.0 ± 1.0	1.9 ± 0.5	1.9 ± 0.3	3.0 ± 1.3*
Hepatocyte enlargement, mild/moderate	0	0	0	24*
Hepatocyte necrosis	3	5	2	2
Inflammatory cell infiltration	30	26	17	21
Basophilic foci	0	2	2	0
Eosinophilic foci	1	1	0	4
Hepatocyte vacuolation, mild/moderate	3	2	7	16*
Hepatocellular adenoma only (No. of mice)	5	0	2	6
Hepatocellular carcinoma (No. of mice)	1	1	0	3

From Hunter et al. (1982a)
*$p < 0.05$
**$p = 0.0013$; Fisher exact test

The NOAEL for non-neoplastic effects was 100 ppm, equal to 10 mg/kg bw per day, on the basis of hepatocellular lesions at 500 ppm. The no-observed-effect level (NOEL) for neoplasia was 500 ppm (equal to 49 mg/kg bw per day) on the basis of a significant ($p = 0.0013$) increase in the combined incidence of liver tumours at 2500 ppm in males.

Groups of 80 Crl:CD1 (ICR) BR male mice were given diets containing propiconazole (purity, 92.4%) at a concentration of 0, 100, 500 or 850 ppm for 18 months. Fifty animals per group were used for evaluation of oncogenic potential. An additional 10 animals per group were designated for blood chemistry investigations, scheduled at weeks—1, 9, 14, 53 and 79. Ten animals per group were used for interim sacrifice at weeks 9 and 53. Animals were observed routinely for mortality, clinical signs, body weight and food con-

sumption. Clinical chemistry investigations for cholesterol concentration, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and sorbitol dehydrogenase activities were performed on 10 males per group. Extensive gross examinations were performed and a wide range of tissues was preserved from all animals. Only samples of liver were examined histologically, but the examinations included an independent peer review. Results were analysed with a range of appropriate statistical tests. Statements of compliance with GLP were provided, but the study did not comply fully with OECD test guideline 451 owing to the limited histopathological examinations undertaken and poor survival.

Homogeneity and achieved intakes were satisfactory. The mean intakes of propiconazole were 11.0, 59 and 108 mg/kg bw per day in groups receiving dietary concentrations of 100, 500 and 850 ppm, respectively. Survival in the main group was relatively poor (30–40% at week 78), but was unaffected by propiconazole. No clinical signs nor behavioural changes indicative of a treatment-related effect were observed. Body-weight gain and food consumption were increased at 850 ppm during the first month, but were subsequently lower than those of the controls; body-weight gain was 15% lower than that of controls at 1 year. Slightly reduced body-weight gain (up to 10%) was evident at 500 ppm. Treatment-related decreases in plasma cholesterol concentrations were recorded throughout the study in animals receiving dietary concentrations of 500 (10–35%) and 850 ppm (20–40%); the decreases at weeks 9 and 14 were statistically significant in the group at 850 ppm. Sorbitol dehydrogenase activities were increased (by approximately 50%) in mice treated at 850 ppm at weeks 9 and 14. Mean liver weights were increased at 500 and 850 ppm (Table 15). Weights of other organs (brain, heart, kidney and testes) were not notably altered by consumption of propiconazole.

Treatment-related macroscopic findings of enlarged livers and more liver nodules/masses were seen at week 78 (Table 15). Evidence of liver changes was seen during histopathological examinations at all time-points. At week 9, fatty change was slightly increased at 100 ppm and greater, and hypertrophy was present in animals at 500 ppm and greater. Hypertrophy was the only clearly treatment-related finding at 500 ppm at 53 and 79 weeks. Animals at the highest dose had a broad pattern of hepatic effects, including hepatocellular adenomas (Table 15). The incidence of adenomas at 500 ppm (3 out of 50) is at the lower end of the control range of 6–18% reported in CD1 mice in the same test facility (Schaetti, 1999) and is not considered to be biologically significant; the incidence at 850 ppm was above that for historical and concurrent controls and was considered to be treatment-related (Gerspach, 1997).

The NOAEL for non-neoplastic effects was 100 ppm (equal to 11 mg/kg bw per day) on the basis of reduced cholesterol, reduced body-weight gain (10%) and increased liver weights/hypertrophy at 500 ppm. The fatty change noted at 9 weeks in 2 out of 10 animals at 100 ppm did not progress and was not considered to be biologically significant.

The NOAEL for tumours was 500 ppm (equal to 59 mg/kg bw per day) on the basis of a significant increase in the incidence of liver adenomas at 850 ppm.

In a follow-up investigation, preserved liver sections from animals in the control group and at the highest dose that were sacrificed at 9 weeks in the study by Gerspach (1997) were stained for proliferating cell nuclear antigen (PCNA). The results showed no difference between animals in the control group (labelling index, 0.05 ± 0.04%) and animals treated with propiconazole (0.06 ± 0.04%) (Weber, 1997).

Table 15. Liver findings in male mice given diets containing propiconazole for up to 79 weeks

Finding	Dietary concentration (ppm)			
	0	100	500	850
Liver weight (g)				
Week 9	2.25	2.12	2.50 (+11%)	2.98* (+32%)
Week 53	2.58	2.55	2.93 (+14%)	2.86* (+11%)
Week 79	2.58	2.58	2.93 (+14%)	3.07* (+19%)
Gross findings in week 79				
Enlarged liver	12/60	9/60	12/60	21/60
Liver mass	1/60	3/60	3/60	4/60
Liver nodule	0/60	0/60	0/60	1/60
Microscopic findings				
Week 9:				
Livers examined	10	10	10	10
Fatty change	0	2	2	9*
Hepatocellular hypertrophy	0	0	6*	10*
Necrosis	2	3	3	5
Necrosis monocellular	0	0	1	3
Lymphohistiocytic infiltration	2	2	1	6
Week 53:				
Livers examined	10	10	10	10
Hepatocellular hypertrophy	1	3	6*	8*
Necrosis	5	3	6	6
Hyperplasia, Kupffer cells	0	1	1	2
Amyloidosis	5	5	8	9
Week 79:				
Livers examined	50	50	50	50
Hepatocellular hypertrophy	15	18	28*	29*
Kupffer cell pigmentation	3	5	3	11*
Focus of cellular change	0	0	1	6*
Fatty change	21	14	15	13
Inflammatory cell infiltration	4	2	2	6
Hemangioma	0	0	1	1
Malignant lymphoma, systemic infiltration	0	1	1	1
Hepatocellular adenoma	1	0	3	10*
Hepatocellular carcinoma	1	3	2	2
Total hepatocellular tumours	2	3	5	12*

From Gerspach (1997)
*$p < 0.05$

Rats

Groups of CD Sprague-Dawley rats (50 of each sex per group for tumorigenic evaluation; 10 of each sex per group for interim sacrifice at 52 weeks; 10 of each sex per group for haematology; and 10 of each sex per group for blood chemistry and urine analysis) were fed diets containing propiconazole (purity, 91.9%; batch P4-60) at a concentration of 100, 500 or 2500 ppm for 107–109 weeks. Routine observations included clinical signs, mortality, body weight, food and water consumption. Haematology and urine analysis, clinical chemistry, ophthalmoscopy and hearing tests were performed at approximately 6, 12, 18 and 24 months; with an additional clinical chemistry analysis at 33 weeks. Extensive gross and microscopic examinations were performed (including special stains). Results were assessed using a range of appropriate statistical tests. The study complied with OECD guideline 453 and statements of compliance with GLP were provided.

Results of diet analyses are not reported, but the procedure used for preparation of diet was shown to be acceptable in other studies. Achieved intakes were 0, 3.6, 18 or 96 mg/kg bw per day in males and 0, 4.6, 23 or 131 mg/kg bw per day in females. Survival of the rats in groups treated with propiconazole, especially at the highest dose, was higher

Table 16. Findings in Sprague-Dawley rats given diets containing propiconazole for up to 2 years

	Dietary concentration (ppm)							
	0		100		500		2500	
	Males	Females	Males	Females	Males	Females	Males	Females
Body-weight gain (g)								
Weeks 0–26	419	189	418	188	409	175*	365*	139*
Weeks 26–52	113	81	118	75	108	78	83*	37*
Weeks 52–104	105	105	120	115	110	116	70*	79
Body weight (g)								
Week 78	794	495	806	496	802	482	701*	361*
Week 104	820	506	840	525	797	512	716*	395*
Food consumption: body-weight gain weeks 0–26[a] *Food intake (% controls)*	10.6	16.8	10.6	16.6	10.7	17.7	11.9	22.0
Weeks 1–26	100	100	99	99	99	98	98	95
Weeks 27–52	100	100	99	97	98	97	96	92
Haemoglobin (g %)								
Week 26	15.3	14.7	15.6	14.7	15.3	15.2	15.0	14.5
Week 52	16.1	16.1	16.7*	16.7*	16.3	16.3	16.0	16.0
Week 78	14.3	13.2	14.3	14.9*	13.9	14.8*	13.0*	13.6
Week 103	13.1	14.6 ± 0.4	14.5	14.2 ± 1.2	14.3	13.7 ± 1.2	14.0	12.8* ± 1.7
Erythrocyte count (10^6/mm^3)								
Week 26	8.8	7.6	8.9	7.6	8.5	8.0*	8.7	7.8
Week 52	7.5	6.3	7.6	6.2	7.6	6.3	7.8	6.5
Week 78	8.0	6.4	8.0	6.8	7.9	6.5	8.0	6.5
Week 103	6.2	7.0 ± 0.3	7.7*	6.8 ± 0.8	7.4*	6.6 ± 0.5	7.6*	6.5 ± 0.7
Chloride (mmol/l)								
Week 26	105	105	103*	101*	100*	100*	99*	103*
Week 52	103	101	101*	101	101	100*	101*	102
Week 78	101	100	101	100	101	99	102	98
Glucose (mg %)								
Week 26	—	130 ± 15	—	128 ± 15	—	108 ± 14*	—	109 ± 10*
Week 33	—	129 ± 12	—	137 ± 12	—	119 ± 15	—	120 ± 18
Week 52	—	111 ± 12	—	108 ± 14	—	102 ± 10	—	98 ± 16*
Week 78	—	131 ± 18	—	123 ± 13	—	114 ± 12*	—	111 ± 9*
Week 104	—	122 ± 15	—	120 ± 8	—	109 ± 19	—	114 ± 8
Albumin : globulin ratio								
Week 26	1.2	1.7	1.3*	1.4*	1.2	1.3*	1.3*	1.2*
Week 52	1.3	1.6	1.2	1.5	1.3	1.4*	1.3	1.3*
Week 78	1.2	1.4	1.1	1.3*	1.2	1.1*	1.2	1.1*
Exocrine pancreas atrophy Liver	—	1/59	—	3/61	—	6/62[#]	—	9*/65
No. of animals examined	64	67	67	69	66	67	65	67
Enlarged hepatocytes	2	1	0	2	2	2	5	13*
Benign liver tumours	2	1	1	1	2	0	2	2
Cholangioma	0	0	0	0	0	0	0	1
Dilatation of uterine lumen	—	4/58	—	10/63	—	9/63	—	17/65*

From Hunter et al. (1982b; 1985)
[a] The only time period calculated
[#] $p = 0.057$
* $p < 0.05$

than that in the control group, possibly because of lower body weight and decreased food consumption in treated animals. Overall survival was >50% in all groups of males at termination and in all groups of females treated with propiconazole. Body-weight gain and food consumption were decreased in females at the highest dose throughout the study and in males at the highest dose during the first year. At 500 ppm, growth and food efficiency was decreased slightly in females during the first 26 weeks (Table 16), but not subsequently and was not considered to be adverse. Urine analysis, ophthalmoscopic and hearing inves-

tigations did not identify any adverse effects. At 2500 ppm, numerous changes in haematology (erythrocyte parameters) and clinical chemistry were observed; some were also present at 500 and 100 ppm, although dose–response relationships were often absent, the direction of changes was reversed between the sexes (Table 16) and values were within normal ranges. At interim kill and termination, increased liver weight (by approximately 20%) was observed in males and females at 2500 ppm. At 52 weeks, relative weights of the heart and ovaries was increased in females at 2500 ppm.

Histopathological examination identified the liver as the primary target organ, with increases in the incidence of enlarged liver cells seen at 2500 ppm, which was statistically significant in females. Increased atrophy of the exocrine pancreas was evident in females at a dietary concentration of 500 ppm and greater. The overall incidence of tumours was increased in males, with no clear dose–response relationship or clear increases in specific tumours, this finding was possibly related to the increased survival in treated animals. Liver cell tumours were slightly higher in animals at the highest dose (Table 16), but were within normal background ranges and when combined with the increased survival are not considered to indicate a tumourigenic response to propiconazole. Reticulum cell tumours of the pancreas were present in three animals in the satellite groups, but were not seen in the main group and are thus not considered to indicate a carcinogenic response to propiconazole (Hunter et al., 1982b; 1985).

The NOAEL for tumours was 2500 ppm (96 mg/kg bw per day) the highest dose tested. Propiconazole is not carcinogenic to rats.

The NOAEL for non-neoplastic effects was 500 ppm (equal to 18 mg/kg bw per day) on the basis of increased luminal dilatation of the uterus in females and liver lesions and reduced body-weight gain in both sexes at 2500 ppm.

Dogs

No long-term studies in dogs were submitted

2.4 Genotoxicity

Propiconazole has been investigated in vitro for its ability to induce gene mutations in bacteria and mammalian cells, chromosomal aberrations in mammalian cells, and unscheduled DNA synthesis in mammalian cells. Propiconazole has also been tested in vivo in two studies of micronucleus induction and a study of dominant lethal mutation (Table 17). The highest concentrations used were justified on the basis of results of screening for toxicity/cytotoxicity. All studies gave negative results and complied with the OECD guidelines extant at the time of performance. The only study performed to GLP was the study of micronucleus induction in mice. Although the studies do not meet current OECD guidelines, the overall extent of the genotoxicity database is considered adequate.

A number of additional studies giving negative results were reviewed by the JMPR in 1987 (Annex 1, reference 52).

The Meeting concluded that propiconazole was unlikely to be genotoxic.

Table 17. Results of studies of genotoxicity with propiconazole

End-point	Test object	Concentration or dose	Purity (%) [Batch]	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, 100, 1535, 1537, 1538	20–5120 µg/plate ± S9; in DMSO	90.7 [103119]	Negative +S9 Negative –S9	Deparade & Arni (1983)
Gene mutation	Mouse lymphoma L5178Y $Tk^{+/-}$ cells	7.8–125 µg/ml ± S9; in DMSO	90.7 [103119]	Negative +S9 Negative –S9	Strasser & Muller (1982a)
Cell transformation	BALB 3T3 mouse embryo cells	1.2–18.5 µg/ml; in DMSO	90.7 [103119]	Negative	Strasser & Muller (1982b)
Chromosomal aberration	Human peripheral lymphocytes (one donor)	1–180 µg/ml ± S9; in DMSO	89.7 [P301064]	Negative +S9 Negative –S9	Strasser & Arni (1984)
Unscheduled DNA synthesis	Tif:RAIf rat hepatocytes	0.7–83 ng/ml; in DMSO	90.7 [103119]	Negative	Puri & Muller (1982)
In vivo					
Micronucleus formation	Bone marrow from Chinese hamsters (eight of each sex per group)	0, 307, 615, or 1230 mg/kg bw by gavage; in arachis oil	90.7 [103119]	Negative	Strasser & Arni (1987)
Micronucleus formation	Bone marrow from Ico:CD1 mice (five of each sex per group)	0, 80, 1600, or 3200 mg/kg bw by gavage; in arachis oil	92.4 [OP303011]	Negative	Deparade (1999)
Dominant lethal mutation	Tif:MAGf (SPF) mice (20 males)	0, 165, or 495 mg/kg bw by gavage; in methylcellulose)	90.7 [103119]	Negative	Hool & Müller (1979) Caresa (1988)

S9, 9000 × *g* supernatant from mouse liver cells induced with Aroclor 1254 or with propiconazole

2.5 Reproductive toxicity

(a) Multigeneration study

In a two-generation study (two litters per generation), groups of CD rats received diets containing propiconazole (purity, 89.7%; batch FL830377) at a concentration of 0, 100, 500 or 2500 ppm during pre-mating, gestation, lactation and weaning. Groups consisted of 15 males and 30 females. Dosing was initiated 12 weeks before mating for F_{1a} and F_{2a} litters. Animals were observed for mortality, clinical signs, body weight, food consumption, mating performance and reproductive outcome. All P_1 parental animals and 10 weanlings of each sex per group received a gross examination, brain and reproductive organs were weighed, and liver and reproductive organs were examined histopathologically. The study complied with OECD guideline 416 (1983) and statements of compliance with GLP were provided.

Dietary analyses showed satisfactory levels of incorporation, and mean intakes of propiconazole during the study were approximately 10, 50 or 250 mg/kg bw per day. There were no signs of toxicity in P_0 or P_1 males other than a slight (approximately 10%) reduction in body-weight gain at 2500 ppm. Females at the highest dose exhibited reduced body-weight gain (approximately 20%) and reduced food consumption during most stages of the study. Females at the intermediate dose showed reductions in body-weight gain (approximately 10%; $p < 0.01$). Mating performance, fertility and duration of gestation were similar in all groups, although the degree of variation in values for controls hindered comparisons between matings. Lower weights of pups at day 21 were often associated with larger litter sizes in the F_{1a} and F_{1b} matings, but total litter weights were lower at 2500 ppm (Table 18). In the second generation, both pup weight and litter size were reduced at 2500 ppm, and in the F_{2b} litters, pup weights were also reduced ($p < 0.01$) at 500 ppm. The main effect on F_2 litter size was poor survival during lactation. Organ weights such as brain and testes/epididymides showed no consistent pattern between matings, with reductions in absolute values or increases in relative values being secondary to body-weight changes. There were no

Table 18. Litter findings (means) in a multigeneration study in rats given diets containing propiconazole

Finding	Dietary concentration (ppm)			
	0	100	500	2500
F_{1a} day 21				
Pup weight (g)	47	43	46	35*
No. of pups	6.8	7.2	7.4	7.4
Litter weight (g)	320	310	340	260
F_{1b} day 21				
Pup weight (g)	42	44	45	34**
No. of pups	6.3	7.7	7.7	7.2
Litter weight (g)	264	339	346	245
F_{2a} day 21				
Pup weight (g)	42	42	41	31**
No. of pups	7.8	7.1	7.0	6.2**
Litter weight (g)	328	298	287*	192
Viable F2a pups				
Day 0	12.5	13.0	10.8	9.2**
Day 4	7.9	7.8	7.5	6.7**
Day 14	7.8	7.2	7.0	6.2**
F_{2b} day 21				
Pup weight (g)	47	47	43**	35**
No. of pups	7.7	8.0	7.8	5.7**
Litter weight (g)	362	376	335	200
Viable F_{2b} pups				
Day 0	12.8	13.4	12.7	11.3
Day 4	7.8	8.0	7.8	7.3
Day 14	7.7	8.0	7.8	5.7*

From Borders et al. (1985)
*$p < 0.05$
**$p < 0.01$

treatment-related gross pathology findings. Histopathological examination of the reproductive organs found no notable effects of treatment. Treatment-related changes (hepatocellular swelling and clear-cell changes) were found in the livers of both parental and weanling animals at 2500 ppm and in parents from groups at 500 ppm.

The NOAEL for parental toxicity was 100 ppm (equivalent to 7 mg/kg bw per day) on the basis of reduced body-weight gain in dams and hepatotoxicity at 500 ppm.

The NOAEL for reproductive outcome was 500 ppm (equivalent to 35 mg/kg bw per day) on the basis of reduced pup survival at 2500 ppm in the F_2 generation.

The NOAEL for offspring toxicity was 100 ppm (equivalent to 7 mg/kg bw per day) on the basis of reduced pup body weights at day 21 in the F_{2b} litters at 500 ppm (Borders et al., 1985).

(b) Developmental toxicity

Rats

Groups of 25 mated female Tif:RaIf rats were given propiconazole (purity, 91.9%; batch P4-6) at a dose of 0, 30, 100 or 300 mg/kg bw per day by gavage in 2% carboxymethyl cellulose on days 6–15 of gestation. One third of the fetuses were evaluated by Wilson sectioning, the rest were stained for skeletal investigation. Three dams in the group receiving

Table 19. Findings in rats given propiconazole by gavage during organogenesis

Parameter	Dose (mg/kg bw per day)							
	0		30		90		360/300	
Body-weight gain (g)								
Days 6–8	7 ± 4		4 ± 5		3 ± 5*		3 ± 5*	
Days 6–16	47 ± 8		44 ± 10		46 ± 7		40 ± 11*	
Food consumption (g)								
Days 0–6	19		21		21		20	
Days 6–7	21		22		19		19	
Days 7–8	21		21		20		17*	
Days 8–9	22		22		20*		19*	
Days 9–10	22		23		21		17*	
Maternal body weight (g)								
Day 6	285		283		292		285	
Day 8	292		287		295		288	
Day 16	331		328		333		326	
Day 20[a]	313		303		315		308	
Litter parameters								
Live fetuses per litter	12.3		13.5		13.7		13.0	
Fetal weight (g)	3.5		3.4		3.4		3.4	
% male fetuses	52		49		48		46	
Fetal findings (fetuses/litters)								
Cleft palate	0	0/22	0	0/21	1	1/22	2	2/22
Cleft/hare lip	0	0/22	0	0/21	2	2/22	0	0/22
Renal papilla short	32/14 1	16/22	27/148	9/20	40/156	18/22	57/148*	19/22
Renal papilla missing	4	4	4	3	8	3	16*	12
Dilated ureter	38	16	21	8	38	16	63*	20
Rudimentary ribs	0/129	0/22	1/136	1/21	4/146*	4/22*	53/137**	16/22**
Sternebrae not ossified	49		54		83*		99**	

Marcsisin et al. (1987)
[a] Minus uterine contents
* $p < 0.05$
** $p < 0.01$

the highest dose died. No malformations were recorded at 300 mg/kg bw per day, but two fetuses at the intermediate dose had malformations. No cleft palates were recorded in the study. Delayed ossification, particularly of the phalangeal nuclei and calcaneum was seen at the highest dose. The level of detail in the report was insufficient to permit a NOAEL to be derived (Fritz, 1979).

Groups of 24 mated female Crl:COBS CD(SD)BR VAF/Plus rats were given propiconazole (purity, 92.1%; batch FL850083) at a dose of 0, 30, 90 or 360/300 mg/kg bw per day by gavage in aqueous 3% corn starch + 0.5% Tween® on days 6–15 of gestation. The highest dose was reduced from 360 to 300 mg/kg bw per day after three to five doses owing to maternal toxicity. Dams were sacrificed on day 20 of gestation and fetuses were examined. Approximately half of the fetuses in each litter were examined for visceral abnormalities, the remainder were examined for skeletal abnormalities after staining with alizarin red. This study complied with OECD guideline 414 (1981) and statements of compliance with GLP were provided.

One control female died due to complications associated with an early birth. Severe compound-related maternal toxicity (lethargy, ataxia, salivation) was observed at the highest dose during the first five days of dosing at 360 mg/kg bw per day. Dams from the group at the highest dose also exhibited reduced body-weight gain (Table 19) at 360 mg/kg bw per

day. The reduced body-weight gain persisted after the dose was reduced to 300 mg/kg bw per day. Reduced body-weight gain was also noted at the intermediate dose during the first days of dosing, but terminal body weights were similar to those of controls. There were no effects on litter sizes, fetal viability, litter size, pup weight or sex ratio. One fetus at the intermediate dose had multiple malformations (cleft lip and palate, micromelia and club foot), which were not considered to be clearly related to treatment. At the highest dose, one fetus had multiple malformations (anasarca, cleft palate, hydromelia and protruding tongue), another fetus at the highest dose had cleft palate. Cleft palate is very rare, but not unknown, in CD rats; incidence in controls ranged from 0% to 0.3%. Evidence of delayed development of the urinary system was seen at the highest and intermediate doses, together with an increase in rudimentary ribs and unossified sternebrae (Table 19). It is uncertain if these findings were secondary to maternal toxicity as fetal weights were similar in all groups.

The NOAEL for maternal toxicity was 90 mg/kg bw per day on the basis of severe signs of toxicity at 300 mg/kg bw per day. Although the maternal body-weight gains were significantly decreased at 90 mg/kg bw per day during days 6–8 of gestation only, this effect was considered to be temporary and secondary to reduced food consumption.

The NOAEL for developmental toxicity was 30 mg/kg bw per day on the basis of reduced ossification and the presence of rudimentary ribs at 90 mg/kg bw per day (Marcsisin et al., 1987).

In a study to investigate the findings of cleft palate reported by Marcsisin et al. (1987), mated female Crl:COBS CD(SD)BR VAF/Plus rats (178 controls; 189 test) were given propiconazole (purity, 92.1%; batch FL850083) at a dose of 0 or 300 mg/kg bw per day by gavage in aqueous 3% corn starch + 0.5% Tween® on days 6–15 of gestation. Dams were sacrificed on day 20 of gestation and the uterine contents were examined. Fetal examinations focused primarily on the palate. Statements of compliance with GLP were provided.

Three animals from the group treated with propiconazole were found dead, and another was sacrificed owing to problems associated with early delivery. Clinical signs

Table 20. Findings in rats given propiconazole by gavage during organogenesis

Parameter	Dose (mg/kg bw per day)			
	0		300	
Body-weight gain (g)				
Days 6–8	9.3 ± 6.1		–2.7 ± 8.8*	
Days 6–16	61 ± 13		41 ± 13*	
Days 16–20	58 ± 13		63 ± 16*	
Litter parameters				
Live fetuses per litter	13.7 ± 3.2		13.1 ± 3.4*	
Fetal weight (g)	3.5		3.3*	
% male fetuses	49		50	
Fetal findings (fetuses/litters)				
Cleft palate	0/2122	0/155	2/2064	2/158

From Mallows (1987)
*$p < 0.05$
**$p < 0.01$

including ataxia, coma, lethargy, abnormal breathing and ptosis were present in animals treated with propiconazole. Food consumption and body-weight gain were reduced during dosing with propiconazole, but partially recovered on cessation of dosing (Table 20). There were no unusual findings at the necropsy examination. Fetal weight and litter size were reduced in the group receiving propiconazole (Table 20). Two fetuses (from different litters) with cleft palate were seen out of 2064 fetuses from dams treated with propiconazole (0.1%), versus zero out of 2122 fetuses in the control group.

This study was not designed to determine a NOAEL.

Cleft palate is a very rare but occasional finding in rats in control groups and there are published data (Khera, 1985) that indicate that testing compounds at maternally toxic doses is associated in some way with the induction of a number of malformations, including cleft palate. The low incidences seen in these two studies cannot be discounted as being related to treatment with propiconazole, but it is considered that they are unlikely to be seen in the absence of maternal toxicity.

Rabbits

Groups of 19 inseminated, female New Zealand White rabbits were given propiconazole (purity, 92.1%; batch FL850083) at a dose of 0, 100, 250 or 400 mg/kg bw per day by gavage in aqueous 3% corn starch + 0.5% Tween® on days 7–19 of gestation. Dams were observed for clinical signs, mortality, body weight and food consumption, and sacrificed on Day 29 of gestation; fetuses were examined after caesarean section. All fetuses were examined for visceral abnormalities by dissection and for skeletal abnormalities after staining with alizarin red. Owing to processing errors, a large number of fetuses (20–40%) were damaged (disarticulated) before skeletal examinations; the damaged fetuses were examined by experienced technicians for evidence of skeletal abnormalities. Overall, the number of fetuses examined in this study is considered to be acceptable. This study complied with OECD guideline 414 (1981) and statements of compliance with GLP were provided.

Two does (one at the lowest dose and one at the intermediate dose) were found dead. One doe in the control group, one at the intermediate dose and five at the highest dose were sacrificed having aborted or delivered early. Maternal toxicity was evident at 400 mg/kg bw per day (reduced food consumption, body-weight loss; abnormal stools; abortion/early delivery) and to a lesser extent at 250 mg/kg bw per day (reduced feed consumption and body-weight loss) (Table 21). Slight, but consistent, reductions in food consumption and body-weight gain were evident at 100 mg/kg bw per day, but were not associated with any adverse effects on fetuses. Food consumption and body-weight gain in groups receiving propiconazole were greater than those of controls after cessation of dosing (Table 21). There were no necropsy findings indicating treatment-related effects. One animal at the highest dose resorbed an entire litter. In does with viable fetuses, litter sizes and fetal weights were similar in all groups (Table 21). One fetus at the intermediate dose had a number of abnormalities. The only treatment-related finding was an increased incidence of fully formed thirteenth ribs at 400 mg/kg bw per day (Table 21). There was also an increase in the number of resorptions at 400 mg/kg bw per day that was associated with resorption of an entire litter by one dam.

The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of body-weight loss at 250 mg/kg bw per day; the slight effects on food consumption and body-weight gain at 100 mg/kg bw per day were not considered to be adverse.

Table 21. Summary of findings of a study of developmental toxicity in rabbits given propiconazole by gavage

Finding	Dose (mg/kg bw per day)			
	0	100	250	400
Food consumption (g)				
Days 7–8	183 ± 24	162 ± 21	136 ± 32*	79 ± 31*
Days 14–15	164 ± 48	121 ± 63	95 ± 60*	69 ± 45*
Day 26–27	90 ± 45	107 ± 37	104 ± 45	151 ± 48*
Body-weight gain (g)				
Days 7–10	18 ± 3	5 ± 3	–47 ± 5*	–111 ± 8*
Day 20–24	46 ± 8	94 ± 4	118 ± 7*	159 ± 6*
Pregnant	15	18	17	18
Litter parameters				
Viable litters	14	17	15	12*
Litter size	7.2	8.6	8.7	7.2
Resorptions	0.7	0.7	0.7	2.1*
Fetus weight (g); male/female	43.0/44.2	44.4/43.1	42.8/41.1	42.8/43.2
% live males/litter	55	49	50	41
Fetal findings—fetal incidence (%)				
Fully formed thirteenth rib	35/101 (35)	63/146 (43)	58/130 (41)	63/93* (68)

From Raab et al. (1986)
* $p < 0.05$

The NOAEL for developmental toxicity was 250 mg/kg bw per day on the basis of increases in fully formed thirteenth ribs and abortions at 400 mg/kg bw per day. The NOAEL for teratogenicity was 400 mg/kg bw per day, the highest dose tested (Raab et al., 1986).

2.6 Special studies

(a) Neurotoxicity

(i) Acute oral neurotoxicity in rats

No studies of acute neurotoxicity were submitted; however, no evidence of neurotoxicity was apparent in any of the available studies.

(ii) Short-term study of oral neurotoxicity

No short-term studies of neurotoxicity were submitted; however, no evidence of neurotoxicity was apparent in any of the available studies.

(b) Assay for initiation/promotion of liver foci

After finding liver tumours in mice, a study was performed to investigate the effect of propiconazole (purity, 89.7%; batch 301064) on focal proliferative changes in the livers of young rats. Groups of 45 male and 45 female Tif:RAIf rats aged 1 day were given *N*-diethylnitrosamine (DEN) at a dose of 15 mg/kg bw by intraperitoneal administration or 0.5 ml of saline by intraperitoneal administration. After weaning on day 22, animals were given control diet or diets containing phenobarbitone at 500 ppm or propiconazole at 2000 ppm. Subgroups of five animals of each sex were sacrificed after 14, 28 or 56 days of treatment and the livers were examined using periodic acid Schiff, haematoxylin/eosin or histochemical staining for γ-glutamyl transpeptidase-positive foci. Treatment with propiconazole or phenobarbital ± DEN produced significant increases (15–30%) in liver weights. Numbers of γ-glutamyltranspeptidase-positive foci were increased by treatment with phenobarbital or propiconazole, with the increases being more marked after an initi-

Table 22. Number of γ-glutamyl transferase-positive foci in livers from rats treated with propiconazole or phenobarbital

Group	Days of treatment					
	14 days		28 days		56 days	
	Male	Female	Male	Female	Male	Female
Saline + control diet	0	14	1	15	0	38
Saline + 500 ppm phenobarbital	0	0	53	69	11	34
Saline + 2000 ppm propiconazole	0	143	142	165	422	244
N-diethylnitrosamine + control diet	55	179	103	71	121	198
N-diethylnitrosamine + 500 ppm phenobarbital	493	612	367	284	613	660
N-diethylnitrosamine + 2000 ppm propiconazole	552	488	382	516	1178	1189

From Fröhlich et al. (1984)

ating exposure to DEN (Table 22). The variable background rate in females confounds any comparisons of the relative responses in the two sexes. The size of the foci was unaffected by treatment with phenobarbital or propiconazole. The results indicate that propiconazole acts in a similar manner to phenobarbital (Fröhlich et al., 1984).

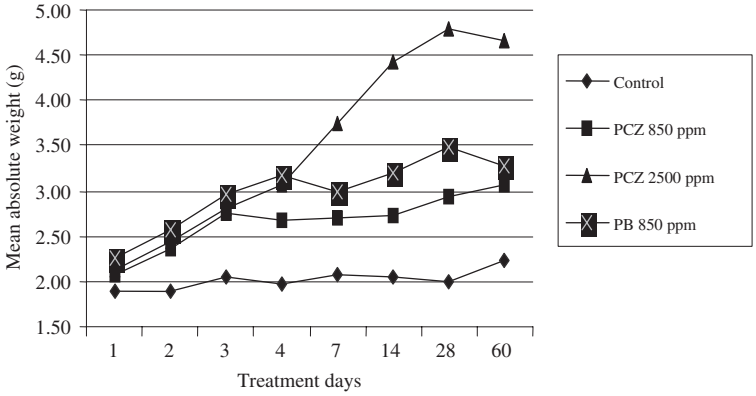
(c) Induction of liver enzymes and cell proliferation

Propiconazole belongs to a class of conazole pesticides, which have complex effects on the hepatic and non-hepatic microsomal monooxygenase systems in vertebrate species. They can act as both inducers and inhibitors of cytochrome P450, depending on the tissue and the specific conazole. A number of studies have been performed to investigate the pattern of microsomal enzyme induction produced by propiconazole.

The pattern of xenobiotic-metabolizing enzymes induced by propiconazole was investigated in a study performed in 1984. Groups of male RAI rats and MAG mice (six in the test groups and eight in the control group) were given propiconazole (purity, 90.7%) at a dose of 0, 20, 80, 160 or 320 mg/kg bw per day by gavage in 2% carboxymethyl cellulose for 2 weeks. After a 24-h fast, the animals were sacrificed and the liver cytosol and microsomal preparations investigated for enzyme activities, protein, nucleic acid and phospholipid contents. Samples from two animals in the control group and two animals at the highest dose were studied by electron microscopy. Animals reciving propiconazole at a dose of 20 mg/kg bw per day showed increases in liver weight. At 80 mg/kg bw per day and above, there were increases in microsomal protein, phospholipid and cytochrome P450 content and activities of ethoxycoumarin deethylase, epoxide hydrolase, UDP-glucuronyltransferase and γ-GT in both rats and mice (Table 23). Cytosolic protein (in mice) and glutathione *S*-transferase (rats and mice) were increased at 160 mg/kg bw per day and above (Table 23). A marked proliferation of smooth endoplasmic reticulum was seen in samples from the animals at 320 mg/kg bw per day (Waechter et al., 1984).

In a study designed to characterize the extent and time-dependency of hepatocyte proliferation and investigate a possible mechanism for the tumorigenic effect observed, groups of five CD1 male mice were given diets containing propiconazole (purity, 92.4%) at a concentration of 0, 850 or 2500 ppm. To examine hepatocyte cell proliferation, each animal received a single intraperitoneal injection of bromodeoxyuridine (BRDU) at a dose of 100 mg/kg bw 2 h before sacrifice. Cell proliferation was assessed by BRDU-immunohistochemistry/image analysis after treatment for 1, 2, 3, 4, 7, 14, 28 or 60 days. Mice treated with phenobarbital (purity, 99%), a known mouse mitogen, at a concentration of 850 ppm were investigated as for those treated with propiconazole.

Figure 1. Mean absolute weight of the liver in mice given diets containing propiconazole or phenobarbital for up to 60 days



From Weber (1999)
PCZ, phenobarbital; PB, phenobarbital

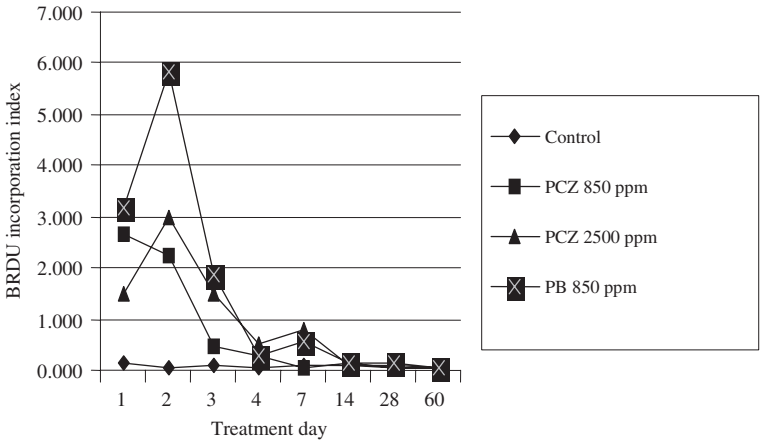
Table 23. Biochemical findings in livers from animals given propiconazole by gavage for 14 days

Finding	Dose (mg/kg bw per day)				
	0	20	80	160	320
Rats					
Liver weight (g)	3.1	3.5*	3.3*	4.1**	4.7**
DNA (mg)	31	NP	NP	NP	36*
Cytosolic protein (mg/g)	66	58	69		
Microsomal protein (mg/g)	9	9	14**	13**	17**
Microsomal phospholipid (mg/g)	6	5	8**	9**	12**
Cytochrome P450 (nmol/g)	12	11	22**	28**	40**
Ethoxycoumarin deethylase (nmol/min per g)	5	4	9**	17**	31**
Epoxide hydrolase (nmol/min per g)	154	132	300**	579**	852**
UDP-glucuronyltransferase (µmol/min per g)	1	1	2**	3**	4**
γ-GT (nmol/min per g)	18	22	24*	17	41**
Glutathione *S*-transferase (µmol/min per g)	87	77*	99	175**	269**
Mice					
Liver weight (g)	4.7	5.2*	5.1*	6.0**	6.8**
DNA (mg)	5	NP	NP	NP	7**
Cytosolic protein (mg/g)	62	59	67	79**	71
Microsomal protein (mg/g)	11	9	12	18**	21**
Microsomal phospholipid (mg/g)	7	7	9	12**	14**
Cytochrome P450 (nmol/g)	16	15	28*	49**	63**
Ethoxycoumarin deethylase (nmol/min per g)	19	24	23	31**	55**
Epoxide hydrolase (nmol/min per g)	63	51	71	160**	212**
UDP-glucuronyltransferase (µmol/min per g)	619	404**	664	890**	1080**
Glutathione *S*-transferase (µmol/min per g)	290	301	380*	421**	422**

From Waechter et al. (1984)
γ-GT, γ-glutamyl transeferase; NP, not performed
* $p < 0.05$
** $p < 0.01$

Dietary incorporation was acceptable, and mean intakes were 127 or 353 mg/kg bw per day for propiconazole and 139 mg/kg bw per day for phenobarbital. No deaths occurred during the study and no treatment-related clinical signs were reported. Body-weight development and food consumption was suppressed during the first days of treatment at 2500 ppm. No effects on body-weight development, food consumption or body weights were noted in groups treated with propiconazole or phenobarbital at 850 ppm. Time- and dose-related increases in absolute and relative weights of the liver were observed in animals treated with propiconazole (Figure 1).

Figure 2. Bromodeoxyuridine incorporation index in mouse hepatocytes after exposure to propiconazole or phenobarbital



From Weber (1999)
PCZ, phenobarbital; PB, phenobarbital

Enlarged and/or speckled livers were found at macroscopic examination in all groups treated with propiconazole or phenobarbital. One hepatic nodule was found in each of the groups treated with propiconazole at a high or low dose. Hepatocellular hypertrophy was seen microscopically in animals treated with propiconazole: the mean severity, increasing with time, ranged from minimal (1 day of treatment) to moderate/marked (28 and 60 days of treatment). Treatment with phenobarbital led to a progression of hepatocellular hypertrophy similar to that observed in the group treated with propiconazole at 2500 ppm. The hypertrophy caused by propiconazole affected all lobular compartments of the liver, but was predominantly centrilobular, while phenobarbital almost exclusively affected centrilobular or midzonal hepatocytes. Hepatocellular necrosis was present in all groups, showing a dose-related increase in incidence and severity in the groups treated with propiconazole. Panlobular hepatocyte vacuolation (minimal) was observed in some animals from all treatment groups at the begining of the study. Centrilobular vacuolation was observed towards the end of the treatment with a high incidence (almost all animals) and severity (minimal to marked) from day 7 to 60 in animals treated with propiconazole at 2500 ppm, but only sporadically in animals treated with propiconazole or phenobarbital at 850 ppm.

Mitotic activity and incorporation of BRDU was increased markedly in most treated animals during days 1–4 of treatment, returning to control values by day 14 (Fig. 2). The increased incorporation of BRDU was primarily in the centrilobular/midzonal hepatocytes. The magnitude of this response was lower with propiconazole at 2500 ppm than with phenobarbital at 850 ppm, contrary to the results for liver weight (Fig. 1), indicating that a short mitogenic response is not the sole mechanism underlying the hepatocellular proliferative effects of propiconazole.

This study showed propiconazole to be a mouse liver mitogen with a degree of similarity to phenobarbital, but of a lower potency in terms of BRDU incorporation (Weber, 1999).

Groups of six male Crl:CD1(ICR)BR mice aged 8 weeks were given diets containing propiconazole (purity, 92.4%; batch No. OP.303011) at a concentration of 0, 850 or 2500 ppm (equal to 0, 149 and 578 mg/kg bw per day) for 14 days. A further group of six

mice was treated with phenobarbital at 850 ppm (equal to 145 mg/kg bw per day). Body weights and food consumption were measured daily and any clinical signs were recorded. After 14 days, the mice were killed and their carcass weights recorded after bleeding, and they were examined macroscopically. The livers of all animals were removed, weighed and stored in liquid nitrogen. The liver samples were separated into microsomal and cytosolic fractions and the following biochemical parameters were investigated: protein content of microsomal and cytosolic fractions; microsomal cytochrome P450; microsomal 7-ethoxyresorufin-*O*-dealkylase activity (EROD); 7-pentoxyresorufin-*O*-dealkylase activity (PROD); microsomal coumarin 7-hydroxylase activity; regio- and stereoselective microsomal hydroxylation of testosterone; microsomal lauric acid 11- and 12-hydroxylation; microsomal UDP-glucuronosyltransferase activity; cystolic glutathione *S*-transferase activity and microsomal epoxide hydrolase activity.

There were no deaths or clinical signs of toxicity. Body weight was similar in all groups. Absolute weights of the liver were increased by 40–100% in all treatment groups (Table 24). A dose-related induction of specific microsomal enzyme activities was seen with a corresponding increase in total protein content (Table 24) in the animals treated with propiconazole. The pattern of enzyme induction produced by propiconazole was similar, but not

Table 24. Induction of microsomal enzymes in male mice given diets containing propiconazole or phenobarbital for 14 days (% of control values)

Parameter	Dietary concentration (ppm)		
	Phenobarbital	Propiconazole	
	850	850	2500
Absolute liver weight	163	140	199
Microsomal cytochrome P450 content	239	300	389
Microsomal pentoxyresorufin *O*-depentylase	3534	3024	5524
Microsomal coumarin 7-hydroxylase	480	534	2384
Microsomal testosterone 2β-hydroxylation	466	298	531
Microsomal testosterone 6α-hydroxylation	779	502	715
Microsomal testosterone 6β-hydroxylation	500	366	524
Microsomal testosterone 15β-hydroxylation	526	316	977
Microsomal testosterone 16β-hydroxylation	5.7[a]	5.2[a]	6.4[a]
Microsomal testosterone oxidation to androstenedione	179	589[b]	652[b]
Microsomal testosterone 2α-hydroxylation	0[a]	2.7[a,b]	2.7[a,b]
Total microsomal testosterone oxidation	356	440	555
Microsomal epoxide hydrolase[d]	179	172	321
Immunoblot goat anti-rat CYP2B1 (Cyp2b) Band 1	2743	2608	3049
Immunoblot goat anti-rat CYP2B1 (Cyp2b) Band 2	581	810	579
Immunoblot goat anti-rat CYP2B1 (Cyp2b) Band 3	351	439	617
Immunoblot mAb p6 (Cyp3a)	577	658	1068
Microsomal ethoxyresorufin *O*-deethylase[c]	232	219	388
Microsomal testosterone 16α-hydroxylation	194	214	262
Microsomal lauric acid 11-hydroxylase	271	267	305
Microsomal lauric acid 12-hydroxylase[d]	163	153	161
Microsomal UDP-glucuronosyltransferase	156	156	139
Cytosolic glutathione *S*-transferase	187	158	184
Microsomal testosterone 7α-hydroxylation	229	175	125
Microsomal protein content	97	109	113
Cytosolic protein content	92	97	94
Immunoblot mAb d15 (Cyp1a)[c]	86	124	89
Immunoblot mAb clo4 (Cyp4a)[d]	129	140	157

From Beilstein (1998)

[a] Absolute values, control value below limit of detection

[b] Notable difference between phenobarbital and propiconazole

[c] Indicative of aromatic hydrocarbon response

[d] Indicative of peroxisomal proliferation

identical, to that produced by phenobarbital. The main differences were in the induction of testosterone oxidation to androstenedione and of testosterone 2α-hydroxylation that were induced by propiconazole but not phenobarbital (Table 24). Within the range of microsomal enzyme parameters measured, the overall response to propiconazole is more consistent with a phenobarbital-type mechanism than peroxisome proliferation or aromatic hydrocarbon mechanism. However, mechanisms other than one similar to that used by phenobarbital cannot be conclusively excluded (Beilstein, 1998).

(d) Studies with metabolites

No studies were submitted. Triazolyl acetic acid and triazolyl alanine are produced from propiconazole by plants, but not by mammals. Triazolyl alanine was reviewed by the JMPR in 1989. Triazolyl acetic acid and triazolyl alanine were considered by the 2004 JMPR during the evaluation of triademefon and triademenol.

3. Observations in humans

Medical surveillance of employees in production, formulation and packaging plants from 1982 to April 2000 revealed four cases (out of a total of 139 individuals) of local skin reactions. All four cases occurred during handling of formulations. It cannot be excluded that formulation ingredients may have contributed to the effects described. The occasional occurrence of local effects during handling of formulations of propiconazole is accordance with other reported cases described in the literature. The consistent finding is of local irritation to the skin or mucosa; as all cases involved the formulated propiconazole product, the reactions could have been associated with constituents other than propiconazole. No cases of serious poisoning with propiconazole or formulations containing propiconazole have been reported to Syngenta or found in the public literature (Jaquet, 1991).

An epicutaneous test with technical-grade propiconazole in 20 human volunteers was conducted in 1991 at the University of Göttingen in Germany. No evidence of sensitization or dermal irritation was reported (Fuchs, 1991).

Comments

After oral administration of radiolabelled propiconazole to rats and mice, the radiolabel is rapidly (C_{max} at 1 h) and extensively (>80% of the administered dose) absorbed and widely distributed, with the highest concentrations being found in the liver and kidney. Excretion of the radiolabel is rapid (80% in 24 h) with significant amounts being found in the urine (39–81%) and the faeces (20–50%), the proportions varying with dose, species and sex. There is a significant degree of biliary excretion and subsequent enterohepatic recirculation. There was no evidence for bioaccumulation with tissue or carcass residues being typically <1% of the administered dose 6 days after dosing. Propiconazole is extensively metabolized and <5% of the dose remains as parent compound; however, many metabolites have not been identified. The primary metabolic steps involve oxidation of the propyl sidechain on the dioxolane ring to give hydroxy or carboxylic acid derivatives. Hydroxylation of the chlorophenyl and triazole rings followed by conjugation with sulfate or glucuronide was also detected. There is evidence for only limited cleavage between the triazole and chlorophenyl rings. The extent of cleavage of the dioxolane ring was significantly different according to species and sex, representing about 60% of urinary radioactivity in male mice, 30% in female mice and 10–30% in male rats. In rats, propiconazole is readily absorbed after dermal application (about 30% within 10 h).

Propiconazole has moderate acute oral toxicity in rats and mice (LD_{50}s, about 1500 mg/kg bw) and low acute dermal (LD_{50}s, >4000 mg/kg bw) and inhalation toxicity (LC_{50}, >5 mg/l of air). Propiconazole is not an eye irritant in rabbits, but is irritating to rabbit skin and is a skin sensitizer in guinea-pigs in the Magnusson & Kligman test.

Decreased body-weight gain was seen in short- and long-term studies of toxicity and studies of developmental and reproductive toxicity, and was often linked with reduced food consumption. In studies of repeated doses, liver was the primary target organ for toxicity attributable to propiconazole. In rats, erythrocyte parameters were reduced and a range of clinical chemistry changes were seen, however, with the exception of reduced chloride and cholesterol concentrations, there was no consistent pattern between sexes and studies, and results were generally within the physiological range.

In two studies, mice given diets containing propiconazole at ≥850 ppm for up to 17 weeks had increases in liver weight, reduced concentrations of serum cholesterol and increased hepatocyte hypertrophy, vacuolation and necrosis. The findings were present after 4 weeks and did not progress with increased duration of dosing. The NOAEL was 500 ppm (equal to 65–85 mg/kg bw per day) in both studies.

Rats given propiconazole at 450 mg/kg bw per day for 28 days by gavage exhibited a range of effects. Males had reductions in body-weight gain, while females had clinical signs of toxicity and reductions in erythrocyte parameters. Both sexes had increased liver weights and hepatocyte hypertrophy, with hepatocyte necrosis also being seen in females. Increases in liver weight with hepatocyte hypertrophy were seen at 150 mg/kg bw per day, but these effects were not considered to be adverse and the NOAEL was this dose. In a 13-week dietary study in rats, reductions in body-weight gain, increased relative liver weight and increased γ-glutamyltranspeptidase activity was seen in both sexes at 6000 ppm. In females, erythrocyte parameters were reduced at this dose. The NOAEL was 1200 ppm (equal to 76 mg/kg bw per day).

Dogs appeared to be sensitive to the local effects of propiconazole as manifested by gastrointestinal tract irritation at ≥8.4 mg/kg bw per day; the NOAELs were 250 ppm (equal to 6.9 mg/kg bw per day) after 90 days and 1.9 mg/kg bw per day after 1 year. No systemic effects were seen in dogs receiving a dose of 8.4 mg/kg bw per day for 1 year or 1250 ppm (equal to 35 mg/kg bw per day) for 90 days, the highest doses tested.

In a 3-week (five applications per week) study of dermal toxicity in rabbits, tremors, dyspnoea and ataxia were increased at ≥1000 mg/kg bw per day. The NOAEL was 200 mg/kg bw per day. In a 13-week (5 days per week; 6 h per day) study in rats treated by inhalation, reduced body-weight gain was seen in females at 0.19 mg/l of air; the NOAEC was 0.085 mg/l of air.

The carcinogenic potential of propiconazole was studied in one study in rats and in two studies in mice. In a 2-year dietary study in male and female mice, and an 18-month dietary study in male mice, the liver was the only target organ. At ≥500 ppm, there were decreases in body-weight gain and serum concentration of cholesterol, and increases in liver weight, hepatocellular hypertrophy and hepatocellular vacuolation. The NOAEL for non-neoplastic effects in both studies was 100 ppm (equal to 11 mg/kg bw per day). Propiconazole was a hepatocarcinogen only in male mice, on the basis of significant increases in the incidence of liver tumours at ≥850 ppm (equal to 108 mg/kg bw per day), with a NOAEL of

500 ppm (equal to 59 mg/kg bw per day). Assays for hepatocyte proliferation (measured by bromodeoxyuridine incorporation) in mice showed qualitative similarities between propiconazole and phenobarbital. The doses that produced increases in tumour incidences (≥850 ppm) also produced cell proliferation, increased liver weight and hepatocyte hypertrophy. Studies of liver enzyme induction in mice showed that propiconazole increased the activity of a number of P450s, particularly Cyp2b, and exhibited similar characteristics to a phenobarbital type inducer of xenobiotic-metabolizing enzymes. The progression from P450 (Cyp2b) induction, initial mitogenic response, hepatocyte hypertrophy and increased liver weight to tumours is consistent with a mode of action similar to that of phenobarbital.

At 2500 ppm (96 mg/kg bw per day) in a 2-year dietary study in rats, there were reductions in body-weight gain in both sexes. Increased incidences of enlarged hepatocytes were present in males and increases in atrophy of the exocrine pancreas and dilatation of the uterine lumen in females. Slight (<10%), transient reductions in body-weight gain, variations in clinical chemistry and haematology parameters that fell within physiological ranges at 500 ppm (equal to 18 mg/kg bw per day) were not considered to be adverse. Propiconazole was not carcinogenic in rats at doses of up to 2500 ppm (equal to 96 mg/kg bw per day). The NOAEL in the 2-year study in rats was 500 ppm (equal to 18 mg/kg bw per day).

Propiconazole gave negative results in an adequate battery of studies of genotoxicity in vitro and in vivo. The Meeting concluded that propiconazole was unlikely to be genotoxic.

On the basis of the above consideration of liver tumours in male mice, the high doses required to induce tumours, the likely mechanism of action, the absence of tumorigenicity in rats and the negative results in studies of genotoxicity, the Meeting concluded that propiconazole was unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, reproductive parameters were not affected by treatment with propiconazole. At 500 ppm (equivalent to 35 mg/kg bw per day) dams had reduced body-weight gains ($p < 0.01$) and both sexes exhibited hepatoxicity, thus the NOAEL for parental toxicity was 100 ppm (equivalent to 7 mg/kg bw per day). The NOAEL for offspring toxicity was 100 ppm (equivalent to 7 mg/kg bw per day) on the basis of decreased pup body weights in the F_{2b} litters ($p < 0.01$). The NOAEL for reproductive effects was 500 ppm (equivalent to 35 mg/kg bw per day) on the basis of reduced pup survival at 2500 ppm (equivalent to 175 mg/kg bw per day).

Three studies of developmental toxicity were conducted in rats and one in rabbits. In the first study in rats, at the highest dose of 300 mg/kg bw per day there was evidence of maternal toxicity and retarded development, but no malformations. In the second study, propiconazole caused developmental delay (incomplete ossification of sternebrae and rudimentary cervical ribs) at a dose of 90 mg/kg bw per day, which also produced a slight, transient reduction in food consumption and body-weight gain at the initiation of dosing. The NOAEL was 90 mg/kg bw per day for maternal effects and 30 mg/kg bw per day for developmental effects. A low incidence of cleft palate was observed at 90 mg/kg bw per day (one fetus; 0.3%) and at 360/300 mg/kg bw per day (two fetuses; 0.7%) in the presence of severe maternal toxicity. The maternal toxicity included lethargy, ataxia, salivation and reductions in food consumption and body-weight gain at the start of the dosing period. The cleft palate finding was also seen at a low incidence in rats in an extensive study that specifically

investigated the palate and jaw at a single dose of 300 mg/kg bw per day. Cleft palates were detected in 2 out of 2064 fetuses of treated animals, versus none in the 2122 fetuses of controls, in the presence of severe maternal toxicity. Marked maternal toxicity was observed throughout the treatment period, included reductions in food consumption and body-weight gain, ataxia, coma, lethargy and prostration, and three treatment-related deaths among 189 dams. Cleft palate is a very rare but occasional finding in control rats and there were published data that indicated testing compounds at maternally toxic doses is associated in some way with the induction of a number of malformations, including cleft palate.

Propiconazole was not teratogenic in rabbits. The NOAEL for fetal effects was 250 mg/kg bw per day on the basis of an increased incidence of the formation of thirteenth ribs at 400 mg/kg bw per day in the presence of maternal body-weight loss, signs of toxicity and abortions. The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of reduced food consumption and body-weight loss at 250 mg/kg bw per day.

No studies of neurotoxicity with propiconazole were available; however, no evidence of neurotoxicity was apparent in any of the available studies.

Humans exposed to formulated products containing propiconazole have shown local irritant reactions. No evidence of sensitization was seen in an epicutaneous test in 20 volunteers.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.07 mg/kg bw based on the NOAEL of 7 mg/kg bw per day in a multigeneration study of reproductive toxicity in rats and a 100-fold safety factor. This value covers all other end-points and is supported by NOAELs of 11 mg/kg bw per day in a 24-month study in mice, and 18 mg/kg bw per day in a 2-year study in rats. This ADI is protective against the local effects seen in the gastrointestinal tract in dogs (NOAEL, 1.9 mg/kg bw per day), which were considered to be concentration-dependent and hence would merit a safety factor of 25.

An ARfD of 0.3 mg/kg bw was established based on the NOAEL of 30 mg/kg bw per day in the study of developmental toxicity in rats and a 100-fold safety factor. The NOAEL was identified on the basis of slight increases in rudimentary ribs and unossified sternebrae at 90 mg/kg bw per day, which could not be discounted. This provides an adequate margin over the maternal toxicity and cleft palate seen at 300 mg/kg bw per day. The Meeting noted that the highest dose tested in dogs was 35 mg/kg bw per day and that the proposed ARfD would be protective for any potentially acute effects observed in dogs.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	24-month study of toxicity and carcinogenicity[a,e]	Toxicity	100 ppm, equal to 11 mg/kg bw per day	500 ppm, equal to 59 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 59 mg/kg bw per day	850 ppm, equal to 108 mg/kg bw per day
Rat	2-year study of toxicity and carcinogenicity[a]	Toxicity	500 ppm, equal to 18 mg/kg bw per day	2500 ppm, equal to 96 mg/kg bw per day
		Carcinogenicity	2500 ppm, equal to 96 mg/kg bw per day[c]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	100 ppm, equivalent to 7 mg/kg bw per day	500 ppm, equivalent to 35 mg/kg bw per day
		Offspring toxicity	100 ppm, equivalent to 7 mg/kg bw per day	500 ppm, equivalent to 35 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity, Embryo- or fetotoxicity	90 mg/kg bw per day 30 mg/kg bw per day	300 mg/kg bw per day 90 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity Embryo- or fetotoxicity	100 mg/kg bw per day 250 mg/kg bw per day	250 mg/kg bw per day 400 mg/kg bw per day
Dog	3-month study of toxicity[a]	Systemic effects	1250 ppm, equal to 35 mg/kg bw per day[c]	—
		Local effects on gastrointestinal tract	250 ppm, equal to 6.9 mg/kg bw per day	1250 ppm, equal to 35 mg/kg bw per day[c]
	12-month study of toxicity[d]	Systemic effects	8.4 mg/kg bw per day[c]	—
		Local effects on gastrointestinal tract	1.9 mg/kg bw per day	8.4 mg/kg bw per day[c]

[a] Diet
[b] Gavage
[c] Highest dose tested
[d] Capsules
[e] Two studies

Estimate of acceptable daily intake for humans

0–0.07 mg/kg bw

Estimate acute reference dose

0.3 mg/kg bw

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

Further observations in humans

Summary of critical end-points for propiconazole

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of oral absorption:	>80% in 48 h
Dermal absorption	About 30% in 10 h (rat)
Distribution:	Widely distributed; highest concentrations in the liver and kidney
Potential for accumulation:	Limited
Rate and extent of excretion:	>95% in the faeces and urine in 48 h; extensive enterohepatic recirculation (68% of administered dose in bile)
Metabolism in animals	Extensive; oxidation of propyl side-chain; hydroxylation of phenyl and triazole rings, plus conjugation; cleavage of dioxolane ring
Toxicologically significant compounds (animals, plants and the environment)	Propiconazole Triazolyl alanine and triazolyl acetic acid are produced in plants but not in animals
Acute toxicity	
Rat, LD_{50}, oral	1517 mg/kg bw
Rat, LD_{50}, dermal	>4000 mg/kg bw
Rat, LC_{50}, inhalation	>5 mg/l of air (4-h; nose only)
Rabbit, dermal irritation	Irritating
Rabbit, eye irritation	Not irritating
Skin sensitization	Sensitizing (Magnusson and Kligman study)
Short-term studies of toxicity	
Target/critical effect	Body weight, liver (mice, rats); erythrocytes (rat); stomach (dog)
Lowest relevant oral NOAEL	50 ppm, equal to 1.9 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	200 mg/kg bw per day (5 days/week)
Lowest relevant inhalation NOAEL	0.085 mg/l (6 h/day; 5 days/week)
Genotoxicity	Not genotoxic in vitro or in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver hypertrophy and tumours (mice) Liver, body weight, uterine lumen dilatation (rats)
Lowest relevant NOAEL	100 ppm, equal to 11 mg/kg bw per day (mice)
Carcinogenicity	Hepatocellular tumours in male mice (≥850 ppm, equal to 108 mg/kg bw per day). Phenobarbital-type mechanism. The NOAEL was 500 ppm (equal to 59 mg/kg bw per day). Unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Reproduction target/critical effect	Reduced pup weight at parentally toxic dose
Lowest relevant reproductive NOAEL	100 ppm, equivalent to 7 mg/kg bw per day (rat)
Developmental target/critical effect	Skeletal variations
Lowest relevant developmental NOAEL	30 mg/kg bw per day (rat)
Neurotoxicity/delayed neurotoxicity	No specific studies; no findings in other studies
Other toxicological studies	
Mechanism of induction of liver tumours	Phenobarbital-type mode of action indicated by cell proliferation, liver weight and microsomal enzyme induction patterns
Medical data	Local irritation associated with exposure to the formulated product

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.07 mg/kg bw	Rats, reproductive toxicity, pup and parental body weight	100
ARfD	0.3 mg/kg bw	Rats, developmental toxicity, embryo- or fetoxicity	100

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