PENCONAZOLE

First draft prepared by
G. Wolterink[1] and S.H. Inayat-Hussain[2]

[1] Centre for Nutrition, Prevention and Health Services, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
[2] Global Toxicology Group Health, Safety and Environment, Petronas, Kuala Lumpur, Malaysia

Explanation

Penconazole is the International Organization for Standardization (ISO)–approved common name for 1-(2,4-dichloro-β-propylphenethyl)-1*H*-1,2,4-triazole (International Union of Pure and Applied Chemistry), which has the Chemical Abstracts Service number 66246-88-6. Penconazole is a systemic triazole fungicide with preventive and curative properties for the control of powdery mildew. It stops the development of fungi by interfering with the biosynthesis of sterols in cell membranes and is used on grapes, pome and stone fruit, cucurbits and strawberries.

Penconazole was previously evaluated for toxicology by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1992, when the Meeting established an acceptable daily intake (ADI) of 0–0.03 mg/kg body weight (bw) on the basis of a no-observed-adverse-effect level (NOAEL) of 3 mg/kg bw per day in a 1-year study in dogs.

In 2008, a group of manufacturers of triazole fungicides formed a task force known as the "Triazole Derivative Metabolite Group" and made a joint submission of toxicological data on the common metabolites 1,2,4-triazole, triazole acetic acid and triazole alanine to JMPR. Triazole alanine and triazole acetic acid residues are primarily associated with plant commodities, whereas 1,2,4-

triazole is mainly associated with animal commodities, lesser amounts of this compound being found in plant commodities.

In 2008, the Meeting established an ADI of 0–0.2 mg/kg bw for 1,2,4-triazole, based on a NOAEL of 16 mg/kg bw per day in a two-generation reproductive toxicity study in rats. The Meeting established an acute reference dose (ARfD) of 0.3 mg/kg bw for 1,2,4-triazole, based on a NOAEL of 30 mg/kg bw per day in a developmental toxicity study in rabbits.

In 2008, the Meeting established a group ADI of 0–1.0 mg/kg bw for triazole alanine and triazole acetic acid (alone or in combination), based on a NOAEL of 100 mg/kg bw per day in a developmental toxicity study in rats administered triazole alanine. The 2008 Meeting concluded that it was unnecessary to establish an ARfD for triazole alanine and triazole acetic acid.

Penconazole was re-evaluated by the present Meeting as part of the periodic review programme of the Codex Committee on Pesticide Residues. Both the new data and previously submitted studies with penconazole were considered by the present Meeting. New data on the common rat and plant metabolites 1,2,4-triazole, triazole acetic acid and triazole alanine were considered by the present Meeting to evaluate whether a revision of the ADIs or ARfDs for these compounds was necessary.

All critical studies contained statements of compliance with good laboratory practice (GLP). The Meeting considered that the database was adequate for the risk assessment.

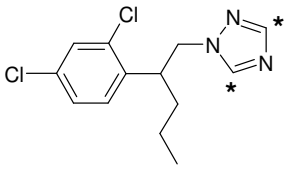
Evaluation for acceptable intake

1. Biochemical aspects

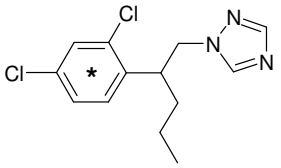
Two ^{14}C-radiolabelled penconazole molecules were used for the toxicokinetic studies (Fig. 1).

Fig. 1. Structure of penconazole with positions of radiolabel

[3,5-^{14}C-triazole]penconazole



[U-^{14}C-phenyl]penconazole



1.1 Absorption, distribution and excretion

Mice

Groups of five male and five female CD-1 (ICR) mice received technical-grade penconazole (purity 98.7%; batch no. FL-840833) in the diet at a dose level of 0, 10, 100, 300, 500, 1000 or 2400 parts per million (ppm) for at least 90 days before a single oral or intravenous dose of 25 µg [^{14}C]penconazole (radiochemical purity > 98%; batch no. GAN-IX-83) in polyethylene glycol (PEG) 200/deionized water (1:1) as dose vehicle. Urine was collected at daily intervals over 48 hours after the ^{14}C dose, and a single 0- to 48-hour collection of faeces was made. Radioactivity in urine and cage wash was determined by direct liquid scintillation counting. Aqueous homogenates of faeces were prepared for scintillation counting by sample oxidation.

The excretion of radioactivity in urine and faeces and recoveries in terminal cage washes following an intravenous or oral dose are presented in Table 1 (intravenous dose) and Table 2 (oral

Table 1. Excretion of radioactivity by mice administered a single intravenous dose of 25 µg [^{14}C]penconazole following pretreatment with dietary doses of unlabelled penconazole for at least 90 days

	% of administered radioactivity					
	10 ppm	**100 ppm**	**300 ppm**	**500 ppm**	**1 000 ppm**	**2 400 ppm**
Males						
Urine						
0–24 h	52.40	42.25	50.95	40.76	50.39	61.28
24–48 h	4.15	5.00	5.46	7.37	4.82	4.49
0–48 h	56.55	47.25	56.41	48.12	55.21	65.78
Faeces						
0–48 h	21.75	31.31	25.35	19.96	25.34	25.69
Total excreted						
0–48 h	78.30	78.56	81.76	68.08	80.55	91.47
Cage wash, 48 h	3.09	5.29	4.34	3.69	4.18	2.07
Total dose recovered, 0–48 h	81.39	83.85	86.10	71.77	84.73	93.54
Females						
Urine						
0–24 h	66.49	64.77	74.55	70.71	69.26	62.84
24–48 h	3.55	2.72	2.32	3.39	2.73	4.42
0–48 h	70.04	67.49	76.88	74.10	71.99	67.26
Faeces						
0–48 h	12.50	11.26	8.65	10.56	10.54	13.90
Total excreted						
0–48 h	82.54	78.75	85.53	84.66	82.53	81.16
Cage wash, 48 h	1.88	2.86	2.65	2.32	3.37	2.61
Total dose recovered, 0–48 h	84.42	81.61	88.18	86.98	85.90	83.77

ppm: parts per million
Source: Hiles (1987a)

dose). For both sexes, there was no marked difference in excretion profiles either across the range of dietary predosing levels or between the oral and intravenous routes of administration of the single radiolabelled dose. Both male and female mice readily absorbed an oral dose of 25 µg [^{14}C]penconazole. There was a difference between the sexes, with females excreting a higher proportion of the dose in urine and less via faeces, compared with males. No change in the distribution of radioactivity was observed between the urine and faecal compartments, from an oral or intravenous dose of [^{14}C]penconazole, following pretreatment with increasing dietary dose levels of technical-grade penconazole from 10 to 2400 ppm (Hiles, 1987a).

Table 2. Excretion of radioactivity by mice administered a single oral dose of 25 µg [^{14}C]penconazole following pretreatment with dietary doses of unlabelled penconazole for at least 90 days

	% of administered radioactivity					
	10 ppm	**100 ppm**	**300 ppm**	**500 ppm**	**1 000 ppm**	**2 400 ppm**
Males						
Urine						
0–24 h	53.07	56.59	54.02	42.74	47.26	54.96
24–48 h	5.75	4.27	7.98	4.05	7.10	6.23
0–48 h	58.82	60.86	62.01	46.79	54.35	61.18
Faeces						
0–48 h	27.73	21.67	18.60	25.29	27.19	23.48
Total excreted						
0–48 h	86.55	82.53	80.61	72.08	81.54	84.66
Cage wash, 48 h	1.83	1.52	1.67	5.58	2.56	2.49
Total dose recovered, 0–48 h	88.38	84.05	82.28	77.66	84.10	87.15
Females						
Urine						
0–24 h	69.73	75.15	71.77	73.24	58.63	53.44
24–48 h	3.24	2.75	3.43	3.23	4.15	12.35
0–48 h	72.97	77.90	75.21	76.48	62.78	65.79
Faeces						
0–48 h	13.07	12.34	13.82	11.46	15.04	16.52
Total excreted						
0–48 h	86.04	90.24	89.03	87.94	77.82	82.31
Cage wash, 48 h	2.33	3.05	4.62	2.17	6.94	4.46
Total dose recovered, 0–48 h	88.37	93.29	93.65	90.11	84.76	86.77

ppm: parts per million
Source: Hiles (1987a)

Rats

The absorption, distribution and excretion of [3,5-^{14}C-triazole]penconazole (radiochemical purity > 98%; batch number not reported), dissolved in ethanol/PEG 200/water (2:3:5 by volume), were studied in groups of two male and two female Tif: RAI f (SPF) rats (a Sprague-Dawley-derived strain) dosed orally by gavage at a single dose of 0.5 or 25 mg/kg bw. Urine, faeces and expired air were collected at 24-hour intervals. After 6 days, the rats were killed, and brain, fat, heart, kidneys, liver, lungs, muscle, ovaries, plasma, spleen, testes, whole blood and residual carcass were sampled and analysed for radioactivity. All excreta and tissue samples were analysed for radioactivity by liquid scintillation counting either directly or following sample oxidation. Urinary metabolite profiles were investigated by thin-layer chromatography (TLC). These data are presented in section 1.2 (Hamböck, 1980, 1985).

Table 3. Group mean excretion data following single oral administration of [3,5-^{14}C-triazole]penconazole to rats

	% of administered dose			
	0.5 mg/kg bw		25 mg/kg bw	
	Males	**Females**	**Males**	**Females**
Urine				
0–24 h	49.86	68.20	47.72	81.05
24–48 h	10.00	3.80	7.74	2.86
48–72 h	3.32	0.74	4.07	0.62
72–144 h	2.43	0.39	2.58	0.27
Subtotal	62.26	73.12	62.10	84.79
Faeces				
0–24 h	17.19	26.38	25.94	10.13
24–48 h	13.17	3.91	6.81	3.08
48–72 h	4.42	0.87	3.93	0.50
72–144 h	2.65	0.37	1.84	0.32
Subtotal	37.32	31.53	38.51	14.20
Expired air				
0–24 h	0.10	0.10	0.04	0.03
24–48 h	0.02	0.02	0.02	0.01
48–144 h	0.02	0.01	0.02	0.00
Subtotal	0.14	0.12	0.08	0.04
Total excretion	99.72	104.77	100.69	99.03
Excretion within 0–24 h	67.05	94.58	73.66	90.68
Tissues	0.09	0.05	0.08	0.04
Cage wash	0.53	0.61	0.63	0.35
Total recovery	100.34	105.41	101.39	99.41

bw: body weight
Source: Hamböck (1980, 1985)

The excretion of radioactivity, expressed as a percentage of the administered dose, is presented in Table 3.

Following a single oral dose of [3,5-^{14}C-triazole]penconazole, absorption was extensive, and the majority of the absorbed dose was excreted rapidly in urine. At both doses, excretion in urine was higher in females, in particular at the high dose. Also, excretion in urine and faeces occurred more rapidly in females than in males. Excretion in expired air was negligible, and excretion was virtually complete by 6 days after dosing. At 6 days after dosing with 0.5 or 25 mg/kg bw, total tissue levels accounted for less than 0.1% of the administered dose. Highest concentrations were found in liver, lungs and kidneys (Hamböck, 1980, 1985).

The blood kinetics and tissue distribution of radioactivity were investigated in Tif: RAI f (SPF) rats administered a single oral dose of [U-^{14}C-phenyl]penconazole, dissolved in a mixture of PEG 200/ethanol/water (5:3:2 by volume) at 50 mg/kg bw. In a group of three males and three

females, blood was collected at 0.5, 1, 2, 4, 6, 8, 12, 24 and 48 hours after dosing. In a second group of rats, one male and one female each were subjected to whole-body radioluminography at 4, 6, 8, 12, 24 and 48 hours after dosing.

The peak blood concentration (8 mg/kg in males and 7 mg/kg in females) was attained 4 and 6 hours after dosing in males and females, respectively. The blood concentration declined to half the maximum value within 13 and 3 hours in males and females, respectively. Radioluminography indicated that most tissue concentrations reached a maximum after 6 hours in males and after 4 hours in females. The highest concentration was found in the penis (probably related to contamination with urinary radioactivity), followed by organs associated with metabolism and excretion (i.e. liver and kidney). Maximum tissue levels and depletion half-lives in tissues of males and females are presented in Table 4.

Table 4. Maximum tissue concentrations and depletion half-lives in male and female rats administered a single oral [^{14}C]penconazole dose of 50 mg/kg bw

	Males		Females	
Tissue	**Concentration (ppm equiv)**	**$t_{½}$ (h)**	**Concentration (ppm equiv)**	**$t_{½}$ (h)**
Adrenal gland	38.3	8	41.3	6
Blood	12.9	11	5.5	4
Bone	4.9	9	2.2	4
Bone marrow	10.0	9	6.1	3
Brain	9.7	8	7.2	3
Fat (abdominal)	27.1	7	24.8	5
Heart	14.7	9	10.5	4
Kidney cortex	53.1	12	28.6	7
Kidney medulla	23.6	8	30.9	3
Liver	73.9	10	36.4	7
Lungs	14.3	12	6.8	8
Muscle (skeletal)	9.2	7	6.0	3
Ovaries (females)	n.a.	n.a.	10.7	3
Penis (males)	115.2	22	n.a.	n.a.
Plasma	13.6	10	7.2	6
Salivary gland	15.8	9	11.5	3
Spinal cord	9.1	8	8.5	4
Spleen	13.1	9	17.7	3
Testes (males)	8.6	8	n.a.	n.a.
Thymus	8.5	8	6.1	4
Thyroid	13.4	8	8.6	4
Uterus (females)	n.a.	n.a.	12.0	4

bw: body weight; equiv: equivalents; n.a.: not applicable; ppm: parts per million; $t_{½}$: half-life
Source: Hassler (1999)

In males, the half-life of elimination from each tissue, assuming first-order kinetics, ranged from 7 to 12 hours, with the single exception of the penis, which had an elimination half-life of 22 hours. In females, half-lives ranged from 3 to 8 hours (Hassler, 1999).

The kinetics of the distribution and elimination of an oral or intravenous dose of [3,5-^{14}C-triazole]penconazole (radiochemical purity > 98%; batch no. GAN-IX-83) was investigated following dietary exposure to unlabelled penconazole (purity 98.7%; batch no. FL-840833) for at least 90 days. Groups of five male and five female Sprague-Dawley: Crl:CD (SD) BR rats received technical-grade penconazole in the diet at a dose level of 0, 10, 100, 300, 500, 1000 or 2400 ppm for at least 13 weeks before a single oral or intravenous dose of 0.1 mg [^{14}C]penconazole in PEG 200/distilled water (1:1) as dose vehicle. The radiolabelled dose was equivalent to a nominal 5 ppm dietary dose. Urine was collected at daily intervals over 48 hours after the ^{14}C dose, and a single 0- to 48-hour collection of faeces was made. Radioactivity in urine and cage wash was determined by direct liquid scintillation counting. Aqueous homogenates of faeces were prepared for scintillation counting by sample oxidation.

The excretion of radioactivity in urine and faeces and recoveries in terminal cage washes following an intravenous or oral dose are presented in Tables 5 and 6, respectively.

Table 5. Excretion of radioactivity by rats administered a single intravenous dose of 0.1 mg [^{14}C]penconazole following pretreatment with dietary doses of unlabelled penconazole for at least 90 days

	% of administered radioactivity					
	10 ppm	**100 ppm**	**300 ppm**	**500 ppm**	**1 000 ppm**	**2 400 ppm**
Males						
Urine						
0–24 h	42.54	44.04	42.28	42.11	39.38	42.43
24–48 h	10.25	8.47	7.84	8.55	9.91	9.91
0–48 h	52.79	52.51	50.12	50.66	49.29	52.34
Faeces						
0–48 h	21.87	25.04	25.31	28.61	27.17	28.81
Cage wash, 48 h	1.80	1.36	1.60	1.09	1.30	1.49
Total recovery, 0–48 h	76.46	78.91	77.03	80.36	77.76	82.64
Females						
Urine						
0–24 h	66.61	68.25	72.11	68.36	66.97	68.30
24–48 h	6.04	5.45	5.25	4.75	7.06	5.77
0–48 h	72.65	73.70	77.36	73.11	74.03	74.07
Faeces						
0–48 h	13.42	11.68	13.12	16.83	15.41	15.26
Cage wash, 48 h	3.14	2.32	2.04	1.29	3.54	3.34
Total recovery, 0–48 h	89.21	87.70	92.52	91.23	92.98	92.67

ppm: parts per million
Source: Hiles (1987b)

Table 6. Excretion of radioactivity by rats administered a single oral dose of 0.1 mg [^{14}C]penconazole following pretreatment with dietary doses of unlabelled penconazole for at least 90 days

	% of administered radioactivity					
	10 ppm	**100 ppm**	**300 ppm**	**500 ppm**	**1 000 ppm**	**2 400 ppm**
Males						
Urine						
0–24 h	45.47	50.74	40.39	45.08	39.55	43.31
24–48 h	7.11	7.83	8.12	9.36	8.20	9.86
0–48 h	52.58	58.57	48.51	54.44	47.75	53.17
Faeces						
0–48 h	28.26	26.45	31.36	25.88	30.59	27.79
Cage wash, 48 h	1.54	1.81	1.70	1.30	1.30	1.90
Total recovery, 0–48 h	82.38	86.83	81.57	81.62	79.64	82.86
Females						
Urine						
0–24 h	69.47	71.52	74.76	70.07	74.17	69.15
24–48 h	4.57	5.55	4.23	4.13	4.62	5.31
0–48 h	74.04	77.07	78.99	74.20	78.79	74.46
Faeces						
0–48 h	13.34	13.30	12.95	15.19	14.63	16.19
Cage wash, 48 h	2.44	3.18	1.31	2.74	1.60	2.98
Total recovery, 0–48 h	87.38	90.37	91.94	89.39	93.42	90.65

ppm: parts per million
Source: Hiles (1987b)

For both sexes, there was no marked difference in excretion profiles either across the range of dietary predosing levels or between the oral and intravenous routes of administration of the single radiolabelled dose. Females excreted a higher proportion of the dose in urine and less via faeces, compared with males. The mean recoveries of administered radioactivity were also higher in females (92%) than in males (81%) over the 48-hour time course of this study. These results show that the pretreatment of male and female rats with dietary doses of penconazole ranging from 10 to 2400 ppm had no apparent effect on the rate or extent of urinary or faecal excretion of a single intravenous or oral dose of 0.1 mg [^{14}C]penconazole (Hiles, 1987b).

The effect of dose level on the kinetics of the distribution and elimination of an oral dose of [^{14}C]penconazole (radiochemical purity 98%; batch no. GAN-IX-83) was investigated in Sprague-Dawley: Crl:CD(SD) BR rats. Groups of five male and five female rats received a single oral dose of technical-grade penconazole equivalent to a dietary dose of 0, 10, 100, 300, 500, 1000 or 2400 ppm. Each rat was then immediately administered a single oral dose of 0.1 mg [3,5-^{14}C-triazole]penconazole in PEG 200/distilled water (1:1 by volume) as dose vehicle. The radiolabelled dose was equivalent to a nominal 5 ppm dietary dose. Urine was collected at daily intervals over 48 hours after the ^{14}C dose, and a single 0- to 48-hour collection of faeces was made. Radioactivity in urine and cage wash was determined by direct liquid scintillation counting. Aqueous homogenates of faeces were prepared for scintillation counting by sample oxidation.

Table 7. Excretion of radioactivity by rats administered a single oral dose of 0.1 mg [3,5-^{14}C-triazole]penconazole, preceded by a single oral dose of unlabelled penconazole across a range of dose levels equivalent to dietary doses of 0–2400 ppm

	% of administered radioactivity						
	0 ppm	**10 ppm**	**100 ppm**	**300 ppm**	**500 ppm**	**1 000 ppm**	**2 400 ppm**
Males							
Urine							
0–24 h	45.28	38.20	43.91	44.76	45.81	39.69	33.85
24–48 h	8.02	7.80	7.80	7.82	9.56	11.69	13.97
0–48 h	53.31	46.00	51.71	52.58	55.37	51.38	47.82
Faeces							
0–48 h	27.28	26.89	26.31	26.51	27.17	24.09	18.82
Cage wash, 48 h	3.21	2.73	2.60	2.65	3.59	2.80	3.78
Total recovery, 0–48 h	83.80	75.62	80.62	81.74	86.13	78.27	70.42
Females							
Urine							
0–24 h	76.93	68.83	77.58	79.48	86.13	79.69	55.58
24–48 h	3.55	3.87	2.39	2.83	4.12	4.00	13.14
0–48 h	80.48	72.20	79.97	82.32	90.25	83.70	68.73
Faeces							
0–48 h	12.68	12.61	11.99	11.07	9.35	10.02	9.98
Cage wash, 48 h	2.25	1.89	2.56	2.14	2.48	2.72	3.35
Total recovery, 0–48 h	95.41	87.20	94.52	95.53	102.08	96.44	82.06

ppm: parts per million
Source: Levan (1987)

The excretion of radioactivity in urine and faeces and recoveries in terminal cage washes are presented in Table 7.

For both sexes, there was no marked difference in excretion profiles across the range of dose levels investigated. There was a difference between the sexes, with females excreting a higher proportion of the dose in urine and less via faeces, compared with males. The mean recoveries of administered radioactivity were also higher in females (93%) than in males (80%) over the 48-hour time course of this study. These results show no apparent relationship between the pretreatment gavage dose level over an equivalent dietary dose range of penconazole from 10 to 2400 ppm and the kinetics of the distribution and elimination of a single 0.1 mg gavage dose of [^{14}C]penconazole between the urine and faecal compartments (Levan, 1987).

The absorption, tissue distribution and excretion of penconazole were investigated in Wistar, KFM-WIST outbred, SPF rats administered a single oral dose of [U-^{14}C-phenyl]penconazole (radiochemical purity 98.2%; batch no. GB-XXIX-57 B1). The identification of penconazole and its metabolites in tissues and excreta is described in section 1.2 (Van Dijk, 1987). The experimental design is presented in Table 8. In the repeated-dose part of the study, rats were treated with unlabelled

Table 8. Experimental design of a study on the absorption, distribution and excretion of penconazole in rats

Group	Number and sex	Route and dose level of [U-^{14}C-phenyl]penconazole	Sample collection
1	5 males	Single oral dose (50 mg/kg bw)	Expired $^{14}CO_2$ collections at 8, 24 and 48 h after dosing Excreta collections at 8, 24, 48, 72 and 96 h after dosing Blood and selected tissues taken for analysis
2	5 males	Single oral dose (0.5 mg/kg bw)	Excreta collections at 8, 24, 48, 72 and 96 h after dosing Blood and selected tissues taken for analysis
3	5 females	Single oral dose (0.5 mg/kg bw)	
4	5 females	Single oral dose (50 mg/kg bw)	
5	3 males (bile duct cannulated)	Single oral dose (0.5 mg/kg bw)	Bile collected at 3 h intervals Urine and faeces collected at 24 and 48 h
6	3 females (bile duct cannulated)	Single oral dose (0.5 mg/kg bw)	Gastrointestinal tract removed, but no tissues taken for analysis
7	5 males	Single oral dose (0.5 mg/kg bw) following 14 consecutive daily 0.5 mg/kg bw oral doses of unlabelled penconazole	Excreta collections at 8, 24, 48, 72 and 96 h after radiolabel dosing Blood and selected tissues taken for analysis
8	5 females		

bw: body weight
Source: Van Dijk (1987)

penconazole (purity > 99%; batch no. P2). At the termination of rats in groups 1–4 and 7–8, the following tissues were taken for radioactivity analysis: bone (femur), brain, fat, heart, intestinal tract (including contents), kidneys, liver, lungs, muscle, ovaries, pancreas, plasma, skin, spleen, stomach, testes, thyroid, uterus, whole blood and residual carcass. All samples were counted for radioactivity by liquid scintillation counting either directly or following tissue digestion or sample oxidation.

The excretion of radioactivity by rats across the eight dose groups is presented in Table 9. Penconazole was very well absorbed by both male and female rats. In bile duct–cannulated rats that were administered a dose of 0.5 mg/kg bw, less than 5% of the dose was excreted in faeces. Males excreted similar proportions of a 0.5 mg/kg bw dose between urine (47%) and faeces (44%), whereas females eliminated a greater proportion of the dose in urine (69%) than in faeces (21%). This difference could be attributed in part to the higher biliary elimination by males (55% of the dose) compared with females (40%). The rate of biliary elimination was fast in both sexes, with 49% of the dose in 0- to 9-hour bile collections in male rats and 29% in female bile over the same interval. The comparative biliary and faecal excretion data of an equivalent dose (0.5 mg/kg bw) in non-cannulated rats indicate that some biliary metabolites were subject to reabsorption. No marked differences in excretion profiles between the low and high dose levels were observed. Furthermore, pretreatment with unlabelled penconazole for 14 days caused no marked difference in excretion profiles. No volatile radiolabelled metabolites were trapped from expired air, which is consistent with the location of the radiolabel in the phenyl ring.

Tissue residues were low by 4 days after dosing, irrespective of sex or dose level (Table 10). Following a 0.5 mg/kg bw dose, most residues were at or below the limit of quantification, with measurable residues in liver, kidneys, blood and residual carcass. Following a 50 mg/kg bw dose level, the highest tissue residues in males were present in liver, kidneys and adrenal glands, with all other tissue concentrations below that found in blood. In females at 50 mg/kg bw, the highest residue appeared in the thyroid; however, thyroid tissue concentrations were below the limit of quantification in all other male and female groups. Progressively lower residues were present in the adrenal glands, liver and kidneys, with concentrations generally lower in females than in males. In the repeated-dose

Table 9. Mean absorption and excretion data following single oral administration of [U-^{14}C-phenyl]penconazole to rats

	% of dose							
	Group 1 (M)	**Group 2 (M)**	**Group 3 (F)**	**Group 4 (F)**	**Group 5 (M)**	**Group 6 (F)**	**Group 7 (M)**	**Group 8 (F)**
	50 mg/kg bw	**0.5 mg/kg bw**	**0.5 mg/kg bw**	**50 mg/kg bw**	**0.5 mg/kg bw**	**0.5 mg/kg bw**	**0.5 mg/kg bw**	**0.5 mg/kg bw**
Urine								
0–8 h	19.4	31.9	56.3	36.5	22.3	46.3	24.8	49.9
8–24 h	15.1	9.6	10.5	31.5	5.9	1.6	14.2	18.7
24–48 h	5.0	3.7	1.7	3.6	n.a.	n.a.	2.0	1.2
48–96 h	1.5	1.7	0.5	0.6	n.a.	n.a.	1.2	0.4
Subtotal	41.1	46.9	69.0	72.2	28.2	47.9	42.2	70.2
Bile								
0–24 h	n.a.	n.a.	n.a.	n.a.	54.6	38.6	n.a.	n.a.
24–48 h	n.a.	n.a.	n.a.	n.a.	0.97	1.6	n.a.	n.a.
Subtotal	n.a.	n.a.	n.a.	n.a.	54.6	40.2	n.a.	n.a.
Faeces								
0–8 h	0.4	2.8	0.9	0.1	3.2	0.9	1.6	2.0
8–24 h	30.7	26.6	16.2	13.5	1.5	1.1	33.5	18.3
24–48 h	9.7	8.7	3.0	3.6	n.a.	n.a.	11.8	2.6
48–96 h	6.1	1.5	1.0	0.9	n.a.	n.a.	4.9	0.8
Subtotal	47.0	43.5	21.1	18.1	4.7	2.0	51.8	23.7
Expired air								
0–48 h	< 0.01	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Cage wash	4.0	8.5	7.7	7.9	0.8	4.2	2.1	3.6
Total excretion	92.1	98.9	97.8	98.2	88.3	94.3	96.1	97.5
GIT	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Tissues	1.6	2.2	0.5	0.2	n.a.	n.a.	1.4	0.2
Digestive tract	n.a.	n.a.	n.a.	n.a.	1.7	0.5	n.a.	n.a.
Carcass	n.a.	n.a.	n.a.	n.a.	0.9	3.5	n.a.	n.a.
Total recovery	93.7	101.1	98.3	98.4	90.9	98.3	97.5	97.7

bw: body weight; F: females; GIT: gastrointestinal tract; M: males; n.a.: not applicable
Source: Van Dijk (1987)

study, most radiolabelled tissue concentrations were below the limit of quantification (Van Dijk, 1987).

1.2 Biotransformation

Rats

The metabolism of [3,5-^{14}C-triazole]penconazole (radiochemical purity > 98%; batch number not reported), dissolved in ethanol/PEG 200/water (2:3:5 by volume), was studied in groups of two male and two female Tif: RAI f (SPF) rats dosed orally by gavage at a single dose of 25 mg/kg bw. In

Table 10. Group mean tissue residues of radioactivity 4 days after a single oral dose of [U-^{14}C-phenyl]penconazole

	Concentration (ppm penconazole equivalents)					
	Group 1 (M)	**Group 2 (M)**	**Group 3 (F)**	**Group 4 (F)**	**Group 7 (M)**	**Group 8 (F)**
	50 mg/kg bw	**0.5 mg/kg bw**	**0.5 mg/kg bw**	**50 mg/kg bw**	**0.5 mg/kg bw**	**0.5 mg/kg bw**
Heart	0.16	< LOQ	< LOQ	0.11	< LOQ	< LOQ
Lungs	0.19	< LOQ	< LOQ	0.17	< LOQ	< LOQ
Liver	1.46	0.019	0.006	0.34	0.015	< LOQ
Spleen	0.16	< LOQ	< LOQ	0.19	< LOQ	< LOQ
Kidneys	0.71	0.007	0.004	0.23	< LOQ	< LOQ
Muscle	0.08	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Bone (femur)	0.07	0.007	0.006	< LOQ	LOQ	< LOQ
Brain	0.06	< LOQ	< LOQ	LOQ	< LOQ	< LOQ
Fat	0.33	< LOQ	< LOQ	0.027	< LOQ	< LOQ
Pancreas	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Stomach	0.10	< LOQ	< LOQ	0.09	< LOQ	< LOQ
Intestinal tract	4.08	0.054	0.006	0.45	0.061	0.005
Adrenal glands	0.56	< LOQ	< LOQ	0.49	< LOQ	< LOQ
Uterus/ovaries	n.a.	n.a.	< LOQ	< LOQ	n.a.	< LOQ
Testes	0.07	< LOQ	n.a.	n.a.	< LOQ	n.a.
Thyroid gland	< LOQ	< LOQ	< LOQ	1.11	< LOQ	< LOQ
Skin chest	0.27	LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Skin back region	0.15	< LOQ	< LOQ	0.05	< LOQ	< LOQ
Carcass	0.19	0.003	LOQ	0.06	LOQ	< LOQ
Blood	0.45	0.006	< LOQ	0.08	0.006	< LOQ
Plasma	0.30	0.004	< LOQ	0.08	LOQ	< LOQ

bw: body weight; F: females; LOQ: limit of quantification; M: males; n.a.: not applicable; ppm: parts per million
Source: Van Dijk (1987)

urine collected over the first 24-hour interval, metabolite profiles were investigated by TLC. The study design and toxicokinetics are described in section 1.1 (Hamböck, 1980, 1985).

Chromatographic analysis of urine samples is presented in Table 11. The chromatographic analysis showed the presence of several polar metabolites and no unchanged parent penconazole. The general pattern of metabolites was qualitatively similar both between the sexes and between the dose levels. However, the relative proportions of radioactivity in metabolite fractions differed considerably between males and females. The polar metabolite fraction (U_{01} and U_{02}) accounted for 45% of the dose in females and just 3% in males. The "medium polar" metabolites (U_{03} and U_{06}) represented 12% of the dose in males, but only 2% in females. These "medium polar" metabolites included free triazole, which accounted for 7% of the dose in males and only 1% in females. No marked differences were apparent in the proportions of other fractionated metabolites (Hamböck, 1980, 1985).

Table 11. Quantitative distribution of urinary metabolites in urine collected over 24 hours after a single oral [3,5-^{14}C-triazole]penconazole dose of 25 mg/kg bw

	% of dose		
TLC fraction code	**Males**	**Females**	**Structural identification**
U_{01}	1	7	Conjugates with glucuronic acid
U_{02}	2	38	
U_{03}	7	1	Free 1,2,4-triazole
U_{06}	5	1	Several carboxylic acid metabolites
U_{07}	21	23	
Other minor fractions	11	11	
Start zone	1	0	
Total	48	81	

bw: body weight; TLC: thin-layer chromatography
Source: Hamböck (1985)

The metabolism of [3,5-^{14}C-triazole]penconazole (radiochemical purity > 98%; batch number not reported), dissolved in ethanol/PEG 200/water (2:3:5 by volume), was studied in 20 male Tif: RAI f (SPF) rats dosed orally by gavage at a single dose of 22.8 mg/kg bw. Urine and faeces were collected over 48 hours. Urinary metabolite profiles were investigated by two-dimensional TLC. The major metabolites were isolated by extraction, column chromatography, preparative TLC and high-performance liquid chromatography (HPLC). Acidic metabolites were acidified and methylated to facilitate purification, and the hydroxylated derivatives were acetylated, thereby preventing potential losses due to chemical instability. Isolated metabolites were analysed by mass spectroscopy and nuclear magnetic resonance (NMR) to elucidate their structures.

In urine and faeces, 62% and 33% of the administered dose were found, respectively. The relative proportions of identified radioactivity are presented in Table 12.

TLC analysis showed an array of metabolites, and the profile was not markedly changed following treatment of metabolites with β-glucuronidase or sulfatase. The amounts of these conjugates were therefore shown to be low.

At least four metabolic reactions were involved in the biotransformation of penconazole:

1. cleavage of the triazole ring (15% of the dose);
2. oxidation of the ω-position of the alkane chain to form the respective carboxylic acid (30% of the dose);
3. oxidation of the 3- or 4-position of the alkane chain to form monohydroxy and dihydroxy derivatives (2.5% of the dose); and
4. oxidation of the triazole ring in the 3- or 5-position (0.7% of the dose).

Secondary metabolic reactions included:

- α-oxidation of the carboxylic acids to form α-hydroxy carboxylic acids (4.4% of the dose);
- decarboxylation following oxidation to α-ketocarboxylic derivative (9% of the dose);
- oxidation of the 3,4-dihydroxy derivatives to produce the corresponding 3- or 4-keto derivatives (0.5% of the dose);
- conjugation of all alkanol derivatives with glucuronic acid (2.5% of the dose); and
- β-oxidation of ω-carboxylic acids, which may also reduce the chain length.

The stepwise reduction of the alkyl chain and the cleavage of the molecule to form free triazole are the two main metabolic reactions. Free triazole was the only metabolite present in both urine and faeces. Oxidation of the triazole ring was a minor metabolic reaction, and no

biotransformation occurred on the dichlorophenyl ring. Sequential methanol extraction of faeces yielded 82% extraction of faecal radioactivity. Free triazole was present and accounted for approximately 1% of the dose, and unchanged penconazole accounted for less than 1% of the dose, which was considered to represent unabsorbed penconazole. Other faecal metabolites (not identified) did not correspond with urinary metabolites.

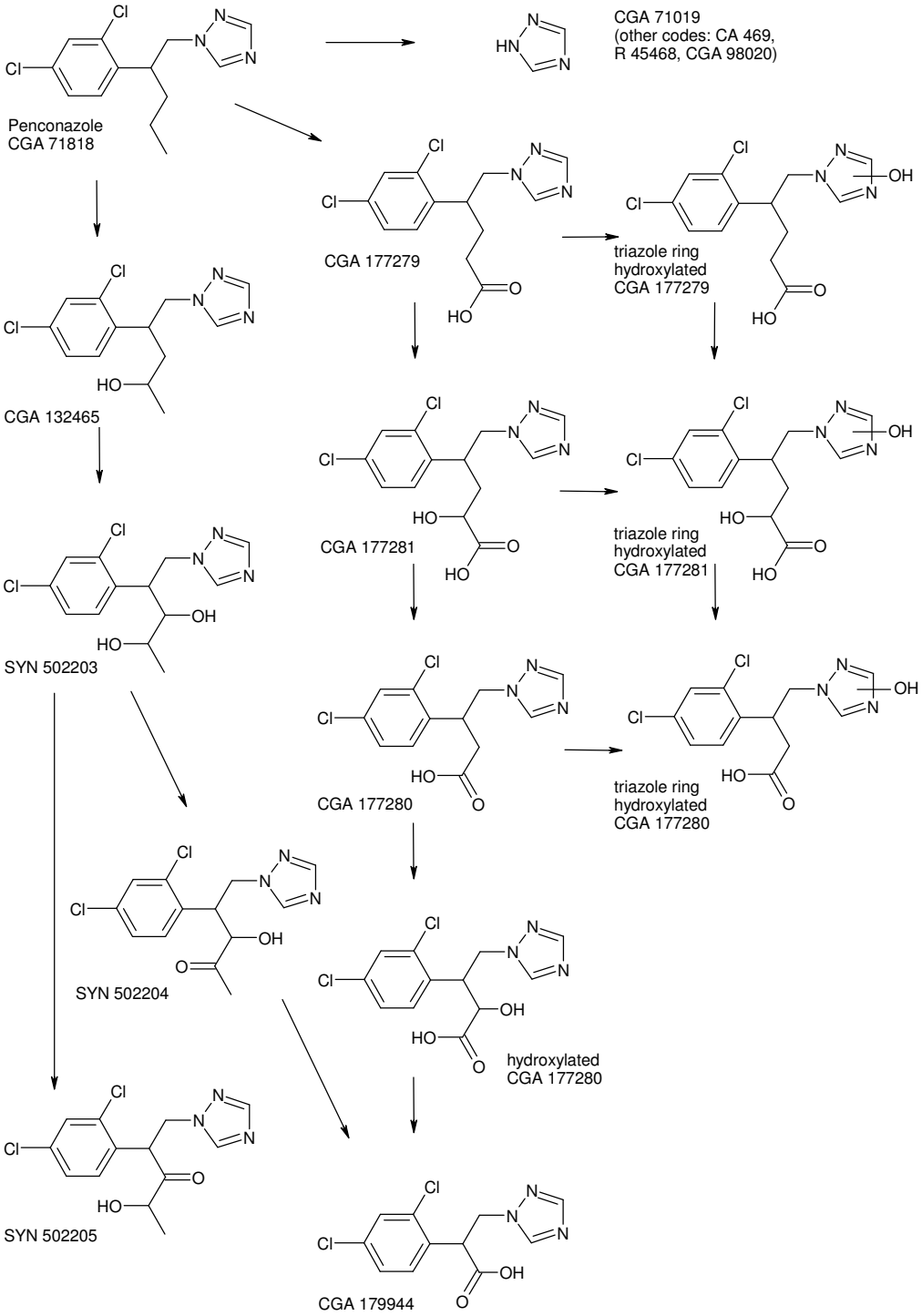
Table 12. Characterization and quantification of metabolites of penconazole in urine and faeces

		% of dose	
Reference code	**Nomenclature**	**Urine**	**Faeces**
CGA 71818	1-[2-(2,4-Dichloro-phenyl)-pentyl]-1*H*-[1,2,4]triazole	–	0.8
CGA 71019 (other codes: CA 469, R 45468, CGA 98020)	1*H*-[1,2,4]Triazole	14	1
CGA 177279	4-(2,4-Dichloro-phenyl)-5-[1,2,4]triazol-1-yl-pentanoic acid	16	–
Triazole ring hydroxylated CGA 177279		0.6	–
CGA 177281	4-(2,4-Dichloro-phenyl)-2-hydroxy-5-[1,2,4]triazol-1-yl-pentanoic acid	4.4	–
CGA 177280	3-(2,4-Dichloro-phenyl)-4-[1,2,4]triazol-1-yl-butyric acid	7.8	–
CGA 177281 (hydroxylated CGA 177280)		< 0.1	–
Triazole ring hydroxylated CGA 177280		Not quantified	–
Triazole ring hydroxylated CGA 177281		0.1	–
CGA 179944	2-(2,4-Dichloro-phenyl)-3-[1,2,4]triazol-1-yl-propionic acid	0.9	–
CGA 132465	4-(2,4-Dichloro-phenyl)-5-[1,2,4]triazol-1-yl-pentan-2-ol		
SYN 502203	4-(2,4-Dichloro-phenyl)-5-[1,2,4]triazol-1-yl-pentane-2,3-diol		
SYN 502204	4-(2,4-Dichloro-phenyl)-3-hydroxy-5-[1,2,4]triazol-1-yl-pentan-2-one	2.5	–
SYN 502205	2-(2,4-Dichloro-phenyl)-4-hydroxy-1-[1,2,4]triazol-1-yl-pentan-3-one		
Not identified	–	16	31

Source: Hamböck (1982, 1984)

The proposed metabolic pathway of penconazole in rats is shown in Fig. 2 (Hamböck, 1982, 1984).

Fig. 2. The proposed biotransformation of penconazole in the rat (urine and faeces)



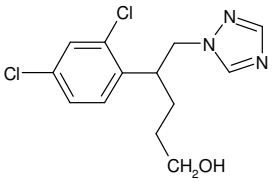
Source: Hamböck (1984)

The metabolism of [U-^{14}C-phenyl]penconazole (radiochemical purity 98.2%; batch no. GB-XXIX-57 B1) was studied in groups of Wistar, KFM-WIST outbred, SPF rats. The experimental designs and toxicokinetics are described in section 1.1 (Van Dijk, 1987). All samples were counted for radioactivity by liquid scintillation counting either directly or following tissue digestion or sample oxidation. Metabolite profiles were investigated by two-dimensional TLC in urine (0- to 24-hour pools), bile (0- to 6-hour, 6- to 12-hour and 12- to 18-hour pools), solvent extracts of faeces (0- to 48-hour pools, except group 8, 0- to 24-hour pool), liver and kidney. Metabolite fractions were treated with enzymes to cleave metabolite conjugates. The relative proportions of the administered dose in resolved metabolite fractions were determined.

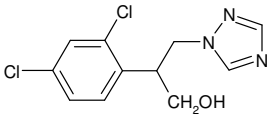
At least eight metabolites were resolved in urine, and 12 in faecal extracts. The solvent extraction of pooled faecal samples yielded about an 85% extraction of radioactivity. No unchanged parent compound was found in excreta. Two of the resolved metabolites in excreta and tissues were characterized as CGA 127841 and CGA 189659 (Fig. 3). Conjugates of these metabolites were also present. All other metabolites remained unidentified in this study. Conjugated CGA 127841 was found in urine, faeces and bile. Unconjugated CGA 127841 was found in faeces, bile, liver and kidney. CGA 189659 was identified in faeces, liver and kidney (Van Dijk, 1987).

Fig. 3. Structural characterization of metabolites of penconazole

CGA 127841



CGA 189659



Source: Van Dijk (1987)

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of studies of acute toxicity with penconazole are summarized in Table 13.

(b) Dermal irritation

In an acute dermal irritation study, the intact or abraded skin of three male and three female New Zealand White rabbits was exposed for 24 hours under occlusion to 0.5 g penconazole (purity 88.4%; batch no. P.2+3) moistened with PEG/water (70:30 by volume). Dermal irritation was scored at 24 and 72 hours after patch removal.

Minimal erythema on the intact and abraded skin was observed at 24 hours. No signs of dermal irritation were observed at 72 hours (Ullmann, 1980).

(c) Ocular irritation

In an acute eye irritation study, 100 mg of penconazole (purity not specified; batch no. FL840833) was instilled into the conjunctival sac of one eye of each of three male and six female New Zealand White rabbits. The untreated eye served as a control. The treated eyes of three female rabbits were washed with deionized water 30 seconds after application. The eyes were examined

Table 13. Results of studies of acute toxicity with penconazole

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50}/LC_{50}	Reference
Mouse	Tif: MAG (SPF)	M/F	Oral	PEG 400	88.4	2 444 mg/kg bw (M/F)	Sarasin (1980)[a]
Rat	Tif: RAI f (SPF)	M/F	Oral	PEG 400	88.4	2 125 mg/kg bw (M/F)	Bathe (1980a)[b]
Chinese hamster	–	M/F	Oral	PEG 400	88.4	~5 000 mg/kg bw (M/F)	Bathe (1980b)[c]
Rabbit	New Zealand White	M/F	Oral	Carboxymethyl cellulose	88.4	971 mg/kg bw (M/F)	Kobel (1981)[d]
Rat	Tif: RAI f (SPF)	M/F	Dermal	PEG 400	88.4	> 3 000 mg/kg bw (M/F)	Bathe (1980c)[e]
Rat	Tif: RAI f (SPF)	M/F	Inhalation	–	96.1	> 4.0 mg/L (M/F)	Hartmann (1987)[f]

bw: body weight; F: female; LC_{50}: median lethal concentration; LD_{50}: median lethal dose; M: male; PEG: polyethylene glycol

[a] Study design resembles Organisation for Economic Co-operation and Development (OECD) Test Guideline 401. The mice were given a dose of 1500, 2000, 3000 or 5000 mg/kg bw. At 3000 and 5000 mg/kg bw, 4/5 and 5/5 males died, respectively. At 1500, 3000 and 5000 mg/kg bw, 1/5, 5/5 and 5/5 females died, respectively. At all doses, sedation, dyspnoea, ruffled fur and curved or ventral/lateral body position were observed. The first onset of symptoms occurred within 1 hour after dosing in all groups. The longest duration of symptoms in surviving animals was 7 days after 1500, 2000 and 3000 mg/kg bw. Recovery was complete in survivors of all groups by day 8 post-dosing. Necropsy revealed no treatment-related findings. Batch no. P.2+3.

[b] Study design resembles OECD Test Guideline 401. The rats were given a dose of 500, 1000, 2000 or 4000 mg/kg bw. At 2000 and 4000 mg/kg bw, 3/5 and 5/5 males died, respectively. At 1000 and 4000 mg/kg bw, 2/5 and 4/5 females died, respectively. At 500 mg/kg bw, sedation, dyspnoea, curved or lateral/ventral body position and ruffled fur were observed. At higher doses, diarrhoea was also observed. At the high dose, symptoms lasted up to 9 days. Gross pathology revealed no treatment-related findings. Batch no. P.2+3.

[c] Study design resembles OECD Test Guideline 401. The hamsters were given a dose of 2000, 4000 or 5000 mg/kg bw. At 4000 and 5000 mg/kg bw, 1/5 and 1/5 males died, respectively. At 4000 and 5000 mg/kg bw, 2/5 and 3/5 females died, respectively. At 2000 mg/kg bw, sedation, dyspnoea, curved body position, ruffled fur and exophthalmos were observed. At higher doses, salivation and lateral/ventral body position were also observed. At the high dose, symptoms lasted up to 8 days. Gross pathology revealed no treatment-related findings. Batch no. P.2+3.

[d] Study design resembles OECD Test Guideline 401. The rabbits were given a dose of 600, 1000 or 2000 mg/kg bw. At 1000 and 2000 mg/kg bw, 2/3 and 3/3 males died, respectively. At 1000 and 2000 mg/kg bw, 2/3 and 3/3 females died, respectively. At 600 mg/kg bw, ataxia and ruffled fur were observed. At higher doses, sedation, dyspnoea, curved or lateral body position, dacryorrhoea and tremor were also observed. At 1000 mg/kg bw, symptoms lasted up to 6 days. Gross pathology of dead rabbits revealed partly congested organs. Batch no. P.2+3.

[e] Study design resembles OECD Test Guideline 402. The rats were given a dose of 2000, 2500 or 3000 mg/kg bw. No deaths occurred. Slight symptoms of toxicity were observed in all treatments. The symptoms included dyspnoea, ruffled fur and curved body position. Gross pathology revealed no treatment-related findings. Batch no. P.2+3.

[f] Study design resembles OECD Test Guideline 403. Rats were exposed nose only to an actual penconazole concentration of 4.0 mg/L. Vehicle was "F1", a powdered mixture of aluminium oxide and Sipernat 50 S (3:1 weight per weight). No mortality was observed. All rats displayed sedation, dyspnoea, ruffled fur and curved body position. In the vehicle control group, the symptoms were graded as slight to moderate, whereas in the penconazole group, they were graded as slight to severe. Macroscopic examination showed mottled lungs and thymus haemorrhages in the vehicle control group. In the penconazole group, no effects were observed. Mass median aerodynamic diameter was 3.5–5.4 (± 2.0) µm. Batch no. EN 603012.

macroscopically for signs of irritation at 1, 24, 48 and 72 hours and at days 4, 7 and 10 post-instillation. The study design resembles Organisation for Economic Co-operation and Development (OECD) Test Guideline 405.

An overview of the mean irritation scores of the unwashed eyes is presented in Table 14.

Table 14. Eye irritation in rabbits – Group mean scores at the 24-, 48- and 72-hour readings (unwashed eye)

	Males (*n* = 3)			Females (*n* = 3)			Males and females (*n* = 6)		
	24 h	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
Corneal opacity	0.33	0.67	0.67	1.0	1.0	1.0	0.67	0.83	0.83
Iris lesions	1.0	0.0	0.33	1.0	0	0	1.0	0.0	0.17
Conjunctival redness	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chemosis	1.0	1.0	0.33	1.0	1.0	0.33	1.0	1.0	0.33

Source: Kuhn (1988)

In the unwashed eyes, partial corneal opacity (grade 1) was seen in the majority of the animals between 24 and 72 hours, lasting for up to 4 days in two rabbits. Grade 1 iris lesions were not noted after 72 hours. Slight swelling (grade 1) of the conjunctiva was observed in all animals up to 48 hours and in two rabbits at 72 hours, whereas redness lasted for up to 7 days in three rabbits. Recovery was complete after 10 days. In washed eyes, no effects on the cornea or iris were observed. Corneal chemosis lasted for 48 hours, and redness for up to 72 hours only. The eyes were free of irritation on day 4 (Kuhn, 1988).

(d) Dermal sensitization

In a dermal sensitization study using the Magnusson and Kligman maximization test, performed in accordance with OECD Test Guideline 406, penconazole (purity 96%; batch no. EN 603012) was tested in 20 female GOHI (Himalayan spotted) guinea-pigs. The vehicle control group consisted of 10 animals. In the induction phase, the animals received 5% penconazole followed by epidermal treatment with 50% penconazole on day 8. The challenge on day 21 was performed with epidermal application of 20% penconazole. Benzocaine was used as a positive control.

After challenge application, skin reactions were evident at the application site in some animals at 24 and 48 hours. The sensitization rate was 15%. Under the conditions of this study, penconazole was not a skin sensitizer (Cantoreggi, 1998).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 90-day dietary study, penconazole (purity 98.7%; batch no. FL-840833) was administered to groups of 15 male and 15 female Crl:CD®-1(ICR)BR mice at 0, 10, 100, 300, 500, 1000 or 2400 ppm (equal to 0, 1.7, 17, 52, 85, 163 and 423 mg/kg bw per day for males and 0, 2.5, 24, 72, 116, 237 and 614 mg/kg bw per day for females, respectively). Mice were observed daily for mortality and clinical signs. A detailed physical examination was performed weekly. Body weight and feed consumption were measured weekly. Ophthalmoscopic examinations were done before study initiation on all animals and on control and high-dose animals near study termination. Prior to termination, blood and urine samples were taken from all animals for haematology, blood biochemistry and urine analysis. All mice underwent complete necropsy. Testes, ovaries, liver, heart, brain, kidneys and spleen were weighed. A wide range of organs of all mice was examined microscopically. The study design resembles OECD Test Guideline 407.

No treatment-related mortality or clinical signs were observed. Body weight, feed consumption, and ophthalmoscopic and haematological parameters were not affected. Males given 1000 or 2400 ppm had lower total protein (7–9%). Alanine aminotransferase (ALAT) activity was significantly increased (+170% versus controls) in the 2400 ppm males. Significant reductions in

gamma-glutamyltranspeptidase (GGT) activity at the 500, 1000 and 2400 ppm levels were considered not toxicologically relevant. Females at 2400 ppm had significantly lower total protein (10%) and albumin levels (14%) and albumin to globulin ratios (13%). Cholesterol level was decreased in both male and female mice at 1000 ppm (31–36%) and 2400 ppm (40–61%). Absolute and relative liver weights were significantly higher in the 2400 ppm male (41%) and female rats (32%) and in males at 500 ppm (10%) and 1000 ppm (16%). No treatment-related macroscopic changes were observed. Histopathological examination revealed centrilobular hypertrophy of hepatocytes in males at 500 and 1000 ppm and in both sexes at 2400 ppm. Degeneration of the hepatocytes around the central vein and hepatocellular vacuolation were observed in males at 2400 ppm. Focal coagulative necrosis was found in the liver of some of the 1000 and 2400 ppm males and in a few females. Frozen sections of liver stained with Oil Red O revealed no difference between control and treated rats in the amount of lipid material present. In the absence of other signs of liver toxicity, the increased hepatocyte hypertrophy in males at 500 ppm is not considered adverse. There were no other microscopic findings that could be related to the treatment.

The NOAEL was 500 ppm (equal to 85 mg/kg bw per day), based on lower total protein and cholesterol levels and focal coagulative necrosis in the liver of both sexes at 1000 ppm (equal to 163 mg/kg bw per day) (Hiles, 1987a).

In another 90-day dietary study, penconazole (purity 97.7%; batch no. WS007001) was administered to groups of 10 male and 10 female C57BL/10JfCD-1 mice at 0, 100, 500, 1500, 3000 or 5000 ppm (equivalent to 0, 14, 69, 229, 437 and 837 mg/kg bw per day for males and 0, 18, 87, 274, 545 and 983 mg/kg bw per day for females, respectively). Mice in the 5000 ppm group were killed for humane reasons during the second week of the study. Mice were observed daily for mortality and clinical signs. A detailed physical examination and body weight measurements were performed daily during the first 2 weeks and weekly thereafter. Feed consumption was measured weekly. At termination during week 2 for the 5000 ppm mice and on day 92 for all other surviving mice, blood was sampled for clinical chemistry. All mice underwent complete necropsy. Testes, epididymides, uterus, liver, heart, brain, kidneys, adrenals and spleen were weighed. Adrenals, brain, epididymides, ovary, kidney, liver and testis from the control and 3000 ppm groups and livers from the 100, 500 and 1500 ppm groups were examined microscopically.

At 5000 ppm, the mice lost weight (8–11%) during the first week of treatment. The mice in this group were killed for humane reasons in week 2. No treatment-related mortality was observed in the other dose groups. At 3000 ppm, the mice initially lost weight (up to 4–5%). At the end of the study, the body weights of males at 1500 and 3000 ppm were 6% and 15% lower than control male values, respectively. Females at 3000 ppm had a 11% lower body weight than control females at termination. Feed consumption was reduced in both sexes at 3000 ppm (25–26%) and 5000 ppm (26–44%) on day 1. Feed utilization was less efficient in both sexes at 3000 ppm and in males at 1500 ppm than in controls. Significant reductions in cholesterol levels were observed at 1500 ppm (42–43%), 3000 ppm (52–54%) and 5000 ppm (67–71%). Small reductions in cholesterol level were seen at 500 ppm (10–29%). In the 3000 ppm group, plasma alkaline phosphatase (ALP) activity was slightly increased (22–25%). At 5000 ppm, ALP activity was increased in both sexes by 51–95%. Plasma albumin and plasma total protein were slightly lower (6–8%) in females at 1500 and 3000 ppm. In females at 5000 ppm, albumin levels were decreased by 17%. There was a slight reduction (20%) in triglyceride levels in both sexes at 3000 ppm and a more marked reduction (42–52%) at 5000 ppm. Relative liver weights were increased by 12%, 33% and 48% for males at 500, 1500 and 3000 ppm, respectively, and by 10% and 27% for females at 1500 and 3000 ppm, respectively. Hepatocyte hypertrophy and increased nuclear pleomorphism were present in all males at 1500 and 3000 ppm. Hepatocyte hypertrophy was also observed in 4/10 females treated with 3000 ppm. A small increase in the incidence of mononuclear cell infiltration of the liver observed in males and females treated with 3000 ppm was considered not to be of toxicological significance in view of it being a common spontaneous change in mice. Adrenal weights adjusted for body weight were higher than control values in females receiving 3000 ppm owing to high values for three females. When adrenal weights

for these animals were excluded, there were no differences between adrenal weights in treated and control animals.

The NOAEL was 500 ppm (equal to 69 mg/kg bw per day), based on reductions in cholesterol levels in both sexes, a reduction in total protein and albumin levels in females, and a reduction in body weight gain and increased nuclear pleomorphism in hepatocytes in males at 1500 ppm (equal to 229 mg/kg bw per day) (Milburn, 2002).

Rats

In a 28-day toxicity study, penconazole (purity 91.7%; batch no. P.11-14) was administered by gavage to groups of 10 male and 10 female Tif: RAI f (SPF) rats at 0, 20, 100 or 500 mg/kg bw per day. The vehicle was aqueous methyl cellulose. From day 8 of the study, doses were increased to 0, 100, 500 and 1000 mg/kg bw per day, respectively. Animals were checked daily for mortality and clinical signs of toxicity. Body weights and feed and water consumption were measured weekly. Ophthalmoscopy was performed before treatment started and towards termination. Blood and urine were sampled at termination for haematology, clinical biochemistry and urine analysis. All rats were necropsied, and weights of liver, spleen, kidneys, heart, brain, thyroid, thymus, gonads and adrenals were recorded. These organs were also examined microscopically.

No deaths were observed. At 1000 mg/kg bw per day, marked apathy and lateral body position after administration of the test substance on day 10 of dosing were observed in three rats. Body weight gain was reduced in both sexes from week 2 onward at 500 mg/kg bw per day (up to 15–17%) and 1000 mg/kg bw per day (up to 21–35%). Feed consumption during weeks 2–4 was decreased at 500 mg/kg bw per day (8–9%) and 1000 mg/kg bw per day (12–19%). Water consumption was increased (31%) in high-dose females. Slight decreases in haemoglobin and haematocrit were observed in females at 500 and 1000 mg/kg bw per day. In males and females at 500 and 1000 mg/kg bw per day, glucose, cholesterol, protein, sodium and calcium levels and the activities of ALP and ALAT were slightly increased, whereas potassium and chloride levels were decreased. In addition, in females at these doses, glucose and inorganic phosphate levels were increased, and creatinine levels were slightly decreased. Urine volume was increased in both sexes at the middle and high doses. Absolute and relative weights of the liver, kidneys and adrenals were dose-dependently increased at 500 and 1000 mg/kg bw per day in both sexes. In high-dose females, absolute and relative thyroid weights were also slightly increased. Enlarged livers and hepatocyte hypertrophy were observed in some mid-dose rats and all high-dose rats.

The data indicate that various adverse effects, in particular liver toxicity, occur at doses above 100 mg/kg bw per day. However, owing to the change in dose levels after week 1, no definite NOAEL could be identified from this study (Basler, 1984).

In a 28-day toxicity study, the toxicities of two batches of penconazole were compared. Penconazole “batch A” (purity 96.2%; batch no. Op.3-23.01.90) or penconazole “batch B” (purity 96.1%; batch no. EN 603012) was administered by gavage to groups of 10 male and 10 female Tif: RAI f (SPF) rats at 0, 100 or 500 mg/kg bw per day. The vehicle was 0.5% aqueous methyl cellulose with 0.1% Tween 80. Animals were checked daily for mortality and clinical signs of toxicity. Body weights and feed consumption were measured weekly. Blood was sampled at termination for haematology and clinical biochemistry. All rats were necropsied, and weights of liver, spleen, kidneys, testes, heart, brain, thyroid, thymus, gonads and adrenals were recorded. These organs (except for the brain and gonads) and the parathyroid glands and lungs were also examined microscopically.

At the high dose, one male and three females were killed in a moribund condition. At 500 mg/kg bw per day in animals treated with batch B, hunch-backed posture, piloerection and laboured breathing were observed. Body weight gain was not affected by treatment. At 500 mg/kg bw per day (batches A and B), feed consumption was reduced by 10–13% in both sexes. A decreased prothrombin time was observed in male and female rats at the low dose (6–10%) and high dose (15–

18%). Slightly higher platelet counts occurred in both sexes at 500 mg/kg bw per day (12–30%), but this was significant in males only. There was an increase in plasma protein concentrations (3–5% at the low dose, 7–9% at the high dose) associated with higher globulin levels and minimally lower albumin to globulin ratios and a decrease (22–28%) in total bilirubin at both doses. At 500 mg/kg bw per day, increased plasma albumin (5–14%, males) and cholesterol levels (25–91%, both sexes) and decreased chloride levels (4–6%, both sexes) were observed. Glucose levels were increased in females at 100 mg/kg bw per day (16–17%) and 500 mg/kg bw per day (39–44%). Urea levels were increased (17–38%) at 500 mg/kg bw per day in both sexes (significant only in the 500 mg/kg bw per day group receiving batch A). Relative liver weights were increased at 100 mg/kg bw per day (8–13%) and 500 mg/kg bw per day (45–60%). Relative kidney weights were increased at 500 mg/kg bw per day (15–22%). Male rats had increased relative thyroid weights at 100 mg/kg bw per day (11–40%) and 500 mg/kg bw per day (34–53%). At the high dose, ovary weights were increased (15–17%). Histopathology showed minimal hypertrophy of the centrilobular hepatocytes in all treated groups in males and at 500 mg/kg bw per day in females, minimal to moderate hepatocellular necrosis at 500 mg/kg bw per day in males and inflammatory cell infiltrations in the liver of both sexes at 500 mg/kg bw per day. At both doses, minimal hypertrophy of the follicle epithelium of the thyroid was seen in both sexes. High-dose females showed cortical atrophy of the adrenal glands and minimal extramedullary haematopoiesis in the spleen. The data showed that both batches induced similar toxicity, although the effects tended to be less accentuated in the rats treated with batch A.

A NOAEL could not be identified. The LOAEL for both batches of penconazole was 100 mg/kg bw per day, based on changes in clinical chemistry and haematology parameters and minimal hypertrophy of the follicle epithelium of the thyroid at all doses (Fankhauser, 1991).

In a 13-week dietary toxicity study, penconazole (purity 91.7%; batch no. P.11-14) was administered to groups of 20 male and 20 female Tif: RAI f (SPF) rats at 0, 30, 300 or 3000 ppm (equal to 0, 2.0, 19 and 202 mg/kg bw per day for males and 0, 2.1, 21 and 209 mg/kg bw per day for females, respectively). Animals were checked daily for mortality and clinical signs (including neurological, oral and behavioural inspection). Ophthalmological examinations and a hearing test were conducted at the beginning and towards the end of the treatment period. Body weights and feed consumption were measured weekly. Water consumption was measured monthly. Blood was sampled for haematology and clinical biochemistry at termination. Urine analysis was not performed. All rats were necropsied, and weights of liver, spleen, kidneys, testes, ovaries, heart, brain, thymus and adrenals were recorded. A wide range of tissues of all rats was examined microscopically.

No mortalities or clinical signs were observed. Ophthalmoscopy and the hearing test revealed no treatment-related effects. Significant reductions in body weight gain (26%) and feed consumption (10%) were found in high-dose females. Occasional changes in haematology and clinical chemistry were small and within the historical control range and were considered not to be toxicologically relevant. Absolute (21–22%) and relative liver weights (27–40%) were increased in both sexes at 3000 ppm. In the absence of other signs of liver toxicity, these increases in liver weight are not considered adverse. In the 3000 ppm males, both the absolute and relative testes weights were slightly higher (5% and 10%, respectively). Necropsy revealed no treatment-related effects. Histopathology revealed minimal hypertrophy of the hepatocytes in both sexes at 3000 ppm.

The NOAEL was 300 ppm (equal to 19 mg/kg bw per day), based on reduced body weight gain and feed consumption in females and increased testes weight observed at 3000 ppm (equal to 202 mg/kg bw per day) (Basler, 1982).

In another 13-week dietary toxicity study, penconazole (purity 91.7%; batch no. P.11-14) was administered to groups of 20 male and 20 female Tif: RAI f (SPF) rats at 0, 10, 30 or 100 ppm (equal to 0, 0.77, 2.1 and 7.1 mg/kg bw per day for males and 0, 0.78, 2.1 and 7.3 mg/kg bw per day for females, respectively). Animals were checked daily for mortality and clinical signs (including neurological, oral and behavioural inspection). Ophthalmological examinations and a hearing test

were conducted at the beginning and towards the end of the treatment period in control and 100 ppm rats. Body weights and feed consumption were measured weekly. Water consumption was measured weekly during the first month and monthly thereafter. Blood was sampled for haematology and clinical biochemistry at termination. Urine analysis was not performed. All rats were necropsied, and weights of liver, kidneys, testes, ovaries, heart, brain, thymus and adrenals were recorded. A wide range of tissues of all rats was examined microscopically.

No mortalities or clinical signs were observed. No treatment-related effects on body weight gain, feed and water consumption, or ophthalmoscopy or in the hearing test were observed. Occasional changes in haematology and clinical chemistry were small and within the historical control range and were considered not to be toxicologically relevant. Absolute and relative liver weights were increased in males at 10 and 30 ppm (up to 23%), but not at 100 ppm. Relative kidney weights were slightly decreased in females at 10 and 30 ppm (11%) and 100 ppm (7%). In the absence of a dose–response relationship and other signs of liver or kidney toxicity, these changes in organ weight were considered not to be adverse. In the 3000 ppm males, both the absolute and relative testes weights were slightly higher (5% and 10%, respectively). Necropsy and histopathology revealed no treatment-related effects.

The NOAEL was 100 ppm (equal to 7.1 mg/kg bw per day), the highest dose tested (Basler, 1983).

In a third 13-week dietary toxicity study, penconazole (purity 98.7%; batch no. FL-840833) was administered to groups of 15 male and 15 female Charles River (Crl:CD(SD)BR) rats at 0, 10, 100, 300, 500, 1000 or 2400 ppm (equal to 0, 0.81, 7.5, 23, 38, 72 and 179 mg/kg bw per day for males and 0, 0.96, 9.1, 28, 45, 86 and 209 mg/kg bw per day for females, respectively). Animals were checked daily for mortality and clinical signs (including neurological, oral and behavioural inspection). Detailed physical examination was performed weekly. Ophthalmological examinations were conducted at the beginning of the study in all rats and towards the end of the treatment period in control and 2400 ppm rats. Body weights and feed consumption were measured weekly. Blood was sampled for haematology and clinical biochemistry at termination. Urine analysis was performed during week 13. All rats were necropsied, and weights of liver, kidneys, testes, ovaries, heart, brain, spleen and adrenals were recorded. A wide range of tissues of all rats was examined microscopically.

No mortalities or clinical signs were observed. Over the entire study duration, decreases in body weight gain (15%) and feed consumption (9%) were observed in females at 2400 ppm. In males at 1000 and 2400 ppm, body weight gain was decreased (10%) during the first week of treatment. Ophthalmoscopy and haematology showed no effects of treatment. Occasional changes in clinical chemistry were small, often not dose related and within the historical control range and were considered not to be toxicologically relevant. In all male treatment groups, urea nitrogen was slightly, but significantly, higher (12–35%) than in the control group. The creatinine level was marginally increased in the 1000 ppm ($P < 0.05$) and 2400 ppm (statistically not significant) males, as well as in the 500, 1000 and 2400 ppm females (not significant). Total protein level was somewhat increased (9%) in males at 2400 ppm, and the albumin to globulin ratio was marginally lower (10%) in the males at 1000 and 2400 ppm. The albumin to globulin ratio was also significantly lower (10%) in females given 2400 ppm because of significantly lower albumin. The changes were not accompanied by other evidence of primary renal toxicity. Absolute and relative liver weights were increased in males at 1000 and 2400 ppm (up to 31%) and in females at 500, 1000 and 2400 ppm (up to 29%). The absolute and relative weights of the left adrenal were significantly lower in males at 100 and 500 ppm, but not at higher doses. No changes in adrenal weights were observed in females. Therefore, the effects on adrenal weights in males were not considered to be related to the treatment. At 2400 ppm, females showed significantly lower body weights, resulting in significantly higher relative weights for the brain, left adrenal, left ovary, and left and right kidney. Histopathological examination showed increased incidences of centrilobular hypertrophy of hepatocytes in the 1000 and 2400 ppm males and females and a slightly increased incidence in males at 500 ppm. Degeneration of the hepatocytes

around the central vein was observed in males and females at 2400 ppm. The incidence of hepatocellular vacuolation was dose-dependently increased in males at 500, 1000 and 2400 ppm.

The NOAEL was 300 ppm (equal to 23 mg/kg bw per day), based on an increased incidence of hepatocellular vacuolation and hypertrophy at 500 ppm (equal to 38 mg/kg bw per day) (Hiles, 1987c).

Dogs

In a 1-year dietary toxicity study, 10 male and 10 female Beagle dogs per dose group received penconazole (purity 91.7%; batch no. P.11-14) at a dietary concentration of 0, 100, 500 or 5000 ppm (equal to 0, 3.1, 16.9 and 133 mg/kg bw per day for males and 0, 3.3, 16.7 and 139 mg/kg bw per day for females, respectively). During week 20, the highest dose was reduced to 2500 ppm (equal to 86 mg/kg bw per day for males and 89 mg/kg bw per day for females) because of excessive reduction in feed consumption and body weight gain for the animals in that group.

Four males and four females per dose group were killed after 13 weeks or 1 year of treatment, whereas two dogs of each sex per dose were killed at the end of a 4-week recovery period following 1 year of treatment. The dogs were checked daily for mortality and clinical signs. Feed consumption was measured daily, and body weights were measured weekly. Ophthalmological and auditory examinations were performed pretreatment, in week 13, in week 52 or 56, and in the week before termination. Haematology, clinical chemistry and urine analysis were performed pretrial and during weeks 13, 26, 52 and 56. All dogs were necropsied, and weights of brain, thyroid, heart, liver, adrenals, kidneys, testes and ovaries were recorded. Histology was performed on a large selection of organs.

None of the dogs died during the study. An increased incidence of vomiting was seen in both sexes at 5000 ppm and in females at 2500 ppm. Ophthalmological and auditory examinations revealed no treatment-related effects.

During the first 13 weeks of treatment, slight reductions in body weight gain (2–27%) were observed at 500 ppm, whereas at 5000 ppm, the dogs lost body weight (10–12%). Feed consumption was drastically reduced during the first 19 weeks of treatment at 5000 ppm (42–53% during week 1). After lowering the dose to 2500 ppm, no difference in feed consumption was observed in males, whereas feed consumption tended to be increased in high-dose females. At 13 weeks, reduced haemoglobin levels (9%) and erythrocyte counts (10%) were observed in males at 5000 ppm. However, similar changes were also observed before treatment started, and therefore these changes are not considered to be toxicologically relevant. At 13 weeks, increased activities of ALP (~400%), GGT (~1000%), aspartate aminotransferase (ASAT) (~150%), ALAT (~800%) and ornithine carbamoyltransferase (OCT, ~450%) were observed in both sexes at 5000 ppm. At 5000 ppm, there was a decrease in glucose levels (8–12%, both sexes), decreased urea nitrogen (females), increased bilirubin (32%, females), increased total globulin (12%, males), increased sodium concentrations (3%, females) and decreased chloride concentration (3%, males). Urine analysis showed no treatment-related changes. Absolute and relative liver weights of males and females were higher at 500 (15–24%) and 5000 ppm (absolute, 30–41%; relative, 75–88%). Kidney weights of both sexes at 5000 ppm were increased (absolute, 16–18%; relative, 54–60%), as was the relative adrenal weight of the 5000 ppm females (38%). Testes weight was reduced at 5000 ppm (absolute, 48%; relative, 27%).

At 13 weeks, all 5000 ppm dogs and one 500 ppm male showed minimal, multifocal changes in the liver in the form of monocellular hepatocyte necrosis associated with minimal inflammatory cell infiltration. Small, circumscribed foci (with loss of hepatocytes, haemorrhage) and inflammatory cell infiltration were also found in some dogs. Two males at 5000 ppm also had vacuolation of the hepatocyte cytoplasm that was considered to be degenerative in nature. All males at 5000 ppm had a moderate to marked reduction in spermatogenic activity, characterized by atrophy of the seminiferous epithelium associated with formation of giant cells and absence of spermatozoa in the epididymis (which contained cellular debris).

After 12 months of treatment, body weight gain over the entire study was not affected at 100 ppm, whereas it was slightly reduced at 500 ppm (−20%) and markedly reduced at 2500 ppm (−40% in males, −60% in females). After reduction of the dose level from 5000 ppm to 2500 ppm in week 20, the high-dose animals gained weight faster than the animals of the other groups, including the controls. After lowering the dose to 2500 ppm, feed consumption returned to normal. No treatment-related changes in haematology were observed at 26 or 52 weeks. After reduction of the highest concentration of 5000 ppm to 2500 ppm, most of the metabolic parameters affected at the 13-week investigation returned to normal (e.g. urea nitrogen, bilirubin, sodium and chloride concentrations). OCT activity remained increased in the 2500 ppm animals (300–1700%, both sexes) until termination of the treatment. Total globulin level was increased (16%) in males at 2500 ppm at weeks 26 and 53. Levels of the electrolytes sodium and chloride showed slight deviations in the 5000 ppm group at 13 weeks, but were comparable with control values after the dietary concentration had been reduced to 2500 ppm. Slight increases in inorganic phosphate levels that were observed in high-dose females throughout the study were within normal biological ranges. At 2500 ppm, increased activities of ALP (~400%), GGT (~300–500%), ASAT (~100%) and ALAT (~400–600%) were observed at 26 and 52 weeks. Liver weights of females were higher at 500 ppm (absolute, 27%; relative, 28%) and in both sexes at 2500 ppm (absolute, 27–46%; relative, 35–63%). Kidney weights of both sexes at 2500 ppm were increased (absolute, 12–25%; relative, 21–39%). In females, increased relative weights of heart and ovaries were observed. In the absence of a dose–response relationship and histopathological changes in these organs, these observations were considered not to be treatment related. Histopathology showed minimal lesions of the liver in all dogs at 2500 ppm and in some dogs at 500 ppm. These lesions were characterized by monocellular hepatocyte necrosis associated with inflammatory cell infiltration. In addition, two 2500 ppm males showed absence of spermatozoa in the epididymis due to atrophy of the seminiferous epithelium.

After 4 weeks of recovery following the 12-month treatment period, most affected clinical chemistry parameters and enzyme activities returned to normal in the high-dose dogs (ALP, GGT, ASAT) or were at least clearly lower than at the end of the treatment (ALAT, OCT). Histopathology in recovery animals showed hepatocyte necrosis associated with inflammatory cell infiltration in one high-dose male. Minimal to moderate bilateral tubular atrophy was still present in both males of the high-dose group after the 4-week recovery period.

The NOAEL was 100 ppm (equal to 3.1 mg/kg bw per day), based on reduced body weight gain, increased absolute and relative liver weights, and slight histopathological changes in the liver (hepatocyte necrosis associated with inflammatory cell infiltration) in males and females at 500 ppm (equivalent to 16.7 mg/kg bw per day) (Gfeller, 1984).

(b) Dermal application

Groups of five male and five female New Zealand White rabbits were dermally exposed to penconazole (purity 91.7%; batch no. P.11-14) moistened with water at a dose of 0, 1000, 1500 or 2000 mg/kg bw per day for 6 hours/day, 5 days/week, for 3 weeks. The test item was applied to the clipped dorsal area (about 10% of the body surface) and held in contact with the skin with gauze patches. Patches were covered with aluminium foil and fastened with adhesive tape. A satellite group of the same size received the 2000 mg/kg bw per day treatment for 3 weeks and was then observed for a further 2-week recovery period without treatment. The rabbits were checked daily for mortality and clinical signs. Body weights were recorded weekly, and feed consumption was recorded twice weekly. Haematology and clinical chemistry were performed at the end of the study. At termination of the study, all animals were killed and necropsied. Weights of heart, spleen, brain, liver, thymus, adrenals, kidneys, testes and ovaries were recorded. Histology was performed on a selection of organs and tissues.

No mortality and no treatment-related clinical signs were observed. No dermal irritation was noted. Body weight gain, feed consumption, haematological and clinical chemistry parameters, and organ weights were not affected. Macroscopic and histopathological examination revealed no effect of treatment with penconazole.

The NOAEL was 2000 mg/kg bw per day, the highest dose tested (Seifert, 1983).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a 2-year dietary carcinogenicity study, performed according to OECD Test Guideline 453, penconazole (purity 91.7%; batch no. P.11-14) was administered to groups of 80 male and 80 female Tif: MAG f (SPF) mice at 0, 5, 75, 150 or 300 ppm (equal to 0, 0.75, 9.8, 19 and 41 mg/kg bw per day for males and 0, 0.67, 8.8, 17 and 36 mg/kg bw per day for females, respectively). Fifty animals of each sex per dose were used for the evaluation of carcinogenic potential, 20 animals of each sex per dose were used for laboratory investigations up to 24 months and 10 animals of each sex per dose were used for an interim kill after 12 months. The mice were checked daily for mortality and clinical signs. Body weight and feed consumption were recorded weekly during the first 3 months and monthly thereafter. Water consumption was measured at weeks 4, 8, 12, 18 and 23 of the study. A hearing test and eye examination were performed on control and high-dose animals pretreatment and on days 177, 360, 568–569 and 716. Blood and urine from the mice destined for laboratory investigations were sampled for haematology, clinical chemistry and urine analysis at weeks 14 (except blood chemistry), 27, 52, 81 and 105. Animals found dead, killed prematurely or killed at the end of the treatment period were weighed and necropsied. Organ weights (adrenals, brain, heart, liver, kidneys, lungs, spleen, thyroid, ovaries, testes, prostate and pituitary) were recorded. A wide range of tissues was examined microscopically.

No effect of treatment with penconazole on mortality or clinical signs was observed. The hearing tests and eye examinations revealed no effect of treatment. Body weight gain, feed and water consumption, and feed efficiency were not affected by treatment. Occasionally observed statistically significant differences in haematological, clinical chemistry and urine analysis parameters relative to controls did not show any systematic or dose-dependent pattern and were attributed to normal spontaneous physiological variations. After 53 weeks, relative liver weights were marginally increased in males at 300 ppm (10%). In the absence of related findings on clinical chemistry and histopathology, this increase was not considered to be adverse. Macroscopic and histopathological examination did not reveal an effect of treatment. There were no treatment-related increases in the incidence of neoplastic lesions.

The NOAEL was 300 ppm (equal to 36 mg/kg bw per day), the highest dose tested. Penconazole was not carcinogenic in Tif: MAG f (SPF) mice under the conditions of the study (Basler, 1985a).

In an 80-week dietary carcinogenicity study performed according to OECD Test Guideline 451, penconazole (purity 97.7%; batch no. WS007001[CH]) was administered to groups of 50 male and 50 female C57BL/10J$_F$CD-1 mice at 0, 25, 200 or 1500 ppm (equal to 0, 2.7, 22 and 178 mg/kg bw per day for males and 0, 3.5, 28 and 222 mg/kg bw per day for females, respectively). The mice were checked daily for mortality and clinical signs. A detailed clinical examination was performed weekly. Body weight was recorded weekly during weeks 1–15 and biweekly thereafter. Feed consumption was recorded weekly during weeks 1–13, in week 16 and every fourth week thereafter. Feed utilization efficiency was calculated every month for the first 13 weeks. Blood smears prepared from blood collected after 53 weeks and at termination were examined. Animals found dead, killed prematurely or killed at the end of the treatment period were weighed and necropsied. Organ weights (adrenals, brain, heart, liver including gallbladder, kidneys, lungs, spleen, ovaries, uterus and cervix, testes, epididymides and prostate) were recorded. A wide range of tissues was examined microscopically.

Mortality at 80 weeks was not affected by treatment. The number of males with thin appearance was increased at 1500 ppm. Body weight gain was decreased at 1500 ppm in males (29%) and females (23%). Feed intake was not affected, but feed utilization was less efficient in both sexes at the high dose. Macroscopic and blood smear examination did not reveal an effect of treatment. In

high-dose males, liver weights were increased (absolute, 11%; relative, 27%). Slight reductions in kidney weights (< 10%) in both sexes at 1500 ppm were not considered treatment related. At 1500 ppm, spleen weights were reduced in males (absolute, 31%; relative, 23%) and females (absolute, 41%; relative, 37%). Histopathological examination showed an increased incidence and severity of hepatocellular vacuolation in both sexes at 1500 ppm. There were no treatment-related increases in the incidence of neoplastic lesions.

The NOAEL was 200 ppm (equal to 22 mg/kg bw per day), based on decreased body weight gain and absolute and relative spleen weights and increased incidence and severity of hepatocellular vacuolation in both sexes and increased absolute and relative liver weights in males at 1500 ppm (equal to 178 mg/kg bw per day). Penconazole was not carcinogenic in C57BL/10J$_f$CD-1 mice under the conditions of the study (Milburn, 2004).

Rats

In a chronic (27-month) dietary toxicity study performed according to OECD Test Guideline 453, penconazole (purity 91.7%; batch no. P.11-14) was administered to groups of 80 male and 80 female Tif: RAI f (SPF) rats at 0, 5, 75, 150 or 300 ppm (equal to 0, 0.30, 3.8, 7.3 and 15 mg/kg bw per day for males and 0, 0.31, 4.0, 8.1 and 17 mg/kg bw per day for females, respectively). Fifty animals of each sex per dose were used for the evaluation of carcinogenic potential, 20 animals of each sex per dose were used for laboratory investigations up to 24 months and 10 animals of each sex per dose were used for an interim kill after 12 months. The rats were checked daily for mortality and clinical signs. Body weight and feed consumption were recorded weekly during the first 3 months and monthly thereafter. Water consumption was measured monthly during the first 5 months. A hearing test and eye examination were performed on control and high-dose animals pretreatment and on days 177, 361, 568–570 and 730–732. Blood and urine from the rats destined for laboratory investigations were sampled for haematology, clinical chemistry and urine analysis at weeks 14 (except blood chemistry), 27, 52, 81, 104 and 116–117. Animals found dead, killed prematurely or killed at the end of the treatment period were weighed and necropsied. Organ weights (adrenals, brain, heart, liver, kidneys, lungs, spleen, thyroid, ovaries, testes, prostate and pituitary) were recorded. A wide range of tissues was examined microscopically.

There were no effects of treatment on mortality or clinical signs. The hearing tests and eye examinations revealed no effect of treatment. Body weight gain, feed and water consumption, and feed efficiency were not affected by treatment. GGT levels were increased (41–120%) in high-dose females at 27 and 52 weeks. Other occasionally observed statistically significant differences in haematological, clinical chemistry and urine analysis parameters relative to controls did not show any systematic or dose-dependent pattern and were attributed to normal spontaneous physiological variations. Relative liver weights were increased in females at 150 and 300 ppm (13–15%) after 52 weeks, and absolute liver weights were increased in 300 ppm females (13%) after 104 weeks. Absolute and relative pituitary weights were decreased in high-dose males (25–29%) at 1 year, but not after 2 years. A trend (statistically not significant) to higher absolute spleen weights (10–16%) was seen in treated male and female rats at 1 year. As this was not confirmed at the 2-year time point and as relative spleen weights were not affected, this finding is not considered to be toxicologically relevant. There were a number of statistically significant differences in a few other organ weights relative to controls, but as these were not dose related, they are considered to be unrelated to the treatment. Histopathological examination revealed no effect of treatment. The incidence and type of tumours were not affected by treatment with penconazole.

The NOAEL was 150 ppm (equal to 8.1 mg/kg bw per day), based on increased absolute and relative liver weights and an increase in GGT levels at 1 year in females at 300 ppm (equal to 17 mg/kg bw per day). Penconazole was not carcinogenic in Tif: RAI f (SPF) rats under the conditions of the study (Basler, 1985b).

2.4 Genotoxicity

The results of the genotoxicity tests with penconazole are summarized in Table 15. All tests gave negative results.

Table 15. Overview of genotoxicity tests with penconazole[a]

Test	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Gene mutations	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537	10–2 560 µg/plate (±S9)	91.7	Negative	Deparade (1984)[b]
Gene mutations	*S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537; *Escherichia coli* WP2 uvrA	12.5–5 000 µg/plate (±S9)	96.1	Negative	Deparade (1999a)[c]
Gene mutations	V79 Chinese hamster cells, HPRT test	5–80 µg/mL (±S9)	96	Negative Study not fully adherent to OECD guideline	Ogorek (1999a)[d]
Chromosomal aberrations	Chinese hamster ovary cells	6.25–50 µg/mL (–S9) 6.25–25 µg/mL (–S9)	96	Negative	Ogorek (1999b)[e]
Unscheduled DNA synthesis	Male Tif: RAI f (SPF) rat hepatocytes	0.32–40 µg/mL	91.7	Negative	Puri (1984)[f]
In vivo					
Micronucleus formation	ICO:CD-1(CRL) mouse bone marrow	Gavage dose of 200, 400 or 800 mg/kg bw in males and 125, 250 or 500 mg/kg bw in females	96.1	Negative	Deparade (1999b)[g]

bw: body weight; DNA: deoxyribonucleic acid; HPRT: hypoxanthine–guanine phosphoribosyltransferase; OECD: Organisation for Economic Co-operation and Development; S9: 9000 × *g* supernatant fraction from rat liver homogenate

[a] Positive and negative (solvent) controls were included in all studies.

[b] Batch no. P.11-14. Study design resembles OECD Test Guideline 471. Without S9, cytotoxicity was observed at 640 and 2560 µg/plate (first assay) or 2560 µg/plate (second assay). With S9 activation, cytotoxicity occurred at 2560 µg/plate.

[c] Batch no. EN 603012. Performed according to OECD Test Guideline 471.

[d] Batch no. EN 602012. Performed according to OECD Test Guideline 476. In preliminary tests, cytotoxicity was complete or excessive at concentrations of 50 µg/mL and above.

[e] Batch no. EN 602012. Performed according to OECD Test Guideline 473. In preliminary tests with and without S9, inhibition of cell growth was complete or almost complete at concentrations of 100 µg/mL and above.

[f] Batch no. P.11-14. Study design resembles OECD Test Guideline 482, which was deleted in 2014. Vehicle was dimethyl sulfoxide. Cytotoxicity data were not reported. In the report, it is stated that "from the results obtained, the highest usable concentration was calculated to be 40 µg/mL".

[g] Batch no. EN 603012. Performed according to OECD Test Guideline 474. Vehicle was 0.5% carboxymethyl cellulose in water. Five male and five female young adult mice were used per group. Groups of animals treated at the highest dose or with the vehicle alone were killed 24 and 48 hours after administration, whereas animals administered the intermediate or lowest dose or the positive control substance were killed 24 hours after administration. The high-dose males showed occasional signs of toxicity (ventral recumbence, hunched posture, reduced locomotor activity). No effect of penconazole on polychromatic erythrocyte/normochromatic erythrocyte ratio was observed. Toxicokinetic studies (Van Dijk, 1987; Hassler, 1999) show that radioactivity does reach the bone marrow, albeit in low concentrations.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a two-generation dietary reproductive toxicity study, Tif: RAI f (SPF) albino rats (20 of each sex per group for the F_0 and F_1 generations) were fed penconazole (purity 91.7%; batch no. P.11-14) at a dietary concentration of 0, 80, 400 or 2000 ppm. The average dietary intakes over the premating, gestation and lactation periods were equal to 0, 5.5, 29 and 146 mg/kg bw per day for males and 0, 7.5, 40 and 202 mg/kg bw per day for females of the F_0 generation and 0, 6.5, 31 and 166 mg/kg bw per day for males and 0, 8.5, 43 and 227 mg/kg bw per day for females of the F_1 generation, respectively. F_0 adults were treated over a 9-week premating period and throughout the 3-week mating period, gestation and 21-day lactation of the F_1 pups. At postnatal day (PND) 28, 20 weanling rats of each sex per dose were selected for producing the F_2 generation. The remaining weanlings were killed and checked for macroscopic anomalies. The F_1 parental rats were treated for 9 weeks before mating. Clinical examination was performed daily. Detailed observations were made on maternal F_0 and F_1 animals on PNDs 0, 4, 7, 14 and 21. Body weights of parental rats were recorded weekly. In addition, females were weighed on gestation days (GDs) 0, 7, 14 and 20 and PNDs 0, 4, 7, 14 and 21. Feed consumption was recorded weekly during the premating period. In addition, feed consumption of parental females was measured on GDs 0, 6, 11, 16 and 21 and on PNDs 1, 6, 11, 16 and 21. Fertility and mating indices of males and females, copulatory interval and the length of gestation were determined. All litters were examined for number of pups, sex of pups, number of stillbirths, number of live births and gross anomalies. Pups were weighed on PNDs 0, 4, 7, 14 and 21. All pups were checked daily for mortality and clinical signs. The following developmental parameters were assessed in all pups: pinna unfolding (PND 4), onset of hair growth (PND 5), incisor eruption (PND 8), eye opening and righting reflex (PND 14) and photophobotaxis (PNDs 21–23). In pups selected for breeding the F_2 generation, the following parameters were assessed: cliff avoidance, palmar grasp ability, negative geotaxis, exploratory behaviour (PNDs 29–32), vaginal opening (PND 30), pupillary reflex (PNDs 35–40), hearing ability and exploratory behaviour (PNDs 40–45). After weaning, the parental rats were killed and examined macroscopically. In males of the F_0 generation, testes and epididymides were weighed. In the F_1 adult generation and in five F_1 and F_2 weanlings of each sex per dose, weights of adrenals (adults only), brain, kidneys, heart, liver, lungs (weanlings only), ovaries, spleen, testes and thymus were recorded. A wide range of organs from these animals was examined histopathologically.

One control F_1 female, two 400 ppm females (one F_0, one F_1) and six 2000 ppm females (three F_0, three F_1) died during the lactation period. The cause of death was not reported consistently (e.g. for one dam, it is reported that it died during delivery, was killed due to spontaneous death of all pups or was killed in a moribund state). For some dams, it is not clear whether litters were delivered successfully. The interpretation of the reported mortalities is therefore difficult. No clinical signs were reported. During the premating phase, body weight gain was slightly decreased at 2000 ppm (8% in F_0 females, 3% in F_1 males, 7% in F_1 females). During gestation, a slight reduction in body weight gain (16%) was observed in F_1 females at 2000 ppm. Feed consumption was slightly reduced (4%) in females at 2000 ppm. These slight reductions in body weight gain and feed consumption were considered not to be toxicologically relevant. Relative liver weights were increased at 2000 ppm in F_1 parental males (13%) and females (37%). Relative testes weight was slightly increased in F_1 males at 2000 ppm; however, absolute testes weight and testes weight relative to brain weight were not affected. In the liver of adult F_1 rats, slight hypertrophy of the hepatocytes was observed at 400 and 2000 ppm, mainly in the centrilobular region. In 2/16 adult F_1 females at 2000 ppm, slight recent necrosis in the liver was found. In the absence of other histopathological changes, the slight increase in hypertrophy in rats at 400 ppm and in male rats at 2000 ppm is not considered to be toxicologically relevant. A slightly lower gestation index (79–84% versus 95–100% in controls) was observed at 2000 ppm. Gestation duration was slightly increased (from 0.7 to 0.8 day) at 2000 ppm. In both generations, the mean number of implantation losses was slightly increased in all treated groups (not dose related). Reproductive performance, mating and fertility indices were not impaired in F_0 or F_1 parents. In both generations, the survival index at PND 4 was not affected by treatment, but the postnatal mortality rates of the pups were markedly increased in all treated and control groups during

the second week of lactation, particularly for F_1 pups from the control group and to a lesser extent in the 80 and 400 ppm groups. Mean body weight gains of F_1 pups appeared to be lower at weaning at the 2000 ppm level (18% and 13% in male and female pups, respectively, compared with controls; not significant). However, this is likely to be a consequence of the higher litter size at weaning at 2000 ppm (13.2 pups/litter) compared with controls (9.9 pups/litter). The general development and behaviour of the offspring were not affected by treatment. In the F_1 and F_2 weanlings, increased relative liver weights (22–29%) were observed. In F_1 and F_2 weanlings, no histopathological changes were seen in treated or control animals. In the absence of histopathological changes, the increase in relative liver weights in weanlings at 2000 ppm is not considered to be toxicologically relevant.

The NOAEL for parental toxicity was 400 ppm (equal to 43 mg/kg bw per day), based on increased relative liver weights and the observation of hepatocellular necrosis in F_1 parental females at 2000 ppm (equal to 227 mg/kg bw per day).

The NOAEL for offspring toxicity was 2000 ppm (equal to 146 mg/kg bw per day), the highest dose tested.

The NOAEL for reproductive toxicity was 400 ppm (equal to 40 mg/kg bw per day), based on a lower gestation index and a longer gestation duration in F_0 and F_1 females at 2000 ppm (equal to 202 mg/kg bw per day) (Fritz, 1983a,b).

In another two-generation dietary reproductive toxicity study, Charles River COBS CD albino rats (30 of each sex per group for the F_0 and F_1 generations) were fed penconazole (purity not specified in the report, but the purity of batch no. FL-840833 in other studies is reported to be 98.7%) at a dietary concentration of 0, 25, 250 or 2500 ppm. The achieved intakes of penconazole by the animals during the premating, gestation and lactation periods are summarized in Table 16.

Table 16. Mean daily test substance intake

	Mean daily test substance intake (mg/kg bw per day)		
Generation	**25 ppm**	**250 ppm**	**2 500 ppm**
Premating period / males			
F_0 parents	2.0	20	191
F_1 parents	2.2	22	219
Premating period / females			
F_0 parents	2.4	24	238
F_1 parents	2.5	25	246
Gestation period / females			
F_0 generation	1.9	18	180
F_1 generation	1.7	17	175
Lactation period / females			
F_0 generation	3.3	34	346
F_1 generation	3.2	33	337

bw: body weight; F_0: parental generation; F_1: first filial generation; ppm: parts per million
Source: Schardein (1987)

F_0 adults were treated over a 9-week premating period and throughout the 3-week mating period, gestation and 21-day lactation of the F_1 pups. On PND 4, litters were culled to eight pups. Upon completion of weaning for each litter, 30 weaned rats of each sex (one male and one female per

litter, if possible) were randomly selected in each dose group to become F_1 parents for the F_2 offspring. The remaining F_1 weanlings were killed and subjected to a gross external examination and necropsy. Treatment of the selected F_1 rats continued for 15 weeks before mating and during the 3-week mating, gestation and lactation periods. Upon completion of weaning for each litter, all surviving F_2 pups were killed and subjected to a complete gross necropsy. After weaning of the F_1 and F_2 pups, the respective F_0 and F_1 parents were killed and necropsied. Clinical examination was performed daily. Detailed observations were made weekly on all parental rats and on maternal F_0 and F_1 animals on GDs 0, 7, 14 and 21 and PNDs 0, 4, 7, 14 and 21. Body weights of parental rats were recorded weekly. In addition, females were weighed on GDs 0, 7, 14 and 20 and PNDs 0, 4, 7, 14 and 21. Offspring were weighed per sex on PNDs 0, 4, 7 and 14 and individually on PND 21. Parental feed consumption was recorded weekly during the premating period. In addition, feed consumption of parental females was measured on GDs 0, 7, 14 and 20 and on PNDs 0, 7 and 14. Fertility and mating indices of males and females, copulatory interval and the length of gestation were determined. All litters were examined for number of pups, sex of pups, number of stillbirths, number of live births and gross anomalies. All pups were checked daily for mortality and clinical signs. The culled pups were examined for cervical, thoracic and abdominal abnormalities. After weaning, the parental rats were killed and necropsied. In all parental animals and 10 pups of each sex per dose of the F_1 and F_2 generations, the testes and ovaries were weighed. The liver, pituitary, ovaries, uterus including cervix, vagina, testes, seminal vesicle, epididymis, coagulation gland and prostate of these animals were examined histopathologically.

One 2500 ppm F_0 male and one 25 ppm F_1 female died. These deaths are not considered to be treatment related. No clinical signs were reported. During the premating phase, body weight gains in F_0 females at 2500 ppm were decreased (18%). In the F_1 parental males and females, body weight gain was slightly reduced at 2500 ppm (7–9%) during the premating phase. Feed consumption was also slightly reduced (7–8%) in F_0 and F_1 females at 2500 ppm. In the F_0 generation, the proportion of rats that mated during the first 4 days was reduced at 2500 ppm (63% versus 93% in controls). The reverse situation was seen in the F_1 generation (73% at 2500 ppm versus 60% in controls). In both generations, the overall mating index after completion of the 3-week mating period was slightly decreased in the 2500 ppm groups (80% versus 90–97% in controls). Values for fertility and gestation indices, parturition and length of gestation were similar to control values in all treated groups of both generations. In both generations, the number of pups that were dead at birth or that died during the first 4 days of lactation (including missing/cannibalized pups) was slightly higher at the 2500 ppm level (n = 14–37) when compared with controls (n = 4–14). After culling, pup survival during lactation was similar to control survival in all treatment groups. The ratio of male to female rats at the 2500 ppm level in the F_1 generation was lower throughout lactation. However, a similar sex ratio was observed in control pups of the F_2 generation, and the sex ratio was not affected at 2500 ppm in the F_2 generation. It is concluded that the sex ratio was not affected by treatment with penconazole. In both generations, slight, but generally significant, decreases in mean pup weights were evident for male and female high-dose pups on PND 14 (6–12%) and PND 21 (6–11%). In both generations, uterine examinations of dams at weaning gave no indication of any treatment-induced deviations in the number of implantation sites, postimplantation loss or litter size. Necropsy and histological examination of the parental animals or the pups revealed no treatment-related effects. In parental F_0 and F_1 females, a slight increase in relative ovary weights was observed. However, as absolute ovary weights were not affected, this is considered not to be toxicologically relevant.

The NOAEL for parental toxicity was 250 ppm (equal to 24 mg/kg bw per day), based on reduced body weight gain and feed consumption during the premating period in F_0 and F_1 females at 2500 ppm (equal to 238 mg/kg bw per day).

The NOAEL for offspring toxicity was 250 ppm (equal to 20 mg/kg bw per day), based on an increased number of pups that were born dead or died during PNDs 0–4 and a decreased body weight gain of pups during lactation at 2500 ppm (equal to 191 mg/kg bw per day).

The NOAEL for reproductive toxicity was 250 ppm (equal to 20 mg/kg bw per day), based on a decreased mating index at 2500 ppm (equal to 191 mg/kg bw per day) (Schardein, 1987).

(b) Developmental toxicity

Rats

In a developmental toxicity study, groups of 25 pregnant female Tif: RAI f (SPF) rats were treated orally, by gavage, with penconazole (purity 88.4%; batch no. P.2+3) in 2% aqueous carboxymethyl cellulose at a dose of 0, 30, 100 or 300 mg/kg bw per day from days 6 through 15 of gestation (day 0 = day on which sperm were detected in the vaginal smear). The selection of the upper dose was based on a preliminary study. In a supplementary study, to further investigate the effect of penconazole on skeletal development, groups of 15 pregnant rats were treated with penconazole at a dose of 0 or 300 mg/kg bw per day from GD 6 to GD 15 or 450 mg/kg bw per day from GD 10 to GD 14. Clinical signs, mortality and weight gain were recorded daily. Feed consumption was measured on GDs 6, 11, 16 and 21. All females were killed on day 21 of gestation and subjected to gross examination. The uterus was examined and weighed, and the numbers of live and dead fetuses, implantations, and early and late resorptions were counted. The number of corpora lutea was not reported. Body weight and sex of the fetuses were recorded. About two thirds of the fetuses from each litter were selected for skeletal examinations, and one third for visceral examinations.

Two high-dose dams died at the end of gestation. Necropsy did not reveal any obvious pathological conditions. No treatment-related clinical signs or abortions were observed. Body weight gain was slightly decreased at 30 and 300 mg/kg bw per day, but was increased at 100 mg/kg bw per day. Feed consumption was not significantly affected by treatment. The number of early resorptions was increased at 300 mg/kg bw per day (9%) compared with controls (5%); however, the increase was not statistically significant. The number of live fetuses was slightly decreased at 100 mg/kg bw per day (5%) and 300 mg/kg bw per day (9%). Fetal body weights and sex ratios were not affected by treatment. The number of skeletal anomalies (mainly irregularly shaped second and fifth sternebrae) was increased at 300 mg/kg bw per day (11/182) compared with the controls (2/187). The number of still unossified phalangeal nuclei of the hindlimbs was slightly increased at 100 mg/kg bw per day (37%) and 300 mg/kg bw per day (25%) compared with controls (15%).

In the supplementary study, four dams at 300 mg/kg bw per day and two dams at 450 mg/kg bw per day died on GD 21. Necropsy did not reveal any obvious pathological conditions. Body weight gain was reduced at 300 mg/kg bw per day, but not at 450 mg/kg bw per day. Body weights of fetuses at 300 and 450 mg/kg bw per day were slightly reduced (6%). Skeletal assessment revealed an increased number of unossified phalangeal nuclei of the forelimbs at 450 mg/kg bw per day and unossified phalangeal nuclei of the hindlimbs and heel bone in both groups treated with penconazole. In 11 fetuses from two litters (mainly in one litter) at 450 mg/kg bw per day, the fronto-parietal region showed “wide sutures”. One skeletal anomaly (irregularly shaped sternebrae) was observed in the vehicle control. The Meeting noted that the 450 mg/kg bw per day group was treated only from GD 10 to GD 14. Therefore, the data from this group are considered to be of limited value.

The NOAEL for maternal toxicity was 100 mg/kg bw per day, based on mortality and reduced body weight gain observed at the end of gestation at 300 mg/kg bw per day.

The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, based on delayed ossification observed at 300 mg/kg bw per day (Fritz, 1981).

In another developmental toxicity study, groups of 25 pregnant female Sprague-Dawley rats were treated orally, by gavage, with penconazole (purity 98.7%; batch no. FL.840833) in corn oil at a dose of 0, 5, 100 or 500 mg/kg bw per day from days 6 through 15 of gestation (day 0 = day on which breeding was confirmed). Clinical signs and mortality were recorded daily. Body weight and feed consumption were recorded on GDs 0, 6, 13 and 19. All females were killed on day 20 of gestation and subjected to gross examination. The uterus was examined and weighed, and the numbers of live and dead fetuses, corpora lutea, implantations, and early and late resorptions were counted. Body weight and sex of the fetuses were recorded. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for visceral examinations.

One dam at 5 mg/kg bw per day died as a result of a dosing injury. Two high-dose dams died, on GD 11 and GD 12, respectively. Based on antemortem observations (damp, yellow fur and discharge around the nose and mouth or exudate around eyes, nose, mouth and perianal region, observed early during treatment) and the finding of stomach lesions (multiple black or distended smooth non-glandular mucosa) in both females and distended colon and dark contents in the small intestine in one female, these deaths were considered to be treatment related. At 500 mg/kg bw per day, crusty eye(s), crusty nose and/or muzzle, damp and yellow/brown-stained fur in perianal and/or abdominal region, staggered gait, emaciation, loose stool, weakness and/or lethargy were noted. The females of the highest-dose group showed a clear and statistically significant reduction in net final body weight gain. Net body weight gain was reduced by 41% at 500 mg/kg bw per day. On GD 6, feed consumption was reduced by 19% and 42% at 100 and 500 mg/kg bw per day, respectively. In the dams that survived to termination, no gross pathological changes were observed. Dams at 500 mg/kg bw per day showed an increase in the mean number of early resorptions (2.2 [14.6%] versus 0.3 [2%] in controls, not statistically significant) and late resorption sites (0.6 [4%] versus 0.0 [0%] in controls, statistically significant) and a slight decrease in the mean number of viable fetuses. Although litter weight was not statistically significantly affected by treatment, individual fetal weight data showed significant decreases for male (6%) and female fetuses (3%) at 500 mg/kg bw per day. Fetal weight data at 5 and 100 mg/kg bw per day were not affected. Slight increases in the number of runts (nine versus two in controls) and the number of litters with runts (56% versus 9% in controls) were observed at 500 mg/kg bw per day. Occasionally observed external and visceral abnormalities were within the normal range for this strain of rats and were considered to be spontaneous. Skeletal examinations revealed no treatment-related malformations. At 500 mg/kg bw per day, there was a slight increase in the occurrence of cervical ribs (eight fetuses from five litters, versus one control fetus). The total number of fetuses/litters with abnormal findings at the high dose showed a statistically significant increase compared with the controls.

The NOAEL for maternal toxicity was 100 mg/kg bw per day, based on mortality observed after 5 and 6 days of treatment, clinical signs observed early during treatment, a reduction in net body weight gain and feed consumption on GD 6, stomach lesions and an increased incidence of late resorptions at 500 mg/kg bw per day.

The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, based on a slight increase in the occurrence of cervical ribs and an increase in the total number of fetuses/litters with abnormal findings at 500 mg/kg bw per day (Salamon, 1985).

Rabbits

In a developmental toxicity study, groups of 20 mated Chinchilla-type rabbits were treated orally, by gavage, with penconazole (purity 91.7%; batch no. P.11-14) in 0.5% aqueous sodium carboxymethyl cellulose at a dose of 0, 25, 75 or 150 mg/kg bw per day from days 6 through 18 of gestation (the day of mating was designated as GD 0). Mortality and clinical signs were recorded daily. Body weight was measured daily from GD 0 to GD 28. Feed consumption was measured on GDs 0, 6, 11, 15, 19, 24 and 28. All females were killed on day 28 of gestation. All does were examined macroscopically for abnormalities. The reproductive tract was examined, and the numbers of live and dead fetuses, corpora lutea, implantations, and early and late resorptions were counted. Body weight and sex of the fetuses were recorded. All fetuses were subjected to skeletal and visceral examinations.

One control dam and one high-dose dam died. These deaths were not considered to be treatment related. No effect of treatment on the incidence of clinical signs was observed. Body weight gains for the overall pregnancy period (GDs 0–28) were comparable for all groups. However, body weight development of high-dose females (150 mg/kg bw per day) showed a different pattern during the treatment period. At 150 mg/kg bw per day, mean body weight gain was clearly lower (50% compared with controls) during GDs 6–11, whereas it was increased during GDs 11–15 (56% compared with controls) and lowered again during GDs 15–18 (40% compared with controls). After cessation of treatment, body weight gain was increased (43% compared with controls) at the high

dose, so that the overall body weight gain during the gestation period was only 8% below the control value. Mean feed consumption at the high dose was decreased (22%) during treatment and increased (16%) during the post-treatment period. At 150 mg/kg bw per day, the numbers of corpora lutea were about 11–14% higher. In this high-dose group, the incidence of embryonic resorptions was slightly increased (9.7% of implantations versus 4.8% in the controls). These deviations were not statistically significant. Fetal weights were not affected by the treatment. In the high-dose group, one fetus with multiple malformations (right forelimb with first and fifth digits missing, cleft lip, cleft palate, microphthalmia, internal hydrocephaly, brachymelia, sternum poorly ossified), one with microphthalmia and another one with microphthalmia associated with internal hydrocephaly were found. Skeletal examinations revealed no treatment-related effects.

The NOAEL for maternal toxicity was 75 mg/kg bw per day, based on reduction of body weight gain and feed consumption during treatment at 150 mg/kg bw per day.

The NOAEL for embryo and fetal toxicity was 75 mg/kg bw per day, based on the increased incidence of microphthalmia and hydroencephalus at 150 mg/kg bw per day (Giese, 1982).

In another developmental toxicity study, groups of 20 artificially inseminated New Zealand White rabbits were treated orally, by gavage, with penconazole (purity 98.7%; batch no. FL.840833) in 3% aqueous cornstarch at a dose of 0, 10, 50 or 200 mg/kg bw per day from days 7 through 19 of gestation (the day of artificial insemination was designated as GD 0). Mortality and clinical signs were recorded daily. Body weight was measured on GDs 0, 7, 10, 14, 20, 24 and 29. Feed consumption was measured daily from GD 0 to GD 29. All females were killed on day 29 of gestation. All does were examined macroscopically for abnormalities. The reproductive tract was examined, and the numbers of live and dead fetuses, corpora lutea, implantations, and early and late resorptions were counted. The body weight and sex of the fetuses were recorded. All fetuses were subjected to skeletal and visceral examinations.

One control dam and one high-dose dam died. These deaths were not considered to be treatment related. In the low-, mid- and high-dose groups, two, two and one dam delivered before scheduled caesarean section on GD 29. This was similar to the historical control incidence. In high-dose dams, decreased defecation and urination were seen. Females at 200 mg/kg bw per day lost weight (123 g) during GDs 7–14, whereas control dams gained weight (87 g) over this period. Feed consumption was decreased (by about 50%) in high-dose dams from GD 7 to GD 14 and increased (by about 38%) from GD 20 to GD 29. The reduction in body weight is considered to be a consequence of the reduced feed intake and is not considered to be toxicologically adverse. After the end of treatment, the control, low-dose and mid-dose dams lost weight, whereas the body weights of the high-dose dams remained virtually constant, so that no significant differences in body weight were observed at termination. The numbers of corpora lutea and implantations were slightly decreased at the high dose. This is not considered to be treatment related. At the high dose, the mean number of early resorptions per dam was increased (1 versus 0.5 in controls). As the increase was small, was not statistically significant and remained within the range of the laboratory historical control data for this animal strain, this was not considered to be toxicologically adverse. At the high dose, the mean number of live fetuses per dam was decreased (4.8 versus 6.9 in controls). Macroscopic examination showed that the numbers of fetal malformations were lower in all treated groups than in the vehicle control group. Slightly increased incidences of fetuses with unossified hyoid body and/or arches in the mid- and high-dose groups (3.4% and 7.8%, respectively) and of fetuses with reduced ossification of the skull in the high-dose group were within the range of the historical control data of the testing laboratory and were considered not to be treatment related. Penconazole was not teratogenic under the conditions of the study.

The NOAEL for maternal toxicity was 200 mg/kg bw per day, the highest dose tested.

The NOAEL for embryo and fetal toxicity was 50 mg/kg bw per day, based on the reduced number of live fetuses at 200 mg/kg bw per day (Nemec, 1985).

2.6 Special studies

(a) Study on liver enzyme induction

Groups of six male albino rats (strain RAI) and six male mice (MAG) per treatment group were administered penconazole (purity 98.7%; batch number not reported) at a dose of 0, 10, 80, 160, or 320 mg/kg bw per day by gavage for 14 consecutive days. Control animals (eight per species) received the vehicle alone (10 mL/kg bw). After the 14th application, the animals were fasted for 24 hours, then weighed and killed, and their livers were removed. The phospholipid and cytochrome P450 contents and activities of the ethoxycoumarin *O*-deethylase (ECOD), uridine diphosphate-glucuronosyltransferase (UGT) and epoxide hydrolase enzymes were determined in the microsomal liver fractions. Protein concentrations were determined in homogenates, microsomal fractions and cytosolic fractions. DNA concentration was determined in liver homogenates from the control and high-dose animals. The activity of the microsomal ECOD was also investigated in the presence and absence of monooxygenase inhibitors (tetrahydrofuran, 10 mmol/L; metyrapone, 100 µmol/L) or activator (7,8-benzoflavone, 10 µmol/L). Glutathione *S*-transferase (GST) was determined in the cytosolic fraction. Electron microscopic observations were conducted in the liver from control (two rats + two mice) and high-dose animals (four rats + three mice).

No deaths occurred throughout the treatment period. There was no effect on the body weight gain of either rats or mice, but relative liver weights were statistically significantly and dose-dependently increased in both species at 80 mg/kg bw per day and higher in rats and from 160 mg/kg bw per day upwards in mice. There were no effects on liver weights at 10 mg/kg bw per day. Total liver DNA contents were increased in both species in the high-dose groups (20% and 25% in rats and mice, respectively), but the DNA concentration per gram liver was lower (17–20%) following treatment with penconazole at 320 mg/kg bw per day. In the rat, relative protein contents in homogenates and in cytosolic fractions remained unchanged, whereas they were slightly increased in the mouse (but not dose dependently). In both species, penconazole caused a strong dose-dependent increase in microsomal protein (up to about 60% relative to controls) and phospholipid contents (practically doubled at 320 mg/kg bw per day). Therefore, the phospholipid/protein ratios were higher at 320 mg/kg bw per day than in the respective controls (Table 17).

Activities of xenobiotic-metabolizing liver enzymes (Table 18) were drastically increased. In both species, the effects were highly significant from the 80 mg/kg bw per day level and generally more pronounced in rats than in mice.

The influence of monooxygenase inhibitors on the activity of the rat liver microsomal ECOD activity in treated animals was more sensitive to inhibition by metyrapone, but less sensitive to inhibition by tetrahydrofuran, compared with the ECOD from the untreated control. In contrast, activation by benzoflavone was higher in untreated (+132%) than in treated samples (+49% versus controls) (Table 19). ECOD is known to be catalysed by several cytochrome P450 isozymes, but to various extents: metyrapone inhibits isozymes that are induced by phenobarbital, whereas benzoflavone inhibits the cytochrome P450 forms that respond to polycyclic aromatic hydrocarbon treatment. These results indicate that in rats, penconazole treatment altered the pattern of microsomal cytochrome P450 isozymes in a manner similar to phenobarbital.

No apparent macroscopic changes of organs were observed at necropsy.

At the 320 mg/kg bw per day dose in both species, there was extensive proliferation of smooth endoplasmic reticulum membranes. In 2/4 rats, a few cytoplasmic structures occurred that showed the morphological aspects of membranous whorls or “fingerprints”. One treated rat showed numerous vacuoles or “blisters” of variable size in the pericanalicular regions of the hepatocytes (which contained flocculent material), whereas the bile canaliculi of the liver had normal structure. The other hepatocyte organelles of treated animals exhibited the morphological features of the corresponding controls.

Treatment of male rats and mice with penconazole at an oral dose of 80, 160 or 320 mg/kg bw per day for 14 consecutive days caused a marked liver enlargement, a proliferation of smooth endoplasmic reticulum membranes and a pronounced induction of the activity of several hepatic

Table 17. Study on liver enzyme induction: mean protein and DNA contents (and per cent deviation relative to controls)

	0 mg/kg bw per day	10 mg/kg bw per day	80 mg/kg bw per day	160 mg/kg bw per day	320 mg/kg bw per day
Rats					
Liver DNA					
mg	26.0	nd	nd	nd	31.1* (+20)
mg/g liver[a]	2.89	–	–	–	2.32 (–20)
Protein (mg/g liver)					
Homogenate	161	169	171	170	164
Cytosolic	56.8	54.9	57.9	56.3	55.3
Microsomal	9.55	10.6 (+11)	14.0* (+47)	15.3* (+60)	14.8* (+55)
Phospholipid (mg/g liver)					
Microsomal	4.79	5.20 (+9)	7.70* (+61)	9.86* (+106)	9.48* (+98)
Mice					
Liver DNA					
mg	4.52	nd	nd	nd	5.65** (+25)
mg/g liver[a]	3.43	–	–	–	2.86 (–17)
Protein (mg/g liver)					
Homogenate	134	136	150*	146	155** (+16)
Cytosolic	72.9	73.8	87.3* (+20)	79.6 (+9)	79.0 (+8)
Microsomal	12.8	14.2 (+11)	18.8* (+47)	16.8* (+31)	19.4* (+52)
Phospholipid (mg/g liver)					
Microsomal	5.55	6.84 (+23)	7.81* (+41)	7.61* (+37)	10.4* (+87)

bw: body weight; DNA: deoxyribonucleic acid; nd: not determined; *: $P < 0.05$; **: $P < 0.01$ (Student's *t*-test)
[a] As calculated from the mean DNA and liver weight values (no statistical evaluation).
Source: Waechter, Bentley & Staeubli (1985)

xenobiotic-metabolizing enzymes as a result of an adaptive response of the liver to an enhanced functional load. The results indicate that penconazole belongs to the phenobarbital class of monooxygenase inducers and that the observed liver growth resulted from a combination of both cell division (hyperplasia) and hypertrophy (Waechter, Bentley & Staeubli, 1985).

(b) Studies with metabolites

1,2,4-Triazole

In a 12-month dietary toxicity study, performed according to OECD Test Guideline 452, 1,2,4-triazole (purity 98.5%; batch no. S4317788) was administered to groups of 30 male and 30 female Wistar: Crl:WI(Han) rats at 0, 125, 375, 1000 or 2000 ppm (equal to 0, 6.9, 21, 58 and 113 mg/kg bw per day for males and 0, 8.3, 26, 71 and 136 mg/kg bw per day for females, respectively). Twenty animals of each sex per dose were used to evaluate the potential for general toxicity, and 10 animals of each sex per dose were used to evaluate the potential for neurotoxicity. The rats were checked daily for mortality and clinical signs. A detailed clinical examination was performed weekly.

Table 18. Study on liver enzyme induction: activities of xenobiotic-metabolizing enzymes (and factor increase with respect to controls[a])

	0 mg/kg bw per day	10 mg/kg bw per day	80 mg/kg bw per day	160 mg/kg bw per day	320 mg/kg bw per day
Rats					
Cytochrome P450 (nmol/g liver)	11.6	15.1* [×1.3]	31.5* [×2.7]	42.2* [×3.6]	51.9* [×4.5]
ECOD (nmol/min/g liver)	4.96	6.51 [×1.3]	13.6* [×2.7]	19.9* [×4.0]	21.1* [×4.3]
Epoxide hydrolase (nmol/min/g liver)	141	179* [×1.3]	381* [×2.7]	517* [×3.7]	618* [×4.4]
UGT (µmol/min/g)	1.53	1.68 [×1.1]	2.82* [×1.8]	3.36* [×2.2]	3.42* [×2.2]
GST (µmol/min/g)	100	110 [×1.1]	132* [×1.3]	179* [×1.8]	194* [×1.9]
Mice					
Cytochrome P450 (nmol/g liver)	17.2	20.0 [×1.2]	30.3* [×1.8]	35.2* [×2.1]	43.1* [×2.5]
ECOD (nmol/min/g liver)	30.7	36.7 [×1.2]	67.1* [×2.2]	75.5* [×2.5]	106* [×3.5]
Epoxide hydrolase (nmol/min/g liver)	62.9	57.7 [×0.9]	72.0 [×1.1]	101* [×1.6]	155* [×2.5]
UGT (µmol/min/g)	1 000	1 010	1 420* [×1.4]	1 290* [×1.3]	1 530* [×1.5]
GST (µmol/min/g)	407	317 [×0.8]	440 [×1.1]	473 [×1.2]	517 [×1.3]

bw: body weight; ECOD: ethoxycoumarin-*O*-deethylase; GST: glutathione *S*-transferase; UGT: uridine diphosphate-glucuronosyltransferase; *: $P < 0.05$ (Student's *t*-test)

[a] Increasing factor versus activity of the controls shown in square brackets.

Source: Waechter, Bentley & Staeubli (1985)

Table 19. Liver enzyme induction – Inhibition of ECOD activity (and per cent deviation relative to controls)

		Enzyme activity (nmol/min/g liver)[a]	
Inhibitor	**Concentration**	**Untreated with penconazole**	**Treated with penconazole**
None	0 (control)	4.5	24.3
Tetrahydrofuran	10 mmol/L	2.03 (−55)	16.3 (−33)
Metyrapone	100 µmol/L	2.66 (−41)	5.83 (−76)
7,8-Benzoflavone	10 µmol/L	10.4 (+132)	36.2 (+49)

ECOD: ethoxycoumarin-*O*-deethylase

[a] Per cent deviation relative to controls shown in parentheses.

Source: Waechter, Bentley & Staeubli (1985)

Body weight and feed consumption were recorded weekly during the first 3 months and monthly thereafter. Ophthalmic examinations were performed before the start of the study and prior to termination. Blood and urine for haematology, clinical chemistry and urine analysis were collected from 10 rats of each sex per dose at 3, 6 and 12 months. The estrous cycle was characterized in 10 females per dose over a 23-week period prior to termination. The rats designated for neurotoxicological examination were subjected to functional observational battery and motor activity testing before treatment started and at 3, 6, 9 and 12 months. At termination, sperm were collected from one testis and one epididymis for assessment of the number of sperm cells and their morphology and motility. Animals found dead or killed prematurely were weighed and necropsied. Gross lesions were examined. At termination, 20 rats of each sex per dose were necropsied, and organ weights (adrenals, brain, heart, liver, kidneys, spleen, thyroid, uterus, ovaries, testes, epididymides, prostate and pituitary) were recorded. A wide range of tissues was examined microscopically. The animals of the neurotoxicology group were subjected to a detailed histological examination of the nervous system.

There were no effects of treatment on mortality or clinical signs. In the toxicology group, body weight gain was reduced at 1000 and 2000 ppm in males (up to 8% at both doses) and females (up to 19% at 1000 ppm and up to 20% at 2000 ppm). Similar reductions were observed in the neurotoxicology groups at these doses. Feed consumption was not affected by treatment. The neurological assessments (functional observational battery and motor activity) revealed no treatment-related effects. Landing foot splay was decreased (22–26%) in high-dose females at 3, 6 and 9 months, but not at termination. As these effects were small, were not statistically significant, were not observed after 12 months of treatment and occurred in one sex only, they are considered not to be treatment related. Occasional changes in the motor activity test were small and not statistically significant and were considered not to be related to treatment. There were no effects of treatment on ophthalmoscopy, haematology, clinical chemistry, urine analysis, estrous cycling, sperm parameters, gross pathology or organ weights. Histopathological examination revealed a statistically significant decrease (minimal to marked severity) in the population of Purkinje cells within the cerebellar vermis of the brain (especially in the dorsal region) in 2000 ppm males and females of both the main toxicology and neurotoxicology groups. In minimally affected animals, only a subtle gap or break in the continuity of the Purkinje cell layer located along the internal granular layer was observed. In severely affected animals, the decrease in Purkinje cells was marked with a variable decrease in the width of the molecular layer and density of the internal granular layer. In a few animals, white fibre tract changes with individual nerve fibre or axonal swelling and/or fragmentation, the presence of a phagocytic macrophage or increased numbers of reactive astrocytic cells were found. No other histopathological changes were observed

The NOAEL was 375 ppm (equal to 21 mg/kg bw per day), based on a reduction in body weight gain at 1000 ppm (equal to 58 mg/kg bw per day) (Wahle, 2010a).

Triazole acetic acid

In a 28-day dietary toxicity study in mice, performed according to OECD Test Guideline 407, triazole acetic acid (purity 98.5%; batch no. RDL 211-8-2) was administered to groups of 10 male and 10 female Crl:CD-1 (ICR) mice at 0, 1000, 3000 or 7000 ppm (equal to 0, 159, 483 and 1067 mg/kg bw per day for males and 0, 183, 542 and 1357 mg/kg bw per day for females, respectively). Animals were checked daily for mortality and clinical signs of toxicity. A detailed clinical examination was performed weekly. Body weights and feed consumption were recorded before the start of treatment, daily during week 1 of the treatment period and twice weekly during weeks 2–4. Blood was sampled prior to termination for haematology and clinical biochemistry. At termination, all mice were necropsied, and weights of liver, lung, spleen, kidneys, heart, brain, pituitary, thyroid, thymus, epididymides, ovaries, testes, prostate, uterus and adrenals were recorded. An extensive range of organs was examined microscopically

No adverse effects of treatment with triazole acetic acid were observed in any of the test groups.

The NOAEL was 7000 ppm (equal to 1067 mg/kg bw per day), the highest dose tested (Shearer, 2011).

In a 29-day dietary toxicity study in rats, triazole acetic acid (purity 98.4%; batch no. CH-476108) was administered to groups of 10 male and 10 female Wistar: Crl:WI(Han) rats at 0, 3250, 6500 or 13 000 ppm (equal to 0, 243, 483 and 993 mg/kg bw per day for males and 0, 260, 519 and 940 mg/kg bw per day for females, respectively). Animals were checked daily for mortality and clinical signs of toxicity. A detailed clinical examination was performed weekly. Five rats of each sex per dose were subjected to a functional observational battery during the fourth week of treatment. Body weights and feed consumption were measured weekly. Ophthalmoscopy was performed before treatment started and prior to termination. Blood and urine were sampled during the fourth week of treatment for haematology, clinical biochemistry and urine analysis. At termination, all rats were necropsied, and weights of liver, lung, spleen, kidneys, testes, heart, brain, thyroid, thymus, epididymides, ovaries, testes, prostate, uterus and adrenals were recorded. An extensive range of organs was examined microscopically.

No adverse effects of treatment with triazole acetic acid were observed in any of the test groups. At 6500 and 13 000 ppm, slightly decreased urinary pH was observed, without any associated histopathological or clinical changes. The decreased urinary pH was attributed to the acidic nature of the test material and was not considered to be toxicologically relevant.

The NOAEL was 13 000 ppm (equal to 940 mg/kg bw per day), the highest dose tested (Wahle, 2010b).

In a 13-week combined dietary toxicity and neurotoxicity study in rats, triazole acetic acid (purity 98.5%; batch no. RDL 211-8-2) was administered to groups of 16 male and 16 female Wistar: Crl:WI(Han) rats. The dietary concentrations were adjusted weekly based on body weight and feed consumption in order to obtain target test substance intakes of 0, 100, 500 and 1000 mg/kg bw per day. Actual mean intakes were 0, 94, 495 and 1002 mg/kg bw per day for males and 0, 119, 627 and 1181 mg/kg bw per day for females, respectively. Ten animals of each sex per dose were designated for general toxicity investigations, and six animals of each sex per dose were designated for the neuropathology investigations. Animals were checked daily for mortality and clinical signs. A detailed physical examination was performed weekly. A functional observational battery and a locomotor activity test were done in 10 rats of each sex per group (overlapping with those assigned for the neuropathology investigations) before the start and during weeks 2, 4, 8 and 13 of treatment. Ophthalmological examinations were conducted at the beginning of the study in all rats and prior to termination. Body weights and feed consumption were measured weekly. At termination, blood and urine were sampled from 10 rats of each sex per dose for haematology, clinical biochemistry and urine analysis. At termination, 10 rats of each sex per dose were necropsied, and weights of liver, lung, spleen, kidneys, heart, brain, thyroid, thymus, epididymides, ovaries, testes, prostate, uterus and adrenals were recorded. An extensive range of organs was examined microscopically. The animals of the neuropathology group were subjected to a detailed histological examination of the nervous system.

No effects of treatment on mortality, clinical signs, body weight, feed consumption, ophthalmology, neurological parameters, locomotor activity, haematology, clinical chemistry, urine analysis, macroscopy, histopathology or organ weights were observed. A slight increase in white blood cell count, accompanied by increases in several absolute differential leukocyte counts, observed in high-dose males was not considered to be treatment related, as the increases were within the historical control range and there were no differences from controls in male relative leukocyte counts or in any haematology parameters in female rats. A very slight decrease in serum potassium concentration in high-dose males was also not considered to be treatment related, as the decrease was small and within the historical control range.

The NOAEL was 1002 mg/kg bw per day, the highest dose tested (Wahle, 2010c).

In a one-generation dietary reproductive toxicity study in rats, performed according to OECD Test Guideline 415, Wistar Crl:WI(Han) rats (25 of each sex per dose group) were fed triazole acetic acid (purity 98.5%; batch no. RDL 211-8-2). The dietary concentrations were adjusted weekly based on body weight and feed consumption in order to obtain target test substance intakes of 0, 100, 300 and 1000 mg/kg bw per day. Actual mean intakes are presented in Table 20.

Table 20. Mean test substance intake in a one-generation reproductive toxicity study in rats

	Mean test substance intake (mg/kg bw per day)		
	100 mg/kg bw per day	**300 mg/kg bw per day**	**1 000 mg/kg bw per day**
Parental males	96	287	959
Parental females (premating)	98	293	976
Parental females			
Gestation period	105	315	1 073
Lactation period	115	353	1 227
F_1 males	93	280	926
F_1 females	78	246	770

bw: body weight; F_1: first filial generation
Source: Schneider (2010)

F_0 adults were treated over at least a 73-day premating period and throughout the 2-week mating period, gestation and 21-day lactation of the F_1 pups. On PND 4, litters were culled to eight pups. Upon completion of weaning for each litter, 25 weaned rats of each sex (one male and one female per litter, if possible) were randomly selected in each dose group. Treatment of the selected pups continued until sexual maturation. The remaining F_1 weanlings were killed and subjected to a gross external examination and necropsy. The rats were checked daily for mortality and clinical signs. Body weights of parental rats were recorded weekly. In addition, females were weighed on GDs 0, 7, 14 and 20 and PNDs 1, 4, 7, 14 and 21. Offspring were weighed on PNDs 1, 4, 7, 14 and 21. Parental feed consumption was recorded on a weekly basis during the premating and gestation periods. In addition, feed consumption of parental females was measured during PNDs 1–4, 4–7, 7–14 and 14–21. Estrous cycle length was evaluated by daily analysis of vaginal smear for all F_0 female parental rats for a minimum of 3 weeks prior to mating. Determination of estrous cycle length was continued throughout the pairing period until the female exhibited evidence of copulation. Fertility and mating indices of males and females, copulatory interval and the length of gestation were determined. At termination of the males, sperm parameters were assessed. All litters were examined for number of pups, sex of pups, number of stillbirths, number of live births and gross anomalies. All pups were checked daily for mortality and clinical signs. All culled pups and pups killed at PND 21 were subjected to gross examination. After the scheduled kill at weaning on PND 21, the brain, spleen and thymus of one pup of each sex per litter were weighed. All selected female F_1 pups (25 per group) were evaluated daily for vaginal patency beginning on PND 27. On the day of vaginal opening, the body weights of the respective animals were determined. All selected male F_1 pups (25 per group) were evaluated daily for preputial separation beginning on PND 38. On the day of preputial separation, the body weights of the respective animals were determined. After weaning, the parental rats were killed and necropsied. In all parental animals, a wide range of organs was microscopically examined. In addition, most of these organs were weighed. Differential ovarian follicle count was performed in females of the control and high-dose groups.

No mortalities or treatment-related clinical signs were observed in the parental animals throughout the treatment period. Feed consumption of the high-dose males was statistically significantly decreased during weeks 4–6 (up to 5%), weeks 7–8 (about 6%), weeks 9–10 (about 7%)

and weeks 14–16 (up to 7%). Feed consumption of the mid-dose F_0 males was statistically significantly decreased by about 6% during weeks 9–10, weeks 11–12 and weeks 15–16. Body weight gain was statistically significantly decreased in males at 1000 mg/kg bw per day during weeks 1–2 (about 7%), weeks 3–6 (up to 20%) and weeks 14–15 (about 47%). During the entire study, the high-dose males gained about 10% less weight than the controls. Body weight gain of the mid-dose males was slightly below control values throughout the study. Only the decrease during weeks 11–12 (about 32%) was statistically significant. Body weight gain of low-dose males and females of all dose groups was not affected. Estrous cycling, female reproduction and delivery parameters, male fertility and sperm parameters were not affected by treatment. Slight increases in relative kidney weights (6% and 11%, respectively) in mid- and high-dose males and in relative liver weights (8%) in high-dose males were attributed to the observed reduction in body weight gain. As these increases were not accompanied by histopathological changes, they were not considered to be toxicologically relevant. Necropsy and histopathological examination, including a differential ovarian follicle count, revealed no effect of treatment. No effects of treatment on mortality, clinical observations, body weight gain, feed consumption, sexual maturation or gross examination of the pups were observed at any dose.

The NOAEL for parental toxicity was 287 mg/kg bw per day, based on reduced body weight gain and feed consumption in males at 959 mg/kg bw per day.

The NOAEL for offspring toxicity was 770 mg/kg bw per day, the highest dose tested.

The NOAEL for reproductive toxicity was 959 mg/kg bw per day, the highest dose tested (Schneider, 2010).

In a preliminary developmental toxicity study, groups of 20 time-mated female Crl:WI(Han) rats were treated orally, by gavage, with triazole acetic acid (purity 98.5%; batch no. AE C619102-01-03) at a dose of 0, 500, 750 or 1000 mg/kg bw per day from days 6 through 19 of gestation. The rats were checked daily for clinical signs and mortality. A detailed examination was performed once weekly. Body weights were recorded daily from GD 6 to GD 20. Feed consumption was measured over the periods GDs 3–6, 6–9, 9–12, 12–15, 15–18 and 18–20. All females were killed on day 20 of gestation and subjected to gross examination. The gravid uterus was weighed and examined, and the numbers of live and dead fetuses, implantations, and early and late resorptions were counted. The number of corpora lutea, placental weight and body weight, sex and external abnormalities of the fetuses were recorded.

No mortalities or clinical signs were recorded. Although body weight gain in high-dose females was slightly reduced over the treatment period, statistical significance was not observed. This reduction in body weight gain was not considered to be toxicologically adverse. No effect of treatment on feed consumption, pregnancy data or necropsy findings was observed. Examination of the fetuses revealed no effects on litter, fetal and placental weight, fetal sex ratio or external malformations (Mercer, 2011a).

In a developmental toxicity study, performed according to OECD Test Guideline 414, groups of 24 time-mated female Crl:WI(Han) rats were treated orally, by gavage (vehicle was 0.5% carboxymethyl cellulose), with triazole acetic acid (purity 98.5%; batch no. AE C619102-01-03) at a dose of 0, 100, 300 or 1000 mg/kg bw per day from days 6 through 19 of gestation (day of mating = GD 0). The rats were checked daily for clinical signs and mortality. A detailed examination was performed once weekly. Body weights were recorded daily from GD 6 to GD 20. Feed consumption was measured over the periods GDs 6–9, 9–12, 12–15, 15–18 and 18–20. All females were killed on day 20 of gestation and subjected to gross examination. The gravid uterus was weighed and examined, and the numbers of live and dead fetuses, implantations, and early and late resorptions were counted. The number of corpora lutea, placental weight and body weight, sex and external abnormalities of the fetuses were recorded. Half of the fetuses were examined for skeletal abnormalities, whereas the other half was examined for visceral abnormalities.

Three high-dose females were killed on GDs 8–9 owing to a deterioration in their condition. These animals displayed decreased activity, noisy and laboured breathing, hunched posture, piloerection and partially closed eyes. Necropsy revealed gaseous distensions of the gastrointestinal tract. No signs of local irritation of the stomach or gut were reported. Body weight gain in this high-dose group was lower than that of the controls during GDs 8–10. As these effects were observed early during the study, treatment of the remaining animals in this group was discontinued, and these animals were not further examined. No effect of treatment on mortality, clinical signs, body weight gain, feed consumption or necropsy findings was observed at 100 or 300 mg/kg bw per day. There was no effect of treatment on the mean number of corpora lutea, the mean number of implantations or the extent of preimplantation or postimplantation losses at the middle and low doses. The percentage of male fetuses was statistically significantly reduced at 300 mg/kg bw per day. However, values were within the historical control range, and therefore this reduction was considered unrelated to treatment. There was no effect of treatment at 100 or 300 mg/kg bw per day on fetal, litter, placental or gravid uterine weights. External, skeletal and visceral examination of the low- and mid-dose fetuses also showed no effect of treatment. As severe clinical signs in the dams at 1000 mg/kg bw per day necessitated early termination, the effect of triazole acetic acid on fetal development could not be assessed at this dose.

The NOAEL for maternal toxicity was 300 mg/kg bw per day, based on mortality, clinical signs, and reduced body weight gain and feed consumption observed early during treatment at 1000 mg/kg bw per day.

The NOAEL for embryo and fetal toxicity was 300 mg/kg bw per day, the highest dose tested It is noted that the effect of triazole acetic acid at 1000 mg/kg bw per day on embryo and fetal development could not be assessed due to early termination of the dams at this dose (Mercer, 2011b).

Rabbits

In a developmental toxicity study, performed according to OECD Test Guideline 414, groups of at least 25 time-mated female New Zealand White (Hra:(NZW)SPF) rabbits were treated orally, by gavage, with triazole acetic acid (purity 98.4%; batch no. CH-476108) at a dose of 0, 100, 750 or 1000 mg/kg bw per day from days 6 through 28 of gestation (day 0 = day of mating). The rabbits were checked daily for clinical signs, mortality, abortions and premature deliveries. Body weights and feed consumption were recorded daily. All females were killed on day 29 of gestation and subjected to gross examination. The liver, kidneys and uterus were weighed. The uterus was examined, and the numbers of live and dead fetuses, implantations, and early and late resorptions were counted. The number of corpora lutea and body weight and sex of the fetuses were recorded. Cavitated organs were evaluated in all fetuses by dissection. A single cross-section was made between the parietal and frontal bones of approximately one half of the fetuses per litter, and the brain was examined in situ. The remaining fetuses were examined for soft tissue alterations (including eyes, brain, nasal passages and tongue). All fetuses were examined for skeletal alterations.

One, six and 10 does died or were killed prior to scheduled termination at 100, 750 and 1000 mg/kg bw per day, respectively. The deaths of one and eight does at 750 and 1000 mg/kg bw per day, respectively, were considered to be caused by localized gastrointestinal tract disturbances due to the strong acidic property (pH 1.9–2.0) of the test substance, and not by systemic toxicity. Most of these animals had stomach lesions, generally described as numerous discoloured (black) erosions or ulcerations (pinpoint to 1.0 cm in diameter) on the mucosal surface. These stomach lesions probably led to decreased feed consumption and markedly decreased body weight gain or body weight loss during the period prior to their death. The other deaths are considered unrelated to the test substance (e.g. from intubation errors). One doe at 750 mg/kg bw per day delivered on GD 29, before scheduled termination. The incidences of scant faeces were increased at 750 and 1000 mg/kg bw per day, and the incidence of rales was increased at 750 mg/kg bw per day. These clinical signs were generally observed in does that died or were killed before scheduled termination. No other treatment-related clinical signs were noted. Apart from the gastrointestinal lesions, necropsy revealed no treatment-related changes. Reductions in feed consumption were observed at 750 and 1000 mg/kg bw per day

on the first day of treatment. In the animals that survived to scheduled termination, body weight gain was reduced at 1000 mg/kg bw per day (+450 g in controls, +310 g at 1000 mg/kg bw per day; corrected for gravid uterine weight: −40 g in controls and −130 g at 1000 mg/kg bw per day). In these animals, feed consumption was reduced at 750 mg/kg bw per day (10%) and 1000 mg/kg bw per day (12%).

At 750 and 1000 mg/kg bw per day, fetal weights in both sexes were significantly reduced (by 9–11%) compared with the control group values. No gross external, soft tissue or skeletal fetal alterations (malformations or variations) were caused by doses up to 1000 mg/kg bw per day.

The NOAEL for maternal toxicity was 100 mg/kg bw per day, based on mortality (first observed on day 4 of treatment), clinical signs, and reduced body weight gain and feed consumption (first observed at/after 1 day of treatment) observed at 750 mg/kg bw per day. These effects are probably caused by a local effect on the gastrointestinal tract.

The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, based on decreased fetal weights at 750 mg/kg bw per day (Hoberman, 2010a).

Triazole alanine

In a 12-month dietary toxicity study in rats, performed according to OECD Test Guideline 452, triazole alanine (purity 98%; batch no. MES 133/1) was administered to groups of 30 male and 30 female Wistar: Crl:WI(Han) rats at dietary levels adjusted to achieve target doses of 0, 30, 100, 300 and 1000 mg/kg bw per day. Actual doses were 0, 28, 93, 278 and 916 mg/kg bw per day for males and 0, 36, 120, 375 and 1273 mg/kg bw per day for females, respectively. Twenty animals of each sex per dose were used to evaluate the potential for general toxicity, and 10 animals of each sex per dose were used to evaluate the potential for neurotoxicity. The rats were checked daily for mortality and clinical signs. A detailed clinical examination was performed weekly. Body weight and feed consumption were recorded weekly during the first 3 months and monthly thereafter. Ophthalmic examinations were performed before the start of the study and prior to termination. Blood and urine for haematology, clinical chemistry and urine analysis were collected from 10 rats of each sex per dose at 3, 6 and 12 months. The rats designated for neurotoxicological examinations were subjected to functional observational battery and motor activity testing before treatment started and at 3, 6, 9 and 12 months. Animals found dead or killed prematurely were weighed and necropsied. Gross lesions were examined. At termination, 20 rats of each sex per dose were necropsied, and organ weights (adrenals, brain, heart, liver, kidneys, spleen, thyroid, uterus, ovaries, testes, epididymides, prostate and pituitary) were recorded. A wide range of tissues was examined microscopically. The animals of the neurotoxicology group were subjected to a detailed histological examination of the nervous system.

There were no effects of treatment on mortality, clinical signs, body weight gain, feed consumption, ophthalmology, haematology, urine analysis or clinical chemistry. A decrease in serum potassium level (up to 16%) and an increase in serum glucose level (up to 18%) observed at 6 months in males of the 2000, 6000 and 20 000 ppm groups were not considered to be treatment related, as they occurred in one sex only and were not observed at 3 and 12 months. The neurological assessments (functional observational battery and motor activity) revealed no treatment-related effects. Occasional findings on posture in the home cage, reaction to handling, approach response and body temperature were considered not to be treatment related, as they lacked time and dose dependency. No effects of treatment on gross pathology, organ weights or histopathology were found in the toxicology or neurotoxicology groups. Slightly enhanced incidence and severity of the mineralization of the intestinal mucosa were observed in both sexes at 20 000 ppm, reaching statistical significance for the colon in males. This was considered not to be toxicologically relevant, as the overall incidence of intestinal mineralization was similar between controls (14/20 males, 18/20 females) and high-dose animals (17/20 males, 18/20 females), clinical signs of intestinal disturbance/function were not observed in any animal and this microscopic change is a common background lesion in the ageing rat.

The NOAEL was 20 000 ppm (equal to 916 mg/kg bw per day), the highest dose tested (Wahle, 2012).

In a developmental toxicity study in rabbits, performed according to OECD Test Guideline 414, groups of 25 pregnant female New Zealand White (Hra:(NZW)SPF) rabbits were treated orally, by gavage, with triazole alanine (purity 98%; batch no. MES 133/1) at a dose of 0, 30, 100 or 250 mg/kg bw per day from days 6 through 28 of gestation (day 0 = day of mating). The rabbits were checked daily for clinical signs, mortality, abortions and premature deliveries. Body weights and feed consumption were recorded daily. All females were killed on day 29 of gestation and subjected to gross examination. The liver, kidneys and uterus were weighed. The uterus was examined, and the numbers of live and dead fetuses, implantations, and early and late resorptions were counted. The number of corpora lutea and body weight and sex of the fetuses were recorded. Cavitated organs were evaluated in all fetuses by dissection. A single cross-section was made between the parietal and frontal bones of approximately one half of the fetuses per litter, and the brain was examined in situ. The remaining fetuses were examined for soft tissue alterations (including eyes, brain, nasal passages and tongue). All fetuses were examined for skeletal alterations.

At 250 mg/kg bw per day, a slight increase in the numbers of does with soft or liquid faeces was found. These findings were first observed after 5 days of treatment. No other treatment-related clinical signs, mortalities or abortions were observed. Macroscopic examination did not reveal treatment-related lesions. At 250 mg/kg bw per day, the terminal body weight was slightly lower (3.3%) than the control value. In these high-dose rabbits, significantly lower body weight gains (29%) and feed consumption (11%) were observed from GD 6 to GD 29.

A significant increase in resorptions and postimplantation loss at 30 mg/kg bw per day was not considered to be treatment related, as the increase did not occur at higher doses and the values were within the historical control ranges. Fetal weights of both sexes were significantly reduced at 250 mg/kg bw per day (10.4% in males, 11.6% in females). Although the fetal weights at the high dose were within the historical control range, the observed reductions in body weight were considered to be treatment related by the study authors. No treatment-related malformations were observed in the fetuses. The litter incidence of hyoid, angulated ala (52%) and ribs thickened (12%) at 250 mg/kg bw per day were just outside the historical control range (0–50% and 0–10%, respectively) and are considered to be related to treatment.

The NOAEL for maternal toxicity was 100 mg/kg bw per day, based on increased incidences of soft or liquid faeces (first observed after 5 days of treatment) and decreased body weight gain and feed consumption observed at 250 mg/kg bw per day.

The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, based on decreased fetal weight and increased incidences of hyoid, angulated ala and thickened ribs observed at 250 mg/kg bw per day (Hoberman, 2010b).

3. Observations in humans

No records of adverse health effects were reported during the manufacture or formulation of penconazole-containing products over a 20-year period. Exposure incidences of penconazole formulations reported between 2004 and 2014 were related to intentional misuse (15 cases), occupational exposure (five cases) and accidental exposure (15 cases). In one case, ingestion of penconazole induced effects of moderate severity (not further specified); in the other cases, exposure (oral, dermal, inhalation or eye) produced no or only minor symptoms (information provided by sponsor).

Comments

Biochemical aspects

Absorption was rapid and extensive following the administration of a single oral dose (0.5 or 25 mg/kg bw) of [^{14}C]penconazole to rats (Hamböck, 1980, 1985). At 50 mg/kg bw, maximum blood concentrations were reached in 4 hours in males and 6 hours in females (Hassler, 1999). At this dose, peak tissue concentrations, observed at about 6 and 4 hours after dosing in males and females, respectively, were generally higher in males; the half-life of elimination was also longer in males than in females. Highest tissue concentrations of radioactivity were found in penis (probably related to contamination with urinary radioactivity), liver, lungs and kidneys (Hassler, 1999). A sex difference was apparent in excretion profiles, with females excreting 73–85% of a 0.5 or 25 mg/kg bw dose of penconazole in urine and 14–32% in faeces over a 6-day period, whereas males excreted 62% of the same dose levels in urine and 37–39% in faeces over the same period (Hamböck, 1980, 1985). Excretion was more rapid in females, irrespective of dose level or position of radiolabel. Biliary elimination was greater in males than in females (55% and 40% of the administered dose, respectively). Less than 5% of the dose was excreted in faeces in bile duct–cannulated rats, indicating enterohepatic circulation of biliary metabolites (Van Dijk, 1987). The excretion profiles in males and females were not affected by dose or predosing the rats with unlabelled penconazole for 14 or 90 days (Hiles, 1987b; Van Dijk, 1987).

Primary metabolic reactions involved in the biotransformation of penconazole included cleavage of the triazole ring (estimated 15% of the dose), oxidation of the ω-position of the alkane chain to form the respective carboxylic acid (30% of the dose), oxidation of the 3- or 4-position of the alkane chain to form monohydroxy and dihydroxy derivatives (2.5% of the dose) and oxidation of the triazole ring in the 3- or 5-position (0.7% of the dose). Cleavage of the penconazole molecule to free triazole was more extensive in males than in females (Hamböck, 1980, 1982, 1984, 1985). Secondary metabolic reactions include α-oxidation of the carboxylic acids to form α-hydroxy carboxylic acids (4.4% of the dose), decarboxylation following oxidation to α-ketocarboxylic derivative (9% of the dose), oxidation of the 3,4-dihydroxy derivatives to produce the corresponding 3- or 4-keto derivatives (0.5% of the dose) and conjugation of all alkanol derivatives with glucuronic acid (2.5% of the dose). A small amount of parent penconazole was identified in faeces and was considered to represent unabsorbed dose (Hamböck, 1982, 1984).

Toxicological data

The acute toxicity of penconazole is low (rat: oral LD_{50} > 2000 mg/kg bw; dermal LD_{50} > 3000 mg/kg bw; inhalation LC_{50} > 4.0 mg/L) (Bathe, 1980a,c; Hartmann, 1987). Penconazole was not irritating to the skin or the eyes of rabbits (Ullmann, 1980; Kuhn, 1988). Penconazole was not a skin sensitizer in a Magnusson and Kligman test in guinea-pigs (Cantoreggi, 1998).

In repeated-dose oral toxicity studies with penconazole in mice, rats and dogs, the main adverse effects were body weight changes and liver toxicity.

In a 90-day study in mice using dietary penconazole concentrations of 0, 10, 100, 300, 500, 1000 and 2400 ppm (equal to 0, 1.7, 17, 52, 85, 163 and 423 mg/kg bw per day for males and 0, 2.5, 24, 72, 116, 237 and 614 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 85 mg/kg bw per day), based on lower total protein and cholesterol levels and focal coagulative necrosis in the liver of both sexes at 1000 ppm (equal to 163 mg/kg bw per day) (Hiles, 1987a).

In a second 90-day study in mice using dietary concentrations of 0, 100, 500, 1500, 3000 and 5000 ppm (equal to 0, 14, 69, 229, 437 and 837 mg/kg bw per day for males and 0, 18, 87, 274, 545 and 983 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 69 mg/kg bw per day), based on reductions in cholesterol levels in both sexes, a reduction in total protein and albumin levels in females and a reduction in body weight gain and increased nuclear pleomorphism in hepatocytes in males at 1500 ppm (equal to 229 mg/kg bw per day) (Milburn, 2002).

In a 28-day gavage study in rats using penconazole doses of 0, 100 and 500 mg/kg bw per day, a NOAEL could not be identified. The LOAEL was 100 mg/kg bw per day, based on changes in clinical chemistry and haematology parameters and minimal hypertrophy of the follicle epithelium of the thyroid (Fankhauser, 1991).

In a 13-week study in rats using dietary penconazole concentrations of 0, 30, 300 and 3000 ppm (equal to 0, 2.0, 19 and 202 mg/kg bw per day for males and 0, 2.1, 21 and 209 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 19 mg/kg bw per day), based on reduced body weight gain and feed consumption in females and increased testes weight observed at 3000 ppm (equal to 202 mg/kg bw per day) (Basler, 1982).

In a second 13-week dietary study in rats using penconazole concentrations of 0, 10, 30 and 100 ppm (equal to 0, 0.77, 2.1 and 7.1 mg/kg bw per day for males and 0, 0.78, 2.1 and 7.3 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 7.1 mg/kg bw per day), the highest dose tested (Basler, 1983).

In a third 13-week dietary study in rats using penconazole concentrations of 0, 10, 100, 300, 500, 1000 and 2400 ppm (equal to 0, 0.81, 7.5, 23, 38, 72 and 179 mg/kg bw per day for males and 0, 0.96, 9.1, 28, 45, 86 and 209 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 23 mg/kg bw per day), based on an increased incidence of hepatocellular vacuolation and hypertrophy at 500 ppm (equal to 38 mg/kg bw per day) (Hiles, 1987c).

In a 1-year study, dogs received a dietary penconazole concentration of 0, 100, 500 or 5000 ppm (equal to 0, 3.1, 16.9 and 133 mg/kg bw per day for males and 0, 3.3, 16.7 and 139 mg/kg bw per day for females, respectively). During week 20, the highest dose was reduced to 2500 ppm (equal to 86 mg/kg bw per day for males and 89 mg/kg bw per day for females), because of excessive reduction in feed consumption and body weight gain. The NOAEL was 100 ppm (equal to 3.1 mg/kg bw per day), based on reduced body weight gain, increased absolute and relative liver weights, and slight histopathological changes in the liver (hepatocyte necrosis associated with inflammatory cell infiltration) in males and females at 500 ppm (equal to 16.7 mg/kg bw per day) (Gfeller, 1984).

In a 2-year carcinogenicity study in mice using dietary concentrations of 0, 5, 75, 150 and 300 ppm (equal to 0, 0.75, 9.8, 19 and 41 mg/kg bw per day for males and 0, 0.67, 8.8, 17 and 36 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 36 mg/kg bw per day), the highest dose tested. No treatment-related tumours were observed in mice in this study (Basler, 1985a).

In an 80-week carcinogenicity study in mice using dietary concentrations of 0, 25, 200 and 1500 ppm (equal to 0, 2.7, 22 and 178 mg/kg bw per day for males and 0, 3.5, 28 and 222 mg/kg bw per day for females, respectively), the NOAEL was 200 ppm (equal to 22 mg/kg bw per day), based on decreased body weight gain and absolute and relative spleen weights and increased incidence and severity of hepatocellular vacuolation in both sexes and increased absolute and relative liver weights in males at 1500 ppm (equal to 178 mg/kg bw per day). No treatment-related tumours were observed in mice in this study (Milburn, 2004).

The overall NOAEL for the long-term toxicity studies in mice was 300 ppm (equal to 36 mg/kg bw per day), and the overall LOAEL was 1500 ppm (equal to 178 mg/kg bw per day).

In a 27-month toxicity and carcinogenicity study in rats using dietary concentrations of 0, 5, 75, 150 and 300 ppm (equal to 0, 0.30, 3.8, 7.3 and 15 mg/kg bw per day for males and 0, 0.31, 4.0, 8.1 and 17 mg/kg bw per day for females, respectively), the NOAEL was 150 ppm (equal to 8.1 mg/kg bw per day), based on increased absolute and relative liver weights and an increase in GGT levels at 1 year in females at 300 ppm (equal to 17 mg/kg bw per day). No treatment-related tumours were observed in rats in this study (Basler, 1985b).

The Meeting concluded that penconazole is not carcinogenic in mice or rats.

Penconazole was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. There was no evidence of genotoxicity (Deparade, 1984, 1999a,b; Puri, 1984; Ogorek, 1999a,b).

The Meeting concluded that penconazole is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that penconazole is unlikely to pose a carcinogenic risk to humans.

In a two-generation reproductive toxicity study in rats using penconazole at dietary concentrations of 0, 80, 400 and 2000 ppm (equal to 0, 5.5, 29 and 146 mg/kg bw per day for males and 0, 7.5, 40 and 202 mg/kg bw per day for females of the F_0 generation and 0, 6.5, 31 and 166 mg/kg bw per day for males and 0, 8.5, 43 and 227 mg/kg bw per day for females of the F_1 generation, respectively), the NOAEL for parental toxicity was 400 ppm (equal to 43 mg/kg bw per day), based on increased relative liver weights and the observation of hepatocellular necrosis in F_1 parental females at 2000 ppm (equal to 227 mg/kg bw per day). The NOAEL for offspring toxicity was 2000 ppm (equal to 146 mg/kg bw per day), the highest dose tested. The NOAEL for reproductive toxicity was 400 ppm (equal to 40 mg/kg bw per day), based on a lower gestation index and a longer gestation duration in F_0 and F_1 females at 2000 ppm (equal to 202 mg/kg bw per day) (Fritz, 1983a,b).

In a second two-generation reproductive toxicity study in rats using dietary penconazole concentrations of 0, 25, 250 and 2500 ppm, premating dietary intakes were equal to 0, 2.0, 20 and 191 mg/kg bw per day for males and 0, 2.4, 24 and 238 mg/kg bw per day for females of the F_0 generation and 0, 2.2, 22 and 219 mg/kg bw per day for males and 0, 2.5, 25 and 246 mg/kg bw per day for females of the F_1 generation, respectively. The NOAEL for parental toxicity was 250 ppm (equal to 24 mg/kg bw per day), based on reduced body weight gain and feed consumption during the premating period in F_0 and F_1 females at 2500 ppm (equal to 238 mg/kg bw per day). The NOAEL for offspring toxicity was 250 ppm (equal to 20 mg/kg bw per day), based on an increased number of pups that were born dead or died during PNDs 0–4 and a decreased body weight gain of pups during lactation at 2500 ppm (equal to 191 mg/kg bw per day). The NOAEL for reproductive toxicity was 250 ppm (equal to 20 mg/kg bw per day), based on a decreased mating index at 2500 ppm (equal to 191 mg/kg bw per day) (Schardein, 1987).

In a developmental toxicity study in rats using gavage penconazole doses of 0, 30, 100 and 300 mg/kg bw per day, the NOAEL for maternal toxicity was 100 mg/kg bw per day, based on mortality and reduced body weight gain observed at the end of gestation at 300 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, based on delayed ossification observed at 300 mg/kg bw per day (Fritz, 1981).

In a second developmental toxicity study in rats using gavage penconazole doses of 0, 5, 100 and 500 mg/kg bw per day, the NOAEL for maternal toxicity was 100 mg/kg bw per day, based on mortality observed after 5 and 6 days of treatment, clinical signs observed early during treatment, a reduction in net body weight gain and feed consumption on GD 6, stomach lesions and an increased incidence of late resorptions at 500 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, based on a slight increase in the occurrence of cervical ribs and an increase in the total number of fetuses/litters with abnormal findings at 500 mg/kg bw per day (Salamon, 1985).

The overall NOAEL for maternal and embryo and fetal toxicity in the two developmental toxicity studies in rats was 100 mg/kg bw per day, and the overall LOAEL was 300 mg/kg bw per day.

In a developmental toxicity study in Chinchilla-type rabbits administered penconazole doses of 0, 25, 75 and 150 mg/kg bw per day by gavage (vehicle was 0.5% aqueous sodium carboxymethyl cellulose), the NOAEL for maternal toxicity was 75 mg/kg bw per day, based on reduction of body weight gain and feed consumption during treatment at 150 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 75 mg/kg bw per day, based on the increased incidences of microphthalmia and hydroencephalus at 150 mg/kg bw per day (Giese, 1982).

In a second developmental toxicity study in New Zealand White rabbits administered penconazole doses of 0, 10, 50 and 200 mg/kg bw per day by gavage (vehicle was 3% aqueous cornstarch), the NOAEL for maternal toxicity was 200 mg/kg bw per day, the highest dose tested. The NOAEL for embryo and fetal toxicity was 50 mg/kg bw per day, based on the reduced number of live fetuses at 200 mg/kg bw per day (Nemec, 1985).

The Meeting concluded that penconazole is teratogenic in rabbits, but not in rats.

No neurotoxicity studies with penconazole were provided. In view of the absence of evidence of neurotoxicity in other acute and repeated-dose toxicity studies, the Meeting concluded that penconazole is unlikely to be neurotoxic.

A special study in rats and mice indicates that penconazole at gavage doses of 10, 80, 160 and 320 mg/kg bw per day for 14 days induces liver enzyme induction, liver enlargement and proliferation of smooth endoplasmic reticulum and shares some characteristics with a phenobarbital class of monooxygenase inducers (Waechter, Bentley & Staeubli, 1985).

Toxicological data on metabolites and/or degradates

1,2,4-Triazole

In a 12-month toxicity study in rats using dietary 1,2,4-triazole concentrations of 0, 125, 375, 1000 and 2000 ppm (equal to 0, 6.9, 21, 58 and 113 mg/kg bw per day for males and 0, 8.3, 26, 71 and 136 mg/kg bw per day for females, respectively), the NOAEL was 375 ppm (equal to 21 mg/kg bw per day), based on a reduction in body weight gain at 1000 ppm (equal to 58 mg/kg bw per day) (Wahle, 2010a).

Triazole acetic acid

In a 28-day toxicity study in mice using dietary triazole acetic acid concentrations of 0, 1000, 3000 and 7000 ppm (equal to 0, 159, 483 and 1067 mg/kg bw per day for males and 0, 183, 542 and 1357 mg/kg bw per day for females, respectively), the NOAEL was 7000 ppm (equal to 1067 mg/kg bw per day), the highest dose tested (Shearer, 2011).

In a 29-day toxicity study in rats using triazole acetic acid at dietary concentrations of 0, 3250, 6500 and 13 000 ppm (equal to 0, 243, 483 and 993 mg/kg bw per day for males and 0, 260, 519 and 940 mg/kg bw per day for females, respectively), the NOAEL was 13 000 ppm (equal to 940 mg/kg bw per day), the highest dose tested (Wahle, 2010b).

In a 13-week combined toxicity and neurotoxicity study in rats, dietary triazole acetic acid concentrations were adjusted weekly based on body weight and feed consumption in order to obtain target test substance intakes of 0, 100, 500 and 1000 mg/kg bw per day. Actual mean intakes were 0, 94, 495 and 1002 mg/kg bw per day for males and 0, 119, 627 and 1181 mg/kg bw per day for females, respectively. The NOAEL was 1002 mg/kg bw per day, the highest dose tested (Wahle, 2010c).

In a one-generation reproductive toxicity study in rats, dietary triazole acetic acid concentrations were adjusted weekly based on body weight and feed consumption in order to obtain target test substance intakes of 0, 100, 300 and 1000 mg/kg bw per day. Actual premating test substance intakes were 0, 96, 287 and 959 mg/kg bw per day for males and 0, 98, 293 and 976 mg/kg bw per day for females of the F_0 generation and 0, 93, 280 and 926 mg/kg bw per day for males and 0, 78, 246 and 770 mg/kg bw per day for females of the F_1 generation, respectively. The NOAEL for parental toxicity was 287 mg/kg bw per day, based on reduced body weight gain and feed consumption in males at 959 mg/kg bw per day. The NOAEL for offspring toxicity was 770 mg/kg bw per day, the highest dose tested. The NOAEL for reproductive toxicity was 959 mg/kg bw per day, the highest dose tested (Schneider, 2010).

In a developmental toxicity study in rats administered triazole acetic acid at a dose of 0, 100, 300 or 1000 mg/kg bw per day by gavage (vehicle was 0.5% carboxymethyl cellulose), the NOAEL for maternal toxicity was 300 mg/kg bw per day, based on mortality, clinical signs, and reduced body weight gain and feed consumption observed early during treatment at 1000 mg/kg bw per day. Although there are indications that the findings may be due to a local effect on the gastrointestinal tract, no signs of local irritation of the stomach or gut were reported. Therefore, the Meeting could not

discount the possibility that the findings were due to a systemic effect of the compound. The NOAEL for embryo and fetal toxicity was 300 mg/kg bw per day. As severe clinical signs in the dams at 1000 mg/kg bw per day necessitated early termination, the effect of triazole acetic acid on fetal development could not be assessed at this dose (Mercer, 2011b).

In a developmental toxicity study in rabbits using gavage triazole acetic acid doses of 0, 100, 750 and 1000 mg/kg bw per day, the NOAEL for maternal toxicity was 100 mg/kg bw per day, based on mortality (first observed on day 4 of treatment), clinical signs, and reduced body weight gain and feed consumption (first observed at/after 1 day of treatment) at 750 mg/kg bw per day. As most of these animals had stomach lesions, generally described as numerous discoloured (black) erosions/ulcerations (pinpoint to 1.0 cm in diameter) on the mucosal surface, these effects are probably caused by a local effect on the gastrointestinal tract. The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, based on decreased fetal weights at 750 mg/kg bw per day (Hoberman, 2010a).

Triazole alanine

In a 12-month toxicity study in rats in which dietary triazole alanine concentrations were adjusted to achieve doses of 0, 28, 93, 278 and 916 mg/kg bw per day for males and 0, 36, 120, 375 and 1273 mg/kg bw per day for females, the NOAEL was 916 mg/kg bw per day, the highest dose tested (Wahle, 2012).

In a developmental toxicity study in rabbits using gavage triazole alanine doses of 0, 30, 100 and 250 mg/kg bw per day, the NOAEL for maternal toxicity was 100 mg/kg bw per day, based on increased incidences of soft or liquid faeces (first observed after 5 days of treatment) and decreased body weight gain and feed consumption observed at 250 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, based on decreased fetal weight and increased incidences of hyoid, angulated ala and thickened ribs observed at 250 mg/kg bw per day (Hoberman, 2010b).

Human data

No adverse health effects in plant personnel during the manufacture or formulation of penconazole-containing products over a 20-year period were reported. In incidents related to intentional misuse, occupational exposure and accidental exposure, generally no or only minor symptoms were reported.

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

Toxicological evaluation

Penconazole

The Meeting reaffirmed the ADI of 0–0.03 mg/kg bw for penconazole on the basis of a NOAEL of 3.1 mg/kg bw per day for reduced body weight gain, increased absolute and relative liver weights, and slight histopathological changes in the liver (hepatocyte necrosis associated with inflammatory cell infiltration) in a 1-year study in dogs, using a safety factor of 100.

The Meeting established an ARfD of 0.8 mg/kg bw for penconazole, on the basis of a NOAEL of 75 mg/kg bw per day for increased incidences of microphthalmia and hydrocephalus in a developmental toxicity study in rabbits. The Meeting concluded that this ARfD applies to the general population on the basis of the NOAEL of 100 mg/kg bw per day for early clinical signs, reduced body

weight gain and mortality, which might be due to systemic effects, observed in dams in a developmental toxicity study in rats. A safety factor of 100 was applied.

1,2,4-Triazole

The present Meeting reaffirmed the ADI of 0–0.2 mg/kg bw, established by JMPR in 2008, based on a NOAEL of 16 mg/kg bw per day for testicular effects (sperm abnormalities, sperm counts) observed at 30.9 mg/kg bw per day in a two-generation study of reproductive toxicity in rats, using a safety factor of 100. This ADI is supported by a new 12-month dietary toxicity study in rats with a NOAEL of 21 mg/kg bw per day, based on a reduction in body weight gain at 58 mg/kg bw per day.

The present Meeting reaffirmed the previously established ARfD of 0.3 mg/kg bw for 1,2,4-triazole, based on a NOAEL of 30 mg/kg bw per day for alterations of the urogenital system that occurred in several fetuses at 45 mg/kg bw per day and clinical signs of neurotoxicity in the dams in a study of developmental toxicity in rabbits, and using a safety factor of 100.

Triazole alanine and triazole acetic acid

The present Meeting reaffirmed the group ADI for triazole alanine and triazole acetic acid (alone or in combination) of 0–1 mg/kg bw, established by JMPR in 2008, based on a NOAEL of 100 mg/kg bw per day for delayed ossification in a developmental toxicity study in rats given triazole alanine, a NOAEL of 100 mg/kg bw per day for increased incidences of soft or liquid faeces and decreased body weight gain and feed consumption in a new developmental toxicity study with triazole alanine in rabbits, a NOAEL of 100 mg/kg bw per day for decreased fetal weight and an increase in hyoid, angulated ala and thickened ribs in a new developmental toxicity study with triazole alanine in rabbits, a NOAEL of 100 mg/kg bw per day for mortality, clinical signs, and reduced body weight gain and feed consumption in a new developmental toxicity study in rabbits with triazole acetic acid, and a NOAEL of 100 mg/kg bw per day based on decreased fetal weights in a new developmental toxicity study in rabbits with triazole acetic acid. A safety factor of 100 was used. This group ADI is expressed as triazole alanine.

The present Meeting established an ARfD of 3 mg/kg bw for triazole alanine and triazole acetic acid, based on a NOAEL of 300 mg/kg bw per day on the basis of mortality, clinical signs, and reduced body weight gain and feed consumption observed early during treatment at 1000 mg/kg bw per day in a new developmental toxicity study with triazole acetic acid in rats. A safety factor of 100 was used.

Levels relevant to risk assessment of penconazole

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year and 80-week studies of toxicity and carcinogenicity[a,b]	Toxicity	300 ppm, equal to 36 mg/kg bw per day	1 500 ppm, equal to 178 mg/kg bw per day
		Carcinogenicity	1 500 ppm, equal to 178 mg/kg bw per day[c]	–
Rat	Twenty-seven-month study of toxicity and carcinogenicity[a]	Toxicity	150 ppm, equal to 8.1 mg/kg bw per day	300 ppm, equal to 17 mg/kg bw per day
		Carcinogenicity	300 ppm, equal to 15 mg/kg bw per day[c]	–
	Two-generation study of	Reproductive toxicity	250 ppm, equal to 20 mg/kg bw per day	2 500 ppm, equal to 191 mg/kg bw per

Species	Study	Effect	NOAEL	LOAEL
	reproductive toxicity[a]			day
		Parental toxicity	250 ppm, equal to 24 mg/kg bw per day	2 500 ppm, equal to 238 mg/kg bw per day
		Offspring toxicity	250 ppm, equal to 20 mg/kg bw per day	2 500 ppm, equal to 191 mg/kg bw per day
	Developmental toxicity studies[b,d]	Maternal toxicity	100 mg/kg bw per day	300 mg/kg bw per day
		Embryo and fetal toxicity	100 mg/kg bw per day	300 mg/kg bw per day
Rabbit	Developmental toxicity study[d]	Maternal toxicity	75 mg/kg bw per day	150 mg/kg bw per day
		Embryo and fetal toxicity	75 mg/kg bw per day	150 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	200 mg/kg bw per day[c]	–
		Embryo and fetal toxicity	50 mg/kg bw per day	200 mg/kg bw per day
Dog	One-year study of toxicity[a]	Toxicity	100 ppm, equal to 3.1 mg/kg bw per day	500 ppm, equal to 16.7 mg/kg bw per day

[a] Dietary administration.
[b] Two or more studies combined.
[c] Highest dose tested.
[d] Gavage administration.

Levels relevant to risk assessment of 1,2,4-triazole[a]

Species	Study	Effect	NOAEL	LOAEL
Mouse	Ninety-day study of toxicity[b]	Toxicity	1 000 ppm, equal to 161 mg/kg bw per day	3 000 ppm, equal to 487 mg/kg bw per day
Rat	**One-year study of toxicity[b]**	**Toxicity**	**375 ppm, equal to 21 mg/kg bw per day**	**1 000 ppm, equal to 58 mg/kg bw per day**
	Multigeneration study of reproductive toxicity[b]	Parental toxicity	250 ppm, equal to 16 mg/kg bw per day	500 ppm, equal to 31 mg/kg bw per day
		Offspring toxicity	500 ppm, equal to 31 mg/kg bw per day[c]	–
	Developmental toxicity[d]	Maternal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
Rabbit	Developmental toxicity[d]	Maternal toxicity	30 mg/kg bw per day	45 mg/kg bw per day
		Embryo and fetal toxicity	30 mg/kg bw per day	45 mg/kg bw per day

[a] Studies in bold are new studies. All other studies are derived from the 2008 JMPR evaluation.
[b] Dietary administration.
[c] Highest dose tested.
[d] Gavage administration.

Levels relevant to risk assessment of triazole acetic acid[a]

Species	Study	Effect	NOAEL	LOAEL
Rat	**Thirteen-week study of toxicity and neurotoxicity[b]**	**Toxicity**	**1 002 mg/kg bw per day[c]**	**–**
		Neurotoxicity	**1 002 mg/kg bw per day[c]**	**–**
	One-generation study of reproductive toxicity[b]	**Parental toxicity**	**287 mg/kg bw per day**	**959 mg/kg bw per day**
		Offspring toxicity	**770 mg/kg bw per day[c]**	**–**
		Reproductive toxicity	**959 mg/kg bw per day[c]**	**–**
	Developmental toxicity study[d]	**Maternal toxicity**	**300 mg/kg bw per day**	**1 000 mg/kg bw per day**
		Embryo and fetal toxicity	**300 mg/kg bw per day[c]**	**–[e]**
Rabbit	**Developmental toxicity study[d]**	**Maternal toxicity**	**100 mg/kg bw per day**	**750 mg/kg bw per day**
		Embryo and fetal toxicity	**100 mg/kg bw per day**	**750 mg/kg bw per day**

[a] Studies in bold are new studies.
[b] Dietary administration.
[c] Highest dose tested.
[d] Gavage administration
[e] The effect of triazole acetic acid at 1000 mg/kg bw per day on embryo and fetal development could not be assessed due to early termination of the dams at this dose

Levels relevant to risk assessment of triazole alanine[a]

Species	Study	Effect	NOAEL	LOAEL
Rat	**Twelve-month study of toxicity[b]**	**Toxicity**	**20 000 ppm, equal to 916 mg/kg bw per day[c]**	**–**
	Multigeneration study of reproductive toxicity[b]	Parental toxicity	10 000 ppm, equal to 929 mg/kg bw per day[c]	–
		Offspring toxicity	2 000 ppm, equal to 192 mg/kg bw per day	10 000 ppm, equal to 929 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	1 000 mg/kg bw per day[c]	–
		Embryo and fetal toxicity	100 mg/kg bw per day	300 mg/kg bw per day
Rabbit	**Developmental toxicity study[d]**	**Maternal toxicity**	**100 mg/kg bw per day**	**250 mg/kg bw per day**
		Embryo and fetal toxicity	**100 mg/kg bw per day**	**250 mg/kg bw per day**
Dog	Ninety-day study of toxicity[d]	Toxicity	8 000 ppm, equal to 345 mg/kg bw per day	20 000 ppm, equal to 850 mg/kg bw per day

[a] Studies in bold are new studies. All other studies are derived from the 2008 JMPR evaluation.
[b] Dietary administration.
[c] Highest dose tested.
[d] Gavage administration.

Penconazole

Estimate of acceptable daily intake (ADI)

0–0.03 mg/kg bw

Estimate of acute reference dose (ARfD)

0.8 mg/kg bw

1,2,4-Triazole

Estimate of acceptable daily intake (ADI)

0–0.2 mg/kg bw

Estimate of acute reference dose (ARfD)

0.3 mg/kg bw

Triazole alanine and triazole acetic acid

Estimate of acceptable daily intake (group ADI), expressed as triazole alanine

0–1 mg/kg bw

Estimate of acute reference dose (ARfD)

3 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to penconazole

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rats: Rapid; > 95% in both sexes at 0.5 mg/kg bw
Dermal absorption	No data
Distribution	Rats: Widespread distribution, highest concentrations found in liver and kidney
Potential for accumulation	Low potential for accumulation
Rate and extent of excretion	Rapid; 67% and 94% in male and female rats, respectively, in 24 h. Higher urinary excretion in females (73–85%) than in males (62%). Higher biliary excretion in males (55%) than in females (40%).
Metabolism in animals	Extensively metabolized (14 metabolites identified)
Toxicologically significant compounds in animals and plants	Penconazole, 1,2,4-triazole, triazole acetic acid, triazole alanine

Acute toxicity	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Rat, LD_{50}, dermal	> 3 000 mg/kg bw
Rat, LC_{50}, inhalation	> 4.0 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Guinea-pig, dermal sensitization	Not sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Reduced body weight gain, liver
Lowest relevant oral NOAEL	3.1 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	2 000 mg/kg bw per day (rabbit; highest dose tested)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver
Lowest relevant NOAEL	8.1 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic in mice or rats[a]
Genotoxicity	
	Unlikely to be genotoxic in vivo[a]
Reproductive toxicity	
Target/critical effect	Decreased mating index
Lowest relevant parental NOAEL	24 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	20 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	20 mg/kg bw per day (rat)
Developmental toxicity	
Target/critical effect	Reduced number of live fetuses
Lowest relevant maternal NOAEL	75 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	50 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	No data
Subchronic neurotoxicity NOAEL	No data
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Studies on toxicologically relevant metabolites	*1,2,4-Triazole* Reproductive toxicity: NOAEL 16 mg/kg bw per day (rat) Developmental toxicity: NOAEL 30 mg/kg bw per day (rabbit) One-year toxicity: NOAEL 21 mg/kg bw per day (rat)

	Triazole acetic acid No toxicity up to 1 002 mg/kg bw per day in a 13-week study of toxicity and neurotoxicity in rats Acute toxicity: NOAEL 300 mg/kg bw per day for mortality, clinical signs, and reduced body weight gain and feed consumption observed early during treatment at 1 000 mg/kg bw per day in a developmental toxicity study in rats No evidence of reproductive or offspring toxicity in rats at highest doses tested (959 and 770 mg/kg bw per day, respectively) No evidence of developmental toxicity in rats at 300 mg/kg bw per day *Triazole alanine* No toxicity up to 916 mg/kg bw per day in a 12-month study of toxicity in rats Embryo and fetal toxicity: NOAEL 100 mg/kg bw per day (rat, rabbit)
Medical data	
	Generally no or only minor symptoms after intentional, accidental or occupational exposure incidents

[a] Unlikely to pose a carcinogenic risk to humans from the diet.

Summary

	Value	Study	Safety factor
Penconazole			
ADI	0–0.03 mg/kg bw	One-year study of toxicity (dog)	100
ARfD	0.8 mg/kg bw	Developmental toxicity study (rabbit)	100
1,2,4-Triazole			
ADI	0–0.2 mg/kg bw	Multigeneration reproduction toxicity study (rat), one-year study of toxicity (rat)	100
ARfD	0.3 mg/kg bw	Developmental toxicity study (rabbit)	100
Triazole alanine and triazole acetic acid			
Group ADI	0–1 mg/kg bw	Developmental toxicity studies (rat, rabbit)	100
ARfD	3 mg/kg bw	Developmental toxicity study (rat)	100

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