TEBUCONAZOLE

First draft prepared by
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Explanation

Tebuconazole is the International Organization for Standardization (ISO)–approved name for (*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol (International Union of Pure and Applied Chemistry [IUPAC]), for which the Chemical Abstracts Service (CAS) No. is 107534-96-3. Tebuconazole is a triazole fungicide that acts by inhibiting sterol biosynthesis in fungi (demethylation inhibitor).

The toxicity of tebuconazole was first evaluated by the 1994 Joint FAO/WHO Meeting on Pesticide Residues (JMPR). That 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 2.9 mg/kg bw per day for histopathological alterations in the adrenal glands seen at 4.4 mg/kg bw per day and above in two 52-week toxicity studies in dogs and using a safety factor of 100.

Tebuconazole was re-evaluated by the present Meeting at the request of the Codex Committee on Pesticide Residues (CCPR). Three new studies (an acute neurotoxicity study, a subacute neurotoxicity study and a developmental neurotoxicity study) since the last review by the JMPR were made available. All pivotal studies with tebuconazole were certified as complying with good laboratory practice (GLP) unless otherwise stated.

Evaluation for acceptable daily intake

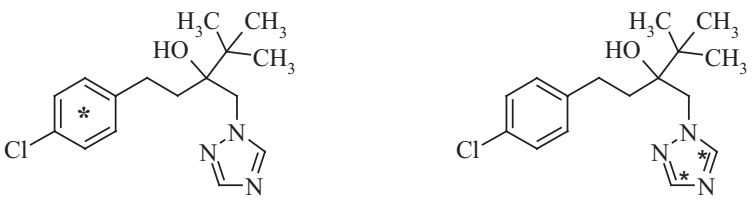
Unless otherwise stated, studies evaluated in this monograph were performed by GLP-certified laboratories and complied with the relevant Organisation for Economic Co-operation and Development (OECD) and/or United States Environmental Protection Agency (USEPA) test guideline(s).

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The absorption, distribution and elimination of tebuconazole were studied in rats after oral dosing of tebuconazole radiolabelled with ^{14}C, as shown in Figure 1.

Figure 1. Position of the radiolabel on tebuconazole used in pharmacokinetic studies in rats



* position of radiolabel

Table 1. Summary of tests performed to investigate biokinetics in rats

Test No.	Administered dose (mg/kg bw)	Sex and number of animals	Kind of test
1	20	5 males	Excretion in expired air, urine, faeces
2	2	5 males	Excretion in bile, urine, faeces
3	2	5 males	Excretion in urine, faeces; plasma levels
4	2	5 females	Excretion in urine, faeces; plasma levels
5	2	5 males	Excretion in urine, faeces; plasma levels, pretreatment[a]
6	2	5 females	Excretion in urine, faeces; plasma levels, pretreatment[a]
7	20	5 males	Excretion in urine, faeces; plasma levels
8	20	5 females	Excretion in urine, faeces; plasma levels
9	20	5 males	Excretion in urine, faeces; plasma levels, repetition of test No. 7

From Weber (1987)

[a] Pretreatment with 2 mg/kg bw non-radiolabelled dose once daily for 14 consecutive days followed by a 2 mg/kg bw radiolabelled dose on day 15.

The absorption, distribution and metabolism (see section 1.2) of tebuconazole uniformly labelled with ^{14}C in the benzene ring (Figure 1) were studied in BOR: WISW (SPF Cpb) Wistar rats. Tebuconazole, both ^{14}C labelled (radiochemical purity 99.5%) and non-radioactive, suspended in 0.5% aqueous tragacanth gel was used in the study. The test substance was administered to male and female rats at an oral dose of 2 or 20 mg/kg bw. In addition, rats of both sexes were first subjected to 14 days of treatment with a daily oral dose of 2 mg/kg bw of unlabelled test substance, followed by a single radioactive dose of 2 mg/kg bw 24 hours after the last of these doses. Furthermore, excretion of the radioactivity in the exhaled air (dose 20 mg/kg bw) and in the bile (dose 2 mg/kg bw) was studied in male rats. The study design and sampling times are summarized in Table 1.

(a) Absorption and distribution

The amounts of radioactivity excreted in the bile (90.7% of the administered dose) and urine (7.4%) by the bile duct–cannulated animals plus the residues in the total body at the time of sacrifice (0.23%) showed that the radioactivity was completely absorbed after oral administration (Table 2). The analysis of the plasma curves (Tables 3 and 4) showed that the test compound was rapidly absorbed from the gastrointestinal tract of male and female rats in all test groups, as indicated by the times at which the maximum plasma radioactivity concentrations were reached (T_{max} values between 0.33 and 1.7 hours). The maximum dose-normalized equivalent concentrations (P_{max}) were achieved between 0.11 and 0.20 hour, indicating good tissue accessibility of the radioactivity administered with the test compound. The values calculated for the terminal half-lives (Table 4) ranged from 31.9 to 52.5 hours and were therefore short in relation to the observation period of 72 hours. The dose-corrected areas under the plasma concentration–time curves (AUC) yielded a relatively wide range of AUC_{total} values (1.7–5.2 hours); correspondingly high total plasma clearances, ranging from 0.6 to 1.9 ml/min, were calculated. The mean residence time of the radioactivity in the plasma ranged from 26.9 to 48.6 hours and was therefore short in relation to the observation period of 72 hours. The relatively high values determined for the distribution volume at steady state of 8.7–17.9 ml/g indicate that the radioactivity continued to be distributed unevenly in the organism at later times after administration.

A statistical analysis of the above-mentioned parameters revealed sex-dependent differences (Table 4) between the test groups. The males in all groups were found to have significantly larger (by a factor of 1.7–3) AUCs, which led to correspondingly lower total clearance values. The males treated with the high dose also exhibited a significantly longer mean residence time; the pretreated males were found to have a significantly shorter terminal elimination half-life. Analysis of the dependence

Table 2. Cumulative excretion of total radioactivity in urine, faeces, bile and exhaled air and radioactive residues in the male rat 48 or 72 hours after oral administration of [phenyl-UL-^{14}C]-tebuconazole

Sample material	Time (h)	% of administered dose	
		Dose (mg/kg bw)	
		20	2
		Test No. 1 (n = 5)	Test No. 2 (n = 4)
Bile	1	—	15.0
	2	—	39.2
	3	—	52.8
	4	—	61.7
	6	—	72.7
	8	—	85.8
	12	—	88.0
	18	—	88.7
	24	—	89.6
	30	—	90.1
	36	—	90.5
	42	—	90.6
	48	—	90.7
Exhaled air	0–72	0.03	—
Urine	3	—	1.0
	4	1.0	2.4
	6	—	4.3
	8	3.2	5.5
	12	—	6.7
	18	—	7.1
	24	13.4	7.3
	32/30	14.5	7.3
	48	15.8	7.4
	56	16.0	—
	72	16.2	—
Faeces	24	62.7	1.5
	48	74.7	1.5
	56	74.9	—
	72	75.8	—
Total excreta	—	92.0	99.0
Total body	—	0.8	0.23
Total recovery	—	92.8	99.2

From Weber (1987)

Table 3. Time course of the dose-normalized equivalent concentrations (P) in the plasma of rats after administration of a single oral dose of [phenyl-UL-^{14}C]tebuconazole

Time post-application (h)	Dose-normalized equivalent concentration (P) in plasma (% of administered dose)						
	Male	Female	Male	Female	Male	Female	Male
	Dose (mg/kg bw)						
	2	2	2[a]	2[a]	20	20	20
	Test No. 3	Test No. 4	Test No. 5	Test No. 6	Test No. 7	Test No. 8	Test No. 9[b]
	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 5)
0.17	0.0986	0.1659	0.0501	0.0781	0.0209	0.0522	0.0450
0.33	0.1493	0.1974	0.0953	0.1081	0.0779	0.0794	0.1025
0.67	0.1481	0.1545	0.1266	0.0973	0.0961	0.0941	0.1622
1	0.1455	0.1392	0.1409	0.1048	0.1627	0.0902	0.1852
1.5	0.1504	0.1128	0.1284	0.1120	0.1471	0.0875	0.1782
2	0.1419	—	0.1285	0.1141	0.1312	0.0781	0.1562
3	0.1295	0.0956	0.1228	0.1170	0.1013	0.0680	0.1265
4	0.1308	0.0908	0.1114	0.0886	0.0933	0.0646	0.1131
6	0.1190	0.0720	0.1040	0.0821	0.1070	0.0560	0.1140
8	0.1118	0.0648	0.0991	0.0306	0.0842	0.0507	0.1111
24	0.0457	0.0225	0.0416	0.0267	0.0439	0.0182	0.0571
32	0.0385	0.0169	0.0737	0.0192	0.0387	0.0113	0.0595
48	0.0231	0.0096	0.0245	0.0118	0.0274	0.0061	0.0317
56	0.0218	0.0087	0.0222	0.0109	0.0240	0.0051	0.0319
72	0.0165	0.0072	0.0155	0.0082	0.0168	0.0044	0.0193

From Weber (1987)

[a] Pretreated with 2 mg/kg bw daily for 14 days.

[b] Repetition of test No. 7.

of the biokinetic characteristics on the size of the dose (Table 4) revealed, for both sexes, significant differences only in those parameters describing the course of the plasma radioactivity concentration. In males, the rise in the plasma radioactivity concentration took place about 2 times more slowly after administration of the high dose. In addition, the terminal elimination of the radioactivity from the plasma was found to proceed significantly more rapidly after the high dose. The females exhibited two significant effects additional to the above. After administration of the high dose, the T_{max} was observed to increase by a factor of 3. In addition, the dose-corrected AUC was significantly smaller in the females after administration of the high dose. Pretreatment with the unlabelled test substance led, in both sexes, to some differences compared with the situation after a single dose. These differences were related mainly to the characteristics derived from the time course of the plasma radioactivity concentrations. Those differences, found to be statistically significant, were a slowdown in the rise of the plasma concentration in females and a shorter terminal half-life in males after pretreatment with unlabelled test substance (Weber, 1987).

(b) Excretion and terminal radioactive residues in tissues

The radioactivity was excreted in exhaled air only to a very small extent (0.03% of the administered dose) (Table 2). The cumulative excretion, radioactivity in the body and total recoveries are shown in Table 5. After 72 hours, between 86.5% and 98.4% of the administered dose (approximately 99% of the recovered dose) was excreted in the urine and faeces. The major route of excretion was faecal (61.5–82.1%). The males of all experimental groups excreted about half as much radioactivity

Table 4. Comparison of biokinetic parameters of plasma radioactivity after oral administration of [phenyl-UL-^{14}C]tebuconazole

Parameter	Male	Female	Male	Female	Male	Female
	Dose (mg/kg bw)					
	2	2	2[a]	2[a]	20	20
	Test No. 3	Test No. 4	Test No. 5	Test No. 6	Test No. 9[b]	Test No. 8
	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 5)
Terminal half-life (h)	48.46	52.46[c]	31.93	43.68	34.45[c]	34.81[d]
AUC_{exp} (h)	3.57	2.00	3.61[c]	1.96	4.24	1.52[c]
AUC_{total} (h)	4.75	2.51	4.35[c]	2.51	5.24	1.74[d]
P_{max} (h)	0.17	0.20	0.14	0.13	0.18[c]	0.11[c]
T_{max} (h)	0.87	0.33	1.70	1.67	1.67[c]	1.06
Clearance total (ml/min)	0.71	1.35	0.71	1.35	0.64[c]	1.85[c]
Clearance renal (ml/min)	0.15	0.55	0.13[c]	0.54	0.13[c]	0.57[d]
Mean residence time (h)	48.63[c]	41.89	41.55	44.27	42.73[c]	26.87[d]
Volume steady state (ml/g)	10.90	16.74	8.71	17.93	8.18[c]	14.87[d]

From Weber (1987)
AUC, area under the curve; exp, experimental
[a] Pretreated with 2 mg/kg bw daily for 14 days.
[b] Repetition of test No. 7.
[c] Values based on four animals.
[d] Values based on three animals.

in the urine as did the females; the proportion of radioactivity excreted in the faeces was correspondingly higher in the males. Bile duct–cannulated rats eliminated, within 48 hours, about 91% of the recovered radioactivity in the bile, about 7% in the urine and only 1.5% in the faeces (Table 2). The large quantities of radioactivity determined in the faeces of the other dose groups must have been, therefore, radioactivity excreted into the intestinal lumen with the bile. The animals with biliary fistulae excreted in the urine, within 48 hours after administration, about half of the quantity of radioactivity, compared with the corresponding males of the other test groups, indicating an enterohepatic recirculation of the radioactivity.

The radioactivity remaining in the body excluding the gastrointestinal tract was low in all test groups. After 72 hours, less than 1% of the applied radioactivity could be detected in the organs, tissues and the remaining carcass. Highest residues were found in the liver and kidney, those organs responsible for metabolism and excretion of the test compound and its metabolites. Sex-dependent differences between the corresponding groups could be observed; the radiolabelled residues determined in most tissues and organs at the end of the study were approximately 1.5–2.5 times higher in males of all groups than in the corresponding females (Weber, 1987).

In a whole-body autoradiography study, [phenyl-UL-^{14}C]tebuconazole (radiochemical purity 99%) was administered in a 0.5% aqueous tragacanth gel solution at a dose level of 20 mg/kg bw to male Bor: WISC SPF Cpb Wistar rats. Seven male rats were used in this study. The animals were sacrificed at 1, 4, 8, 24, 48 and 72 hours after administration of the test substance. After deep-freezing, sagittal sections of the animals (50 µm thick) were cut with a microtome and placed onto an X-ray film. The autoradiographs were visually inspected to estimate the relative concentrations of radioactivity in the various tissues and organs of the rats.

Table 5. Cumulative excretion of total radioactivity in urine and faeces and radioactive residues in the rat 72 hours after oral administration of [phenyl-UL-^{14}C]tebuconazole

Sample material	Time (h)	% of administered dose						
		Male	Female	Male	Female	Male	Female	Male
		Dose (mg/kg bw)						
		2	2	2[a]	2[a]	20	20	20[b]
		Test No. 3 (n = 5)	Test No. 4 (n = 5)	Test No. 5 (n = 5)	Test No. 6 (n = 5)	Test No. 7 (n = 5)	Test No. 8 (n = 5)	Test No. 9 (n = 5)
Urine	4	3.2	8.6	1.8	5.1	1.2	2.1	1.9
	8	6.6	14.2	5.0	9.9	3.4	8.0	5.0
	24	13.9	28.1	12.8	25.8	11.7	23.3	14.4
	32	14.9	29.9	13.9	28.5	12.6	25.2	15.3
	48	15.9	32.0	14.5	31.1	13.9	27.8	16.6
	56	16.1	32.5	14.8	31.7	14.1	28.3	16.7
	72	16.3	32.9	15.0	32.3	14.4	28.8	17.0
Faeces	24	71.1	52.2	64.3	47.1	61.3	50.8	63.8
	48	79.9	60.9	75.0	58.7	70.8	60.8	77.3
	56	81.3	61.0	76.9	61.2	71.1	61.3	77.7
	48–72	82.1	62.5	78.8	61.5	72.1	62.7	78.7
Total excreta	—	98.4	95.4	93.8	93.8	86.5	91.5	95.7
Total body	—	0.81	0.69	1.01	1.38	0.81	0.52	1.03
Total recovery	—	99.2	96.1	94.8	95.2	87.3	92.0	96.7

From Weber (1987)

[a] Pretreated with 2 mg/kg bw daily for 14 days.

[b] Repetition of test No. 9.

One hour after administration, tebuconazole-derived radioactivity was detectable in almost all tissues and organs, with the exception of compact bone substance. The radioactivity of the parent compound was unevenly distributed in the animal body. Very high concentrations of radioactivity were discernible in the contents of the stomach and some portions of the small intestine, in the preputial gland as well as in areas of the mucosa of nose and tongue and the epithelium of the oesophagus. High concentrations were found in the liver, the cortex of the adrenal gland, the infraorbital gland and the hair follicles of the dorsal skin. Low-level radioactivity was detected in the fatty tissues, renal papilla, musculature, thymus, bone marrow and skin. During the entire investigation, the ratio of the radioactivity concentrations among the tissues and organs showed only slight alterations. With increasing time after administration, the concentrations declined more rapidly in the mucosa of the nasopharyngeal tract, the fat tissues, the brain and spinal marrow as well as the infraorbital gland and the preputial gland, compared with the mean body concentration. At the end of the investigation, radioactivity concentrations were relatively low in most organs and tissues; only the cortex of the adrenal gland showed a high level of radioactivity. Additionally, the evaluation of the autoradiographs showed high biliary excretion combined with a long-lasting enterohepatic circulation as well as a relatively low renal elimination rate (Weber, 1988).

1.2 Biotransformation

The metabolism of the test substance after administration of either [phenyl-UL-^{14}C]tebuconazole (purity > 99%) or [triazol-3,5-^{14}C]tebuconazole (purity 98.4%) to several groups of BOR:

Table 6. Summary of tests performed to investigate the metabolism of tebuconazole in rats

Test No.	Administered dose (mg/kg bw)	Sex and number of animals	Kind of test
1	2	5 males	Single oral low dose, phenyl label
2	2	5 females	Single oral low dose, phenyl label
3	2	5 males	Oral low dose, pretreatment,[a] phenyl label
4	2	5 females	Oral low dose, pretreatment,[a] phenyl label
5	20	5 males	Single oral high dose, phenyl label
6	20	5 females	Single oral high dose, phenyl label
7	20	5 males	Single oral high dose, triazole label (excretion experiment)
8	20	5 males	Single oral high dose, triazole label (metabolism)
9	20	5 females	Single oral high dose, triazole label (metabolism)

From Ecker et al. (1987)

[a] Pretreatment with 2 mg/kg bw of non-radiolabelled dose once daily for 14 consecutive days followed by a 2 mg/kg bw radiolabelled dose on day 15.

WISW (SPF Cpb) rats under varying experimental conditions was assayed. The dose groups were a single oral low dose of 2 mg/kg bw; 14 daily single oral non-radioactive doses of 2 mg/kg bw, followed by a radioactive dose of 2 mg/kg bw on the 15th day; and a single oral high dose of 20 mg/kg bw (Table 6).

In the main study, [phenyl-UL-^{14}C]tebuconazole was used. Each group consisted of five male and five female animals. In addition to these trials, the high dose of the triazole-labelled test substance was orally administered to both sexes. Because more than 90% of the recovered radioactivity was excreted via faeces and urine within 48 hours, pooled samples from the sampling intervals 0–48 hours were prepared in order to include all major metabolites. The metabolites were extracted and purified from urine and faeces with suitable solvents. The identification was conducted by comparative high-performancee liquid chromatography and gas chromatography using authentic reference compounds, as well as by employing gas chromatography with mass spectrometry and nuclear magnetic resonance spectroscopic techniques. The radioactivity of urine and extracts of faeces and urine was determined by liquid scintillation counting; solid samples were combusted, and the radioactivity of the trapped carbon dioxide was measured by liquid scintillation counting. The quantitative distribution of the identified metabolites in urine and faeces is given in Table 7.

The excretion balances showed nearly identical patterns compared with those in the biokinetic study (Weber, 1987). Approximately 14–33% of the dose was eliminated in the urine, and 61–82% of the dose was eliminated in the faeces, with no apparent differences between the two dose groups. After dosing with the [^{14}C]phenyl-labelled compound, males excreted significantly less radioactivity in the urine (14–17% of the dose) than did females (29–33% of the dose); in the case of the [^{14}C]-triazole-labelled compound, there were no sex-dependent differences in the urine to faeces ratio of radioactivity excretion. The parent compound was not detected in the urine; only between 0.5% and 2.2% could be found in the faeces of all dose groups. Regarding the excreta as a whole, tebuconazole-1-hydroxy (M 03) and tebuconazole-carboxylic acid (M 06) were the main metabolites in all test groups and accounted for 15.7–28.2% (M 03) and 14.1–36.2% (M 06) of the administered radioactivity, with a slight tendency towards higher amounts in females. Sex-related differences between the test groups were found in the quantitative distribution of some of the minor metabolites in the excreta. One compound, tebuconazole-1,5-di-OH-glucuronide (M 12), was detected in the excreta of male animals only and accounted for 0.7–1.3% of the administered radioactivity. Two further

Table 7. Quantitative distribution of metabolites in urine and faeces after oral administration of [phenyl-UL-^{14}C]tebuconazole or [triazole-3,5-^{14}C]tebuconazole to the rat

Metabolites[a]	% of administered dose							
	Male	Female	Male[b]	Female[b]	Male	Female	Male	Female
	Dose (mg/kg bw)							
	2	2	2	2	20	20	20	20
	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Test No. 5	Test No. 6	Test No. 2	Test No. 3
	Urine/ faeces (0–48 h)	Urine/ faeces (0–48 h)	Urine/ faeces (0–48 h)	Urine/ faeces (0–48 h)	Urine/ faeces (0–48 h)	Urine/ faeces (0–48 h)	Urine (0–48 h)	Urine (0–48 h)
	Phenyl	Phenyl	Phenyl	Phenyl	Phenyl	Phenyl	Triazole	Triazole
Tebuconazole	0.46	0.57	0.69	0.49	2.2	0.47	—	—
M 02	2.4	3.1	3.4	3.1	4.7	5.1	—	—
M 03	15.7	18.9	16.8	21.6	19.7	28.2	2.2	0.30
M 04	1.3	0.47	2.2	0.78	5.6	0.37	—	—
M 06	32.6	36.2	27.1	35.3	14.1	29.8	1.6	9.7
M 07	3.4	1.1	5.6	0.78	2.3	1.0	3.4	0.7
M 10	—	2.0	0.10	2.2	0.09	2.3	0.20	2.7
M 11	0.46	4.8	0.29	3.0	0.19	3.7	0.30	2.9
M 12	1.3	—	0.70	—	1.0	—	0.50	0.20
M 14	0.65	0.66	0.69	0.88	1.1	0.28	—	—
M 26	—	—	—	—	—	—	5.4	1.5
Unknown 1	1.4	1.1	1.2	0.98	1.0	0.65	—	—
Unknown 2	2.0	0.57	1.5	0.59	3.5	0.19	—	—
Unknown 3	1.8	0.09	0.98	0.20	2.3	0.19	—	—
Unknown 4	0.93	0.66	1.3	0.59	2.2	0.84	—	—
Unknown 5	0.56	0.28	0.88	0.29	1.6	0.28	—	—
Total identified[c]	**58.3**	**67.9**	**57.6**	**68.1**	**51.0**	**71.2**	**13.6**	**18.0**
Sum unknown 1–5	6.7	2.7	5.9	2.7	10.6	2.2	—	—
Not assigned[d]	19.8	19.9	26.0	21.4	20.4	13.7	10.3	6.4
Total unidentified[c]	26.5	22.6	32.0	24.1	31.0	15.9	10.3	6.4
Unextracted solids	6.9	3.7	7.7	4.3	9.5	4.4	—	—
Faeces	—	—	—	—	—	—	70.7	72.7
Body[e]	0.83	0.57	1.2	1.1	1.6	1.8	5.9	3.0
Total recovery[c]	**92.5**	**94.8**	**98.3**	**97.5**	**93.2**	**93.4**	**100.6**	**100.2**

From Ecker et al. (1987)

[a] For chemical names and codes, see Figure 2 below.

[b] Pretreated with 2 mg/kg bw daily for 14 days.

[c] Any lack of correspondence between the sum of the individual values and the "total" values is due to rounding.

[d] Radioactivity not in discrete fractions.

[e] Values included for balance reasons.

compounds, tebuconazole-1,5-dihydroxy (M 04) and tebuconazole-ketocarboxylic acid (M 07), were detected in higher amounts in the excreta of the males compared with those of the females. The corresponding values for the males were 1.3–5.6% (M 04) and 2.3–5.6% (M 07) compared with 0.4–0.8% (M 04) and 0.8–1.1% (M 07) of the administered radioactivity in the females. Two compounds were found in greater amounts in the excreta of the females. Tebuconazole-1-hydroxysulfate (M 10) accounted for 2.0–2.3% and tebuconazole-1-OH-glucuronide (M 11) 3.0–4.8% of the administered radioactivity in females. Both compounds were detected in the excreta of the males in amounts of less than one tenth of these values. Two additional compounds, tebuconazole-*o*-hydroxy (M 02) and tebuconazole-desmethyl (M 14), were detected in minor amounts and showed no significant dose- or sex-dependent differences. Neither the dose level nor the pretreatment showed a significant influence on the metabolic pattern in any of the dose groups. Five unidentified compounds were detected in all dose groups, none of them exceeding 3.5% of the administered radioactivity. Sex-related differences between the test groups were detected in the distribution of these compounds. In general, females excreted less than half as much of these compounds as did males. In total, 15.9–32.0% of the administered dose remained unidentified after extraction. This unidentified activity consisted of the above-mentioned unidentified compounds and of background activity not assigned to specific metabolites or fractions. Between 3.7% and 9.5% of the administered radioactivity remained unextractable in the faeces. The rate of identification was high; after administration of [phenyl-UL-^{14}C]tebuconazole at doses of 2 or 20 mg/kg bw, between 51.0% and 71.2% of the administered radioactivity could be identified. The identification balance did not take into account the amount of 1,2,4-triazole ("free triazole", M 26) found in the study with [triazole-3,5-^{14}C]tebuconazole. In a total material balance, the amount of identified radioactivity should include the figures for 1,2,4-triazole as well. The triazole-labelled metabolites from faecal extracts were not quantified in this study because the comparison of the metabolic profiles in faecal extracts shows identity regardless of the label. Comparison of the metabolic profiles in urine of the animals treated with differently labelled tebuconazoles raised one significant difference. One additional metabolite, identified as 1,2,4-triazole (M 26), accounted for 5.4% of the administered radioactivity in males and 1.5% in females. The metabolic profiles revealed sex-related differences similar to those already observed in the animals treated with the phenyl-labelled test substance (Ecker et al., 1987).

The proposed metabolic pathway is given in Figure 2. The first step in the biotransformation of tebuconazole was the hydroxylation of the *t*-butyl group, resulting in tebuconazole-1-hydroxy. This compound was either excreted or further transformed via oxidation to the carboxylic acid, sulfonylation or conjugation with glucuronic acid at the *t*-butyl group. Further steps of minor importance involve hydroxylation of tebuconazole-1-hydroxy in the benzylic position followed by conjugation with glucuronic acid at the *t*-butyl group, hydroxylation of the parent compound's phenyl ring, oxidation in the benzylic position, decarboxylation at the *t*-butyl group and cleavage of the triazole moiety (Neumann & Hartmann, 2009).

2. Toxicological studies

2.1 Acute toxicity

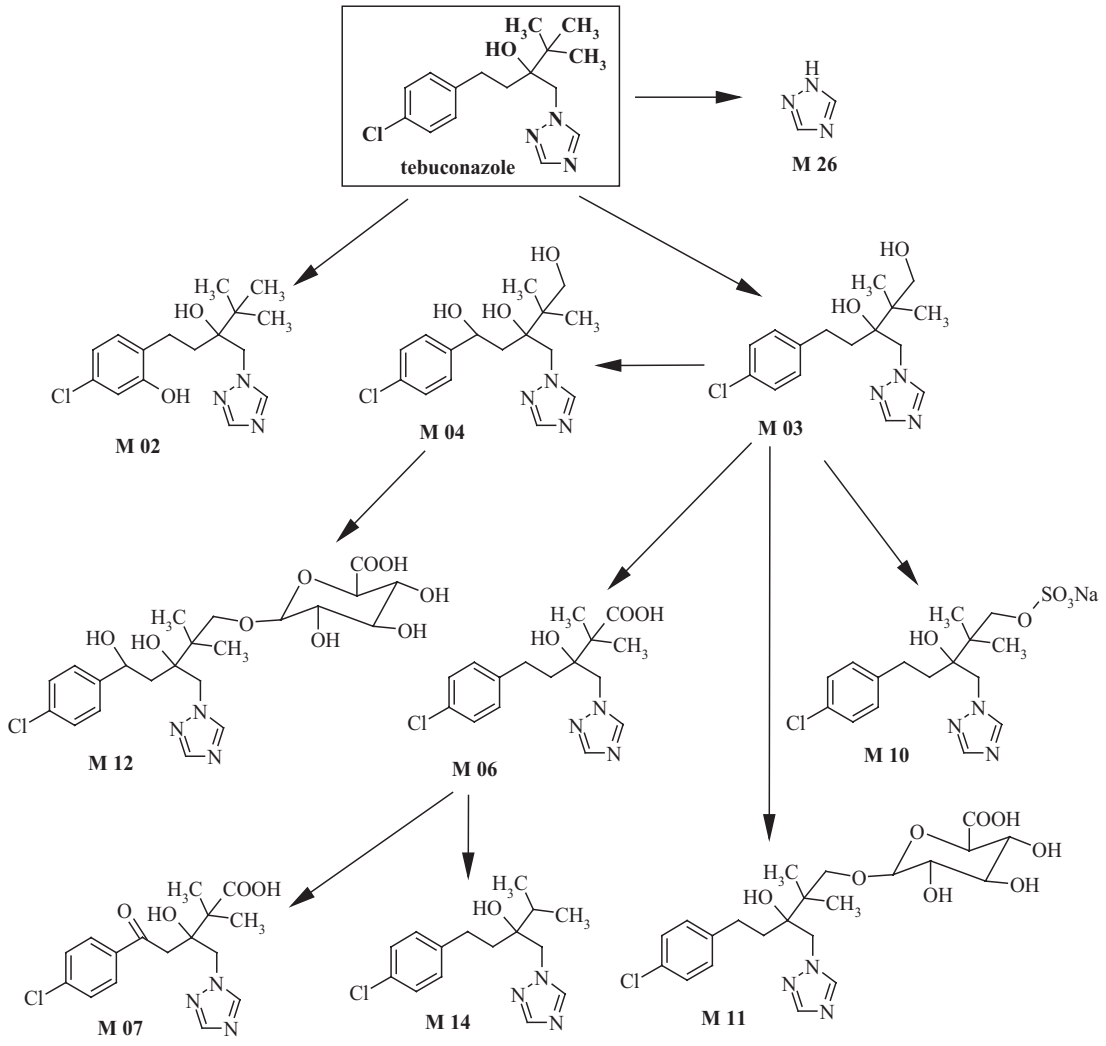
The acute toxicity of tebuconazole is summarized in Table 8.

(a) Oral administration

Mice

Groups of male and female young adult Crj:CD ICR mice (five of each sex per dose) were given tebuconazole (purity 98.0%) as a single dose of 0 mg/kg bw or in the range 1600–5000 mg/kg

Figure 2. Proposed metabolic pathway of tebuconazole in rats



M 02: tebuconazole-*o*-hydroxy
M 03: tebuconazole-1-hydroxy
M 04: tebuconazole-1,5-dihydroxy
M 06: tebuconazole-carboxylic acid
M 07: tebuconazole-ketocarboxylic acid
M 10: tebuconazole-1-hydroxysulfate
M 11: tebuconazole-1-OH-glucuronide
M 12: tebuconazole-1,5-di-OH-glucuronide
M 14: tebuconazole-desmethyl
M 26: 1,2,4-triazole

bw by gavage. The test material was formulated in polyethylene glycol 400. The dose volume was 10 ml/kg bw. Animals were fasted overnight prior to dosing. Animals were observed frequently for clinical signs on day 1 and once or twice a day thereafter. Body weights were recorded on days 1, 7 and 14. Necropsy was performed on all animals.

Sedation and abnormal gait were observed between 1 minute and 1 hour after administration. The main clinical signs observed were sedation, abnormal gait, paralytic gait, hypnosis, half-closed eyes, rough coat, abnormal breathing and chick-like vocalization. The clinical signs disappeared within 3 days post-dosing in surviving animals except for rough coat in females of the 5000 mg/kg bw dose group, which disappeared 5 days after dosing. Changes in the digestive system (mucosal redness, dark reddish brown focus in the stomach, dilated lumen, yellowish contents and mucosal redness in the small intestine), lungs (dark reddish brown) and testis (atrophy) were observed in

Table 8. Acute toxicity of tebuconazole

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/m^3)	Reference
Mouse	Crj:CD ICR	M, F	Oral	Fasted M: 2800 F: 5200	—	Ohta (1991b)
	NMRI	M, F	Oral	Fasted M: 1615 F: 3023	—	Heimann & Pauluhn (1983)[a]
Rat	Crj:CD Sprague-Dawley	M, F	Oral	Fasted M: 4000 F: 1700	—	Ohta (1991a)
	Bor:WISW (SPF Cpb) Wistar	M, F	Oral	Fasted M: > 5000 F: 3933 Non-fasted M: 4264 F: 3352	—	Heimann & Pauluhn (1983)[a]
	Bor:WISW (SPF Cpb) Wistar	M	Oral	> 5000	—	Flucke (1987)
Rabbit	HC:NZW	M, F	Oral	Fasted M and F > 1000	—	Heimann & Pauluhn (1983)[a]
Rat	Bor:WISW (SPF Cpb) Wistar	M, F	Intraperitoneal	Fasted M: 751 F: 395	—	Heimann & Pauluhn (1983)[a]
Rat	Bor:WISW (SPF Cpb) Wistar	M, F	Dermal	M and F > 5000	—	Heimann & Pauluhn (1983)[a]
	Crj:CD SPF Sprague-Dawley	M, F	Dermal	> 2000	—	Ohta (1991c)
Rat	Bor:WISW (SPF Cpb) Wistar	M, F	Inhalation	—	> 818	Heimann & Pauluhn (1983)[a]
	Bor:WISW (SPF Cpb) Wistar	M, F	Inhalation	—	> 5093 (dust) > 371 (aerosol)	Pauluhn (1988)
	Hsd:Cpb:WU Wistar	M, F	Inhalation	—	> 2118	Pauluhn (2007)
Rabbit	HC:NZW	M	Dermal	Non-irritating	—	Heimann & Pauluhn (1983)[a]
	New Zealand White	M, F	Dermal	Non-irritating	—	Sheets (1988)
Rabbit	HC:NZW	M	Ocular irritation	Non-irritating	—	Heimann & Pauluhn (1983)[a]
	New Zealand White	M, F	Ocular irritation	Mildly irritating	—	Eigenberg & Sheets (1988)

Table 8 (continued)

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/m^3)	Reference
Guinea-pig	Pirbright White	F	Dermal sensitization (mazimization test)	Non-sensitizing	—	Heimann (1983)
	Hsd Poc:DH (SPF-bred)	F	Dermal sensitization (mazimization test)	Non-sensitizing	—	Stropp (1996)
	DHPW (SPF-bred)	M	Dermal sensitization (Buehler test)	Non-sensitizing	—	Heimann (1987)
	Dunkin-Hartley	M	Dermal sensitization (Buehler test)	Non-sensitizing	—	Sheets (1990)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male
[a] Non-GLP study.

animals that died during the observation period. Under the study conditions utilized, the oral median lethal dose (LD_{50}) of tebuconazole in mice was calculated to be 2800 and 5200 mg/kg bw for males and females, respectively (Ohta, 1991b).

Groups of male and female young adult NMRI mice (five of each sex per dose) were given tebuconazole (purity 97.1%) as a single dose of 0 mg/kg bw or in the range 100–3350 mg/kg bw for males and 500–5000 mg/kg bw for females by gavage formulated in Cremophor EL/water. The dose volume was 10 ml/kg bw. Animals were fasted overnight prior to dosing. The treated animals were observed for 14 days after dosing. Animals were observed for clinical signs and mortality frequently on day 1 and once per day thereafter. Body weights were recorded on days 0, 7 and 14. A gross necropsy was performed on all animals.

The primary clinical signs noted in the study included behavioural and motility disturbances, dyspnoea, staggering, spastic gait, stiff posture, rolling, reduced reflexes, prostration on side and stomach, occasional twitching and weight loss (males only). Animals that died during the post-treatment observation period exhibited spotted and distended lungs; patchy, pale and enlarged liver and liver lobulation; and reddened glandular stomach. No treatment-related findings were observed in animals sacrificed at termination. Under the study conditions utilized, the oral LD_{50} of tebuconazole in fasted mice was calculated to be 1615 and 3023 mg/kg bw for males and females, respectively (Heimann & Pauluhn, 1983).

Rats

Groups of male and female young adult Crj:CD Sprague-Dawley rats (five of each sex per dose) were given tebuconazole (purity 98.0%) as a single dose of 0 mg/kg bw or in the range from 1600 to 5000 mg/kg bw for males and from 730 to 5000 mg/kg bw for females by gavage formulated in polyethylene glycol 400 suspension. Animals were fasted overnight prior to dosing. Animals were observed for mortality and clinical signs at 1-hour intervals for the first day and twice daily thereafter for 13 days. Body weights were recorded on days 0, 7 and 14. A gross necropsy was performed on all animals.

No treatment-related effects on body weight were observed. Clinical signs of toxicity were manifested as sedation, abnormal gait, paralytic gait and emaciation. The symptoms were observed from 20 minutes to 4 days after administration in both sexes. Generally, the abnormal gait, paralytic gait and marked emaciation were observed 2 days post-dosing. At termination, abnormal findings in the liver (yellow-white patchy areas) and the testis (atrophy) for males were observed. Changes in the urinary bladder (reddish content), the adrenals (redness and hypertrophy) and the trachea (retention of foamy fluid) were observed in animals that died during the observation period. Under the study

conditions utilized, the oral LD_{50} of tebuconazole in rats was calculated to be 4000 and 1700 mg/kg bw for males and females, respectively (Ohta, 1991a).

In a second study, fasted young adult Bor:WISW (SPF Cpb) Wistar rats (five of each sex per dose) were given tebuconazole (purity 97.1%) as a single dose by gavage in Cremophor EL/water at a dose of 0 mg/kg bw or in the range from 1000 to 5000 mg/kg bw for fasted male and female rats and from 500 to 5000 mg/kg bw for non-fasted male and female rats. Animals were observed for 14 days after dosing. Animals were observed for clinical signs and mortality frequently on day 1 and once per day thereafter. Body weights were recorded on days 0, 7 and 14. A gross necropsy was performed on all animals.

Body weight losses were noted in the first week of the post-dosing period but were normalized at the end of the study. The primary clinical signs noted in the study included behavioural, breathing and motility disturbances, staggering, spastic gait, sternal or lateral recumbency, cramped posture, increased urine excretion and poor reflexes. Under the study conditions utilized, the oral LD_{50} of tebuconazole in fasted rats was calculated to be greater than 5000 and 3933 mg/kg bw for males and females, respectively. The oral LD_{50} of tebuconazole in non-fasted rats was calculated to be 4264 and 3352 mg/kg bw for males and females, respectively (Heimann & Pauluhn, 1983).

Rabbits

Groups of male and female young adult HC:NZW albino rabbits (three of each sex per dose) were given tebuconazole (purity 97.1%) as a single dose of 500 or 1000 mg/kg bw by gavage formulated in Cremophor EL/water. The dose volume was 5 ml/kg bw. Animals were fasted overnight prior to dosing. The treated animals were observed for 14 days after dosing. Animals were observed for clinical signs and mortality frequently on day 1 and once per day thereafter. Body weights were recorded on days 0, 7 and 14. A gross necropsy was performed on all animals.

No mortality occurred. A general loss of appetite was observed; however, body weights were not affected by the treatment. In animals sacrificed at termination, slightly distended and spotted lungs and slightly patchy kidneys were observed. Under the study conditions utilized, the oral LD_{50} of tebuconazole in fasted rabbits was greater than 1000 mg/kg bw for males and females, respectively (Heimann & Pauluhn, 1983).

(b) Intraperitoneal administration

Rats

Groups of young adult Bor:WISW (SPF Cpb) Wistar rats (five of each sex per dose) were given tebuconazole (purity 97.1%) intraperitoneally in Cremophor EL/water as a single dose in the range from 50 to 1000 mg/kg bw for fasted males and from 50 to 560 mg/kg bw for fasted females. The dose volume was 10 ml/kg bw. Animals were observed for 14 days. Animals were observed for clinical signs and mortality frequently on day 1 and once a day thereafter. Body weights were recorded on days 0, 7 and 14. A gross necropsy was performed on all animals.

Behavioural, breathing and motility disturbances, staggering, spastic gait, uncoordinated movements, poor reflexes, narcosis, convulsions, and lateral or sternal recumbency were observed. Animals dying during observation exhibited patchy to dark red, distended lungs; patchy, sometimes pale spleen and kidneys; patchy liver, slightly swollen from 500 mg/kg bw onwards, with the individual lobes adhering to each other and to pancreas, diaphragm, stomach and fatty tissue; reddened glandular stomach; thin, unpatterned walls of stomach; clear fluid in abdomen; and whitish deposits on all abdominal organs. Animals sacrificed at the end of the observation period exhibited swollen liver, with the liver lobes adhering to each other, and spleen covered with a white coat. Under the

study conditions utilized, the intraperitoneal LD_{50} of tebuconazole in fasted rats was calculated to be 751 and 395 mg/kg bw for males and females, respectively (Heimann & Pauluhn, 1983).

(c) Dermal application

Rats

Five male and five female young adult Bor:WISW (SPF Cpb) Wistar rats were exposed dermally to tebuconazole (purity 97.1%) at 5000 mg/kg bw applied to a shaved dorsal area of the body surface. The test substance was mixed with physiological saline solution. The exposure period was 24 hours. The treated area was covered by means of occlusive dressings and aluminium foil. The treated site was rinsed with water and soap 24 hours after the treatment. Animals were observed for mortality and clinical signs several times a day on day 1 and once a day for the remainder of the 14-day observation period. Body weights were recorded on days 1, 7 and 14. A gross necropsy was performed on all animals.

No treatment-related mortality, clinical signs, body weight changes, skin irritation or pathological findings were observed. The dermal LD_{50} of tebuconazole in rats was greater than 5000 mg/kg bw for males and females (Heimann & Pauluhn, 1983).

Five male and five female young adult Crj:CD SPF Sprague-Dawley rats were exposed dermally to tebuconazole (purity 98.0%) at 2000 mg/kg bw applied to a shaved dorsal area of the body surface. The test substance was mixed with polyethylene glycol 400 (5 ml/kg bw). The exposure period was 24 hours. The treated area was covered with gauze and sponge held in place by a non-irritating bandage. The treated site was rinsed with warm water 24 hours after the treatment. Animals were observed for mortality and clinical signs several times a day on day 1 and once a day for the remainder of the 14-day observation period. Body weights were recorded on days 1, 7 and 14. A gross necropsy was performed on all animals.

No treatment-related mortality, clinical signs, body weight changes, skin irritation or pathological findings were observed. The dermal LD_{50} of tebuconazole in rats was greater than 2000 mg/kg bw for males and females (Ohta, 1991c).

(d) Exposure by inhalation

Rats

Groups of 5–10 male and female young adult Bor:WISW (SPF Cpb) Wistar rats were exposed by nose only to tebuconazole (purity 97.1%) for 4 hours at nominal concentrations of 100, 250, 2500 or 5000 mg/m^3 (equivalent to analytical concentrations of 16, 49, 387 and 818 mg/m^3) for 4 hours. Another group was exposed to tebuconazole for 6 hours per day for 5 days at nominal concentrations of 100, 300 or 1000 mg/m^3 (equivalent to analytical concentrations of 24, 60 and 240 mg/m^3). The vehicle used in this study was ethanol:polyethylene glycol 400 (1:1). Rats were observed for 14 days. The animals were observed for clinical signs of toxicity several times during the first day and once daily thereafter. Individual body weights were recorded at day 1 (pretest), day 8 and day 15 of the test. Necropsy was performed on all animals.

The particle size was determined in the repeated-exposure study. The mass median aerodynamic diameter (MMAD) was 7.1, 5.0 and 4.6 μm for the 100, 300 and 1000 mg/m^3 dose groups, respectively.

There were no treatment-related effects on mortality or body weights in either study group. In the single-exposure study, reduced motility (lassitude) was observed in the 250, 2500 and 5000 mg/m^3 dose groups. In the repeated-dose study, nonspecific disturbed behaviour (lassitude) was observed in all groups. At necropsy, there were no indications of concentration-related grossly apparent lung

or organ damage. Under the study conditions utilized, it can be concluded that the inhalation median lethal concentration (LC_{50}) of tebuconazole following a single 4-hour exposure and five daily 6-hour exposures in rats was greater than 818 mg/m^3 and greater than 240 mg/m^3, respectively (Heimann & Pauluhn, 1983).

In a second study, groups of five male and five female young adult Bor:WISW (SPF Cpb) Wistar rats were exposed by nose only to tebuconazole (purity 96.2%) for 4 hours at a concentration of 0 or 4000 mg/m^3 (dust and aerosol) for 4 hours. The analytical concentrations were 371 mg/m^3 and 5093 mg/m^3 for aerosol and dust, respectively. The vehicle used in this study was ethanol:polyethylene glycol 400 (1:1). Control and vehicle control groups were also included in the study. Rats were observed for 14 days. The animals were observed for clinical signs of toxicity several times during the first day and daily thereafter. Individual body weights were recorded on the day of treatment and 3, 7 and 14 days following exposure. Necropsy was performed on all animals.

The particle size for the aerosol exposure was less than or equal to 5 μm. For the exposure via dust, approximately 8% of the particles were less than or equal to 5 μm.

No clinical signs of toxicity were observed in this study. No mortality occurred in the study. No treatment-related effects were observed in the group exposed to dust. In aerosol-treated groups, slight body weight loss was observed on day 3. The rats sacrificed at the end of the observation period did not provide any indications of grossly apparent lung or other organ damage. Under the study conditions utilized, it can be concluded that the inhalation LC_{50} of tebuconazole following a single 4-hour exposure was greater than 5093 mg/m^3 for dust exposure and greater than 371 mg/m^3 for aerosol exposure (Pauluhn, 1988).

In a third study, groups of five male and five female young adult Hsd Cpb:WU (SPF) Wistar rats were exposed by nose only to tebuconazole (purity 97.1%) for 4 hours at an aerosol concentration of 0 or 5000 mg/m^3 (target concentration). The gravimetrically measured concentration was 2118 mg/m^3. All animals were observed for mortality, signs of gross toxicity and behavioural changes at least once daily for 14 days after dosing. Body weights were recorded prior to administration and again on days 1, 3, 7 and 14. At the end of the scheduled period, the animals were killed and subjected to a gross examination.

Atmospheres generated had mean aerodynamic particle sizes of 2.76 μm, with a geometric standard deviation of 1.84 μm.

No mortality was observed in the study. All rats tolerated the exposure without specific signs but displayed an ungroomed hair-coat on post-exposure days 1–2. From post-exposure day 3 onwards, all rats appeared to be indistinguishable from the control. Transient body weight loss was observed in females. Statistically significant decreased body temperature was observed in treated groups compared with the controls; however, the extent of the change was too small to be of any toxicological significance. Necropsy findings were unremarkable between the control and the treated groups. Under the study conditions utilized, it can be concluded that the inhalation LC_{50} of tebuconazole in rats was greater than 2118 mg/m^3 (Pauluhn, 2007).

(e) Dermal irritation

In a study of primary dermal irritation, three young adult male HC:NZW rabbits were dermally exposed to 0.5 g of tebuconazole (purity 97.1%) mixed to a paste in water applied to 6 cm^2 of skin and fastened with an elastic adhesive tape. The test material was in contact with the skin for 4 hours. The other side of the back region was treated with water in a similar manner to serve as the control. The treated area was washed with water following a 4-hour exposure. Dermal irritation was scored according to the method of Draize after 30 minutes and then daily for 3 days.

No irritation was observed on any rabbits following application of tebuconazole. Under the conditions of this study, it is concluded that tebuconazole is non-irritating to the skin of rabbits (Heimann & Pauluhn, 1983).

In a second study of primary dermal irritation, three young adult male and female New Zealand White rabbits were dermally exposed to 0.5 g of tebuconazole (purity 96.6%) mixed to a paste in water applied to 6 cm^2 of skin and covered with gauze that was secured with hypoallergenic tape, then covered with a square of plastic and secured with adhesive bandage. Plastic covers were also placed on rabbits to prevent access to the exposure site. The application site was wiped with a paper towel following a 4-hour exposure period. Dermal irritation was scored at 0.5 hour, 1 hour and 1, 2 and 3 days using the method of Draize.

No irritation was observed in this study. No mortality or any adverse clinical signs were observed in the study. Under the conditions of this study, it is concluded that tebuconazole is non-irritating to the skin of rabbits (Sheets, 1988).

(f) Ocular irritation

In a study of primary eye irritation, 100 µl (approximately 50 mg) of tebucobazole (purity 97.1%) was instilled into the conjunctival sac of one eye of each of three male HC:NZW rabbits. The eyes were washed with saline 24 hours after the instillation. Irritation was scored by the method of Draize at 1 hour and 1, 2, 3, 7, 14 and 21 days after exposure.

There were no signs of corneal opacities or lesions involving the iris in any animal during the study. Reddening of conjunctiva was observed in one animal (average score 0.3; reversible at 48 hours). No chemosis was observed. Under the conditions of this study, it is concluded that tebuconazole is non-irritating to the eyes of rabbits (Heimann & Pauluhn, 1983).

In a second study of primary eye irritation, 100 mg of tebucobazole (purity 96.3%) was instilled into the conjunctival sac of one eye of each of three male and female New Zealand White rabbits. The treated eyes were not washed. Irritation was scored by the method of Draize at 1, 24, 48 and 72 hours after exposure.

There were no signs of corneal opacities or lesions involving the iris in any animal during the study. All six rabbits developed redness (grade 1), chemosis (grades 1 and 2) and discharge (grades 2 and 3) of the conjunctiva 1–24 hours after dosing. Chemosis and discharge had resolved in five animals by 72 hours after dosing and in all animals by day 7. Redness had resolved in four rabbits by 72 hours after dosing and in the one remaining animal by day 8. Under the conditions of this study, it is concluded that tebuconazole is mildly irritating to the eyes of rabbits (Eigenberg & Sheets, 1988).

(g) Dermal sensitization

In a study of dermal sensitization with tebuconazole (purity 97.1%), young male Pirbright White guinea-pigs were tested using the maximization method of Magnusson & Kligman. Twenty guinea-pigs were assigned to the test group, and another 20 served as the controls. In this study, the test concentrations chosen were 1% for intradermal induction and 25% for epidermal induction and challenge. The test material was formulated in 1% Cremophor EL in distilled water for intradermal induction and challenge. The topical induction was performed 1 week after the intradermal induction with 25% test substance (test group) (not irritating to the skin) and without test substance (control group) in a 48-hour exposure period. The challenge was performed 3 weeks after the intradermal induction with 25% test substance to the test and control group in a 24-hour exposure period.

Observations on skin effects, clinical signs and body weights were performed. The skin reactions were assessed after 24 and 48 hours.

Five animals in the control group and two animals in the treatment group died during the study. No treatment-related changes in body weights or any other clinical signs were observed. Evaluation revealed the same number of positive skin reactions on flanks in the test compound group and the control group. Under the study conditions utilized, it is concluded that tebuconazole is not a skin sensitizer in male guinea-pigs as determined by the method of Magnusson & Kligman (Heimann, 1983).

In a second study of dermal sensitization with tebuconazole (purity 96.9%), young female Hsd Poc:DH (SPF-bred) guinea-pigs were tested using the maximization method of Magnusson & Kligman. Twenty guinea-pigs were assigned to the test group, and another 10 served as the controls. In this study, the test concentrations chosen were 5% for intradermal induction, 50% for epidermal induction and 40% for challenge. These doses were selected based on the results of the range-finding study conducted on five animals. The test material was formulated in 2% Cremophor EL in saline for intradermal induction, topical induction and challenge. The topical induction was performed 1 week after the intradermal induction with 50% test substance (test group, in vehicle) and without test substance (control group, vehicle only) in a 48-hour exposure period. The challenge was performed 3 weeks after the intradermal induction with 40% test substance to the test and control groups in a 24-hour exposure period. Observations on skin effects, clinical signs and body weight were performed. The skin reactions were assessed after 24 and 48 hours. The sensitivity of the test was assessed using 2-mercaptobenzothiazole.

Five animals in the control group and two animals in the treatment group died during the study. The stability and homogeneity of the test material were confirmed analytically. No treatment-related mortality, changes in body weight or any other clinical signs were observed. No skin effects were observed in the treatment or control groups. Under the study conditions utilized, it is concluded that tebuconazole is not a skin sensitizer in female guinea-pigs as determined by the method of Magnusson & Kligman (Stropp, 1996).

In a third study of dermal sensitization with tebuconazole (purity 97.4%), young adult male DHPW (SPF-bred) guinea-pigs were tested using the method of Buehler (closed-patch test). Three groups of guinea-pigs were randomly established (test group and two control groups consisting of 12 animals each). For the main study, the concentration of the test substance was 25% by weight in 2% Cremophor in sterile water for three weekly induction and challenge exposures. The test concentrations were selected based on the results of the pilot study. For the dermal induction, animals were dermally treated with patches containing 25% test substance formulation (hypoallergenic dressing loaded with the test substance formulation) 3 times at intervals of 7 days. This was the highest usable concentration. After 6 hours of exposure, the patches were removed and the skin was visually assessed. The animals from the control group were exposed to hypoallergenic patches moistened with physiological saline solution. The first challenge was performed 5 weeks after the dermal induction, and patches containing 25% test substance formulation were applied to animals in the control and test groups. Control patches were also applied to the test group. After 6 hours of exposure, the patches were removed. The skin reactions were assessed at 48 and 72 hours after patch removal. The animals were observed for clinical signs at least once daily throughout the entire study period. The body weights of the animals were recorded before initiating the study and weekly thereafter, as well as at the end of the study.

No treatment-related mortality, changes in body weight or any other clinical signs were observed. No skin effects were observed in the treatment or control groups. The second challenge

was not performed because of lack of a response following the first challenge. Dinitrochlorobenzene (DNCB) was used as a positive control, which produced skin reactions as expected. Under the study conditions utilized, it is concluded that tebuconazole is not a skin sensitizer in male guinea-pigs as determined by the method of Buehler (Heimann, 1987).

In a fourth study of dermal sensitization with tebuconazole (purity 94.6%), young adult male Dunkin-Hartley guinea-pigs were tested using the method of Buehler (closed-patch test). Four groups of guinea-pigs were randomly established: a test group (15 animals), a control group (5 animals), a DNCB positive control group (5 animals) and a DNCB control group (5 animals). A dose range–finding study was performed to estimate doses for the induction (highest dose that causes mild irritation) and first challenge (highest non-irritating dose). Animals were dermally treated with patches containing 0.4 g test substance formulation (moistened with deionized water) 3 times at intervals of 7 days. After 6 hours of exposure, the patches were removed and the skin was visually assessed. The animals from the control group were exposed to patches moistened with deionized water. The challenge was performed 4 weeks after the dermal induction, and patches containing 0.4 g test substance formulation were applied to animals in the control and test groups. Control patches were also applied to the test group. DNCB test and control groups were included as positive and non-induced controls. After 6 hours of exposure, the patches were removed. The skin reactions were assessed at 48 and 72 hours after the patch removal. The animals were observed for clinical signs at least once daily throughout the entire study period. The body weights of the animals were recorded before initiating the study and at the end of the study.

No treatment-related mortality, changes in body weights or any other clinical signs were observed in the test or DNCB-treated groups. No skin effects were observed in the treatment or control groups. The DNCB test group showed an average dermal score of 1.0/0.9 after the third induction and 1.0/1.3 after the challenge. Under the study conditions utilized, it is concluded that tebuconazole is not a skin sensitizer in male guinea-pigs as determined by the method of Buehler (Sheets, 1990).

2.2 Short-term studies of toxicity

(a) Oral administration

Rats

In a non-GLP, 28-day dose range–finding study of toxicity, groups of 20 male and 20 female Bor:WISW (SPF Cpb) Wistar rats were given tebuconazole (purity 97.0%) at a dose of 0, 30, 100 or 300 mg/kg bw per day by gavage in Cremophor EL and deionized water (0.2 ml Cremophor diluted with water to 10 ml) for 4 weeks. Ten rats of each sex per dose served as a recovery group for 4 weeks. Rats were observed for clinical signs of toxicity daily. All animals were weighed at the beginning of each week of the study and prior to necropsy. Blood, liver tissue and urine samples were evaluated at the end of the 28-day treatment period and at the end of the recovery period (five rats of each sex per dose). All animals were necropsied, and selected organs were weighed. A complete macroscopic and microscopic examination was performed.

Mild lethargy was observed in a few animals of the 300 mg/kg bw per day dose group. One control female and one high-dose female were found dead on days 19 and 21 of the treatment. The cause of death was not established for either rat. Statistically significant decreases in body weights or body weight gains were observed for males and females of the high-dose group (weeks 1–4) during the treatment period. Doses of 100 and 300 mg/kg bw per day were associated with decreased haemoglobin concentration and haematocrit values. In females at 300 mg/kg bw per day, the leukocyte count was increased. All haematological parameters had normalized after the recovery period.

Clinical chemistry revealed slight (not statistically significant) increases in the activities of glutamate–oxalate transaminase (aspartate aminotransferase [AST]) and glutamate–pyruvate transaminase (alanine aminotransferase [ALT]) in males at 300 mg/kg bw per day and marked increases in liver enzyme activities (glutamate–oxalate and glutamate–pyruvate transaminases and alkaline phosphatase) in females at the same dose. Treatment at 100 and 300 mg/kg bw per day induced the microsomal enzyme system, and the activities of *N*- and *O*-demethylases and cytochrome P450 and the triglyceride concentration in liver were increased. All these changes were reversible. Urinalyses revealed no abnormal findings. At 100 and 300 mg/kg bw per day, absolute and relative weights of the liver and spleen were increased in animals of both sexes, and the absolute weight of the kidney was increased in females. Histopathological findings at 300 mg/kg bw per day consisted of fatty changes in the liver and bile duct proliferation in females; enlargement of the centrilobular hepatocytes was found in male rats. Histopathological changes were also found in the adrenal cortex, consisting of proliferated endothelial cells and an increased incidence of fat vacuoles. Sclerosis of the red pulp of the spleen, associated with sideropenia, was observed in males at 300 mg/kg bw per day; sideropenia was also found in females at 100 mg/kg bw per day.

The NOAEL in the 28-day gavage study in rats was 30 mg/kg bw per day on the basis of changes in haematological and clinical chemical parameters and organ weights seen at the lowest-observed-adverse-effect level (LOAEL) of 100 mg/kg bw per day and above (Heimann & Kaliner, 1984).

In an oral study of toxicity in rats, tebuconazole (purity 93.4%; 4.8% symmetrical isomer) was administered for 13 weeks to Bor:WISW Wistar rats (10 of each sex per dose) in the diet at a dose level of 0, 100, 400 or 1600 parts per million (ppm) (equal to 0, 8.6, 34.8 and 171.7 mg/kg bw per day for males and 0, 10.8, 46.5 and 235.2 mg/kg bw per day for females, respectively). Diets were analysed for homogeneity, stability and concentrations. The analytical data indicated that the mixing procedure was adequate (pilot study) and that the variation between nominal and actual dosage to the animals was acceptable (within 10% of the nominal). Animals were inspected at least twice daily (once daily on weekends) for signs of morbidity and mortality; detailed physical examinations were performed weekly. Body weights, feed consumption and water consumption were recorded at weekly intervals. Ophthalmological examinations were performed on 10 males and 10 females in the control and 1600 ppm dose groups at week 4 and prior to termination. Blood was collected at 1 and 3 months for haematology and clinical analyses from five rats of each sex per group (1 month) and all survivors at 3 months. Urine was analysed at week 4 and at termination. At study termination, all animals were subjected to a detailed gross pathological examination. Organs were collected, weighed and prepared for histopathology.

The treatment had no effect on appearance, behaviour or the findings of haematological examinations or urinalyses. Feed intake was increased in animals of each sex at 1600 ppm. One male in the control group died at week 4, and a second control group male died at week 12; deaths were reported to be associated with blood sampling. In the high-dose group, one male died during the first week of dosing, and one female died during week 4; the authors concluded that both deaths were probably due to haemorrhagic diathesis and considered them to be compound related. Retardation of body weight gain was observed at 400 ppm in females during the first 6 weeks and at 1600 ppm in animals of both sexes. There were no eye abnormalities at the 4-week examination. The authors stated that there was no indication of substance-induced damage to the eyes in the 1600 ppm dose group; however, one high-dose male exhibited corneal erosion, and one high-dose female had a right corneal lens cataract. Few of the changes in clinical chemistry showed a dose-related or time-consistent pattern, and all changes were regarded as toxicologically insignificant. An increase in urea concentration at 1600 ppm and a decrease in triglyceride concentration in animals at 400 ppm and above were seen only after the first 4 weeks and not at the end of the study. Pronounced increases in *N*-demethylase

activity and cytochrome P450 content were found in male animals at 1600 ppm, and increased liver weights were seen in females at this dose. Histopathological examination revealed an increased incidence of intraplasmatic vacuoles in the cells of the zona fasciculata of the adrenals (probably lipid accumulation) in some females at 400 ppm and in all females at 1600 ppm. This effect was less pronounced in males because of a higher background incidence of adrenal vacuole formation in control animals.

The NOAEL in this 90-day dietary toxicity study in rats was 100 ppm, equal to 10.8 mg/kg bw per day, on the basis of retardation of body weight gain and histopathological changes in the adrenals at higher doses seen in females at the LOAEL of 400 ppm, equal to 46.5 mg/kg bw per day (Bomhard & Schilde, 1986).

Dogs

In a 90-day study of toxicity, groups of four male and four female Beagle dogs were given diets containing tebuconazole (purity 93.4%; 4.8% symmetric isomer) at a dose of 0, 200, 1000 or 5000 ppm (equal to 0, 8.5, 41.0 and 212 mg/kg bw per day for males and females, combined) for 13 weeks. The dietary levels were calculated by the formula (compound intake per animal per day)/[(body weight week −1 + body weight week 13)/2]. Test diets were prepared weekly. Homogeneity and stability of the diets were assessed at regular intervals. The test diets were homogeneous (97–104%) and were stable for 14 days. Animals were inspected for signs of morbidity and mortality several times daily. Body temperatures were measured, and reflex tests (pupil reaction, corneal reflex, patellar tendon reflex, stretch, bending and righting reflex) were conducted prior to study initiation and during study weeks 3, 7 and 13. Body weights were recorded weekly. Feed consumption was calculated on a weekly basis. Ophthalmological examinations were performed prior to study initiation and during study weeks 3, 7, 10 and 12; mid- and high-dose dogs were also examined during study week 14. Haematology, clinical chemistry and urinalysis were conducted prior to initiation of the study and at weeks 3, 7 and 13. At the end of the study, a complete gross postmortem was done. Selected organs were weighed, and a comprehensive range of tissues was preserved and examined microscopically.

One dog at 5000 ppm was found dead after the first dose, with no previous clinical signs. All remaining dogs survived until the scheduled terminal sacrifice. Body temperature, pulse rates and reflexes were similar in dosed and concurrent control dogs. At 1000 ppm and above, the mean body weight gain was retarded from week 7 to study termination. Mean body weight gains calculated over 13 weeks were decreased in mid- and high-dose males (25% and 46%, respectively) and females (19% and 48%, respectively) compared with controls. Body weights and body weight gains of low-dose animals were similar to those of concurrent controls. Most of the animals' feed consumption at 5000 ppm was repeatedly incomplete, as the dogs did not eat all the feed served to them. A few dogs at 1000 ppm and most of those at 5000 ppm had a deteriorated nutritional status. Ophthalmic examination revealed lens opacities in all animals at 5000 ppm, which first appeared after 7 weeks of treatment. These results were confirmed by histopathology, where morphological degeneration was observed. Anisocytosis and a change in erythrocyte morphology were found at study termination and were accompanied by a histological increase in siderosis of the liver and spleen in high-dose animals. Increased platelet counts and increased spleen weights were also exhibited in these animals. Effects on the liver were indicated by increased alkaline phosphatase, decreased albumin, increased globulin and increased cytochrome P450 activity at the high dose, as well as a dose-related increase in *N*-demethylase activity. The *N*-demethylase activity in the liver had increased slightly by the end of the study in animals at 1000 ppm. No treatment-related effects on triglyceride, ALT or AST levels were observed. Urinalyses revealed no treatment-related effects. Changes in organ weights did not follow a consistent pattern, except that an increase in spleen weights was seen in animals of both sexes at 5000 ppm. The increased level of iron pigment accumulation in conjunction with mechanisms for

adapting to the increased metabolic rate are considered to be the reasons for the higher mean absolute and relative spleen weights in the highest-dose group. Histopathological examination confirmed lens degeneration, indicating the induction of cataracts in animals at 5000 ppm. Other histopathological alterations observed in this group included slightly increased accumulation of ferriferous pigments in Kupffer cells of the liver and of siderocytes in spleen.

The NOAEL was 200 ppm, equal to 8.5 mg/kg bw per day, on the basis of reduced body weight gain and feed consumption and liver enzyme induction at 1000 ppm, equal to 41 mg/kg bw per day, and higher. The NOAEL for cataract induction was 1000 ppm, equal to 41 mg/kg bw per day (von Keutz & Schilde, 1987a).

In a 1-year study of oral toxicity, groups of four male and four female Beagle dogs were given diets containing tebuconazole (purity 96.9%) at a dietary concentration of 0, 40, 200 or 1000 ppm (weeks 1–39) and 2000 ppm (weeks 40–52), equal to 0, 1.5, 7.5 and 47 mg/kg bw per day in males and females combined, for 53 weeks. Diets were prepared at weekly intervals. Homogeneity and stability of the diets and dietary concentrations were confirmed analytically. The homogeneity and stability of diets were within the acceptable range. The dogs were inspected several times a day for mortality, moribundity and clinical signs. Body weights were recorded weekly. Feed consumption was measured daily for each animal. Ophthalmological examination was performed on all animals 2 weeks prior to study initiation and at weeks 13, 26, 32, 46 (controls and high dose) and 52. Blood was collected from all animals 2 weeks before treatment and at weeks 6, 13, 26, 39, 46 (controls and high dose) and 52 for haematology and clinical chemistry analysis. Urinalysis was conducted on all animals 2 weeks prior to the initiation of the study and at weeks 6, 13, 26, 39, 46 (controls and high dose) and 52. At the end of the study, a complete gross postmortem examination was done. Selected organs were weighed, and a comprehensive range of tissues was preserved and examined microscopically. Liver enzyme activities and protein electrophoresis were evaluated.

No treatment-related effects were observed on survival rate, appearance, behaviour, organ weights, haematological examination, urinalyses, body temperature, pulse rates, reflexes, feed and water consumption or body weight gain. Ophthalmic examination revealed lens opacities in two dogs at 200 ppm and one at 1000 ppm; the opacities appeared between weeks 26 and 32 and were of the same intensity at all subsequent examinations. In the single dog at 1000 ppm that showed this ocular change, corneal opacity was also found, which persisted until the end of the study, whereas the lens opacities disappeared after week 32 of treatment. The lack of a dose–response relationship with regard to lens opacities does not preclude an association with treatment but may be due to the small number of animals in the group and differences in individual sensitivities. None of the other animals in this group had lens stars (physiological structures found occasionally in juvenile dogs); in single animals, faint lens stars were already present before treatment started but did not become more pronounced with the treatment. Incipient lens stars observed in animals at 40 and 200 ppm also remained stable, and most disappeared before the end of the treatment period. Clinical observation did not reveal impairment in any animal's vision. The findings of the haematological examination did not reveal any damage to the red blood cells. Nevertheless, the histopathological examination at the end of the study detected a slightly increased siderin level in the spleen in five of eight animals at 1000/2000 ppm. The incidence of this finding may point to an increased rate of breakdown of the red blood cells, which was so marginal, however, that it was not apparent from the haematological data. Clinical chemistry analyses revealed slight, dose-related changes in the activity of alkaline phosphatase: whereas the age-dependent reduction in activity was similar in control animals and in those at 40 and 200 ppm, the mean activity in animals at 1000/2000 ppm indicated slight induction, resulting in a retardation in the physiological fall in alkaline phosphatase activity. The activity of *N*-demethylase and the triglyceride content of the liver were slightly increased in animals at 1000/2000 ppm. Most of the gross pathological findings, such as a dose-related increase in the incidence of livers with

marked lobulation in animals treated with 200 ppm and above, were not correlated with histopathological alterations. Histopathological findings included intracytoplasmic vacuoles in cells of the zona fasciculata of the adrenals in animals at 200 and 1000/2000 ppm and slight siderosis in the spleen in most animals at 1000/2000 ppm.

The NOAEL was 40 ppm, equal to 1.5 mg/kg bw per day, on the basis of histopathological changes in the adrenals seen at the LOAEL of 200 ppm, equal to 7.5 mg/kg bw per day (von Keutz & Schilde, 1987b).

In a second 1-year study of oral toxicity, groups of four male and four female Beagle dogs were given diets containing tebuconazole (purity 96.0%) at a dietary concentration of 0, 100 or 150 ppm (equal to 0, 2.96 and 4.39 mg/kg bw per day for males and 0, 2.94 and 4.45 mg/kg bw per day for females, respectively) for 53 weeks. Diets were prepared every 2–4 weeks. Homogeneity and stability of the diets and dietary concentrations were confirmed analytically. The homogeneity and stability of the diets were within the acceptable range. The dogs were inspected daily for mortality, moribundity and clinical signs. Body weights were recorded weekly. Feed consumption was measured daily for each animal. Ophthalmological examination was performed on each dog at 3 and 6 months and prior to terminal sacrifice. Blood and urine were collected at 3 and 6 months and prior to terminal sacrifice. At the end of the study, a complete gross postmortem examination was done. Selected organs were weighed, and a comprehensive range of tissues was preserved and examined microscopically.

The treatment did not affect mortality, body weight gain, feed consumption, biochemical, haematological or urinary parameters, ophthalmoscopic findings (including cataracts), gross pathological appearance or organ weights. One control female stopped eating during the 8th week of the study and showed a body temperature of 40.6 °C and elevated white blood cell count. The animal was isolated in a separate room and sacrificed during the 10th week of the study. Another female from the same shipment of animals replaced this female on day 70. The only histopathological alteration was slight hypertrophy of adrenal zona fasciculata cells in all animals at 150 ppm; only one control animal had similar changes. The enlargement was accompanied by an increased incidence of large fatty vacuoles.

The NOAEL was 100 ppm, equal to 2.94 mg/kg bw per day, on the basis of histopathological alterations in the adrenals seen at the LOAEL of 150 ppm, equal to 4.39 mg/kg bw per day. The NOAEL for cataract induction was 150 ppm, equal to 4.39 mg/kg bw per day (Porter et al., 1989, 1993).

(b) Dermal application

Rabbits

In a non-GLP repeated-dose dermal toxicity study, groups of six New Zealand White rabbits of each sex per dose (abraded and intact skin) received a dermal application of tebuconazole (purity 97.1%) formulated in Cremophor EL in Lewatit water at a dose of 0, 50 or 250 mg/kg bw per day, 6 hours per day, 5 days per week, for 3 weeks. The test material was applied to previously shaved skin (abraded and intact skin). The appropriate volume (0.5 ml/kg bw) of tebuconazole suspension was applied to the skin. The treated area was left uncovered. After 6 hours, the treated sites were rinsed with soap and water. Animals were examined for mortality and signs of toxicity daily except on weekends and holidays. Body weights were recorded weekly. The skin irritation was scored according to the method of Draize. Blood and urine samples were collected from all rabbits before the start of the study and at study termination. At termination, all animals were examined externally and internally for macroscopic changes. Selected organs were weighed, and a comprehensive range of tissues was preserved and examined microscopically.

There were no treatment-related deaths or clinical signs and no biologically significant treatment-related effects on body weight, body weight gain or feed consumption. Abraded skin showed slight redness for the first 3 days; however, this was also present before the treatment. No treatment-related effects were observed on the urinalysis, haematological or clinical chemistry parameters evaluated. No induction of microsomal enzymes was observed. Slightly distended lungs, swollen spleens, livers with slight lobulation and patchy kidneys were observed upon macroscopic examination; however, these observations were not corroborated by histopathological findings. Slightly higher absolute and relative spleen and kidney weights were observed in males at 250 mg/kg bw per day. This was a result of two animals having high organ weights and an infestation. Histopathological examination did not reveal treatment-related abnormalities.

The NOAEL in this 21-day dermal toxicity study in rabbits was 250 mg/kg bw per day. A LOAEL was not observed (Heimann & Schilde, 1984).

In a second repeated-dose dermal toxicity study, groups of five New Zealand White rabbits of each sex per dose received a dermal application of tebuconazole (purity 97.4%) formulated in Cremophor EL (2% by volume) in demineralized water at a dose of 0 or 1000 mg/kg bw per day, 6 hours per day, 5 days per week, for 3 weeks. The test material was applied to previously shaved skin (11 cm × 12 cm). The treated area was covered with a non-occlusive dressing for 6 hours. After 6 hours, the treated sites were rinsed with soap and water. Animals were examined for mortality and signs of toxicity daily except on weekends and holidays. Body weights were recorded weekly. The skin irritation was scored according to the Draize method. Blood and urine samples were collected from all rabbits before the start of the study and at study termination. At termination, all animals were examined externally and internally for macroscopic changes. Selected organs were weighed, and a comprehensive range of tissues was preserved and examined microscopically.

No treatment-related effects were observed on clinical signs of toxicity, mortality, body weight, body weight gain, feed consumption, urinary, haematological and clinical chemistry parameters and gross macroscopy. No treatment-related inductions of liver enzymes (microsomal enzymes) were observed in this study. Slightly increased liver weights (absolute and relative) were observed in the high-dose females; however, they were not considered to be an adverse effect, as no corroborative clinical or histological findings were noted. The histopathological examination of the treated skin revealed minimal thickening of the epidermis, in comparison with the untreated skin, in two males and four females. In the case of four females, minimal hyperkeratosis was also noted. The alterations are presumably attributable to mechanical irritation of the skin, as the test compound formulation was a suspension of viscous consistency or slurry, and the pressure of the occlusive dressing presumably resulted in skin friction.

The NOAEL in this 21-day dermal toxicity study in rabbits was 1000 mg/kg bw per day. A LOAEL was not established (Heimann & Schilde, 1988).

(c) Exposure by inhalation

Rat

In a 21-day study of inhalation toxicity after repeated doses, groups of 10 male and 10 female Wistar rats (Bor:WISW) were exposed to nominal aerosol concentrations of tebuconazole (purity 96.2%) at 0, 5, 50 or 500 mg/m^3 in polyethylene glycol by head and nose exposure for 6 hours per day, 5 days per week, for 3 weeks (15 days). The analytical concentrations were 0, 1.2, 11 and 156 mg/m^3 (0, 0.0012, 0.011 and 0.156 mg/l). About 90% of the particle mass had an aerodynamic diameter of less than 5 µm. During the 3 weeks of exposure, body weights, clinical signs and mortality were recorded. At the end of the study, clinical chemistry, haematology, urinalysis, gross pathological and histopathological examinations were performed.

The treatment had no effect on mortality rate, body weight gain, haematological or clinical chemical parameters or organ weights; urinalysis showed no abnormal findings. Rats treated with 156 mg/m^3 had piloerection after each exposure. Mixed-function oxidases in the liver were induced. At the end of the study, *N*-demethylase activity in the liver was increased in animals of both sexes at the highest dose. Males in this group also had increased *O*-demethylase activity. No treatment-related gross pathological or histopathological alterations were observed. The changes in enzyme levels were considered to be an adaptive response and not adverse.

The no-observed-adverse-effect concentration (NOAEC) was greater than or equal to 156 mg/m^3 ($\geq$ 0.156 mg/l). The study author established a no-observed-effect concentration (NOEC) of 0.11 mg/m^3 on the basis of liver enzyme induction (Pauluhn, 1985, 1987).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Two combined chronic toxicity and carcinogenicity studies were performed using mice (Bomhard & Ramm, 1988; Bomhard, 1991; Sander, 1992).

In the first study (Bomhard & Ramm, 1988), groups of 50 male and 50 female NMRI mice were given tebuconazole at a concentration of 0, 20, 60 or 180 ppm (equal to 0. 5.9, 18 and 53 mg/kg bw per day for males and 0, 9.0, 26 and 81 mg/kg bw per day for females) for 21 months in the diet. Groups of 10 similarly treated male and female animals (satellite groups) were sacrificed after a period of 12 months. The tebuconazole doses were based on the results of two previous feeding studies lasting 4 and 8 weeks, respectively, in NMRI mice of the same strain (Ramm & Karbe, 1986; Ramm & Schilde, 1986). The animals were inspected for any clinical signs of toxicity. Individual body weights were recorded weekly for the first 13 weeks and once every 2 weeks thereafter. Feed and water intakes were determined groupwise once a week from the start of the study up to and including week 13 and every 2 weeks from week 15. Haematology, clinical chemistry, urinalysis, measurement of organ weights, and macroscopic and microscopic examinations were conducted after 12 and 21 months. Animals that died spontaneously during the study or were moribund and sacrificed were dissected and their organs/tissues subjected to detailed gross pathological examination.

Clinical signs, feed and water intakes, growth and mortality were unaffected in both sexes of all treated groups, including 180 ppm. In the haematological examination, erythrocytes, haemoglobin and haematocrit concentration were decreased in females of the satellite group at 180 ppm in 12 months, but not at 21 months. At the same dose, the male mice showed significantly lower erythrocyte counts at 12 and 21 months (Table 9). In clinical chemistry, the total bilirubin concentration was significantly increased in females at 180 ppm at 12 months and at 20 ppm and higher at 21 months. Any changes indicating hepatotoxicity were not detected in the liver from animals of both sexes at 20 or 60 ppm at 12 months. Slight increases in total bilirubin at 20 and 60 ppm at 21 months in females are considered to be incidental findings, because no changes were observed in these animals (20 and 60 ppm dose groups) at 12 months, and the increases may have been statistically significant as a result of the unusually low value in the control group at 21 months. The plasma cholesterol levels were significantly lower in males and females in the 180 ppm satellite group and showed a lower tendency in males at 21 months (Table 9).

In both sexes at 180 ppm, absolute and relative liver weights were increased compared with the controls at 12 and 21 months. However, the variations were statistically significant only for the relative liver weights in males at 21 months. The means in this dose group for females were greatly affected by three extreme figures at the end of the study, whereas values for the other animals were largely in the range for control females.

Table 9. Clinical chemistry and haematological examinations in the carcinogenicity study of tebuconazole in mice

Parameter	Sacrifice time	Sex	Dietary concentration (ppm)			
			0	20	60	180
Bilirubin (µmol/l)	12 months	Male	3.9	3.8	3.8	3.9
		Female	2.7	3.2	3.3	3.7**
	21 months	Male	3.3	3.2	3.3	3.4
		Female	2.2	2.6*	3.4**	3.6**
Cholesterol (mmol/l)	12 months	Male	4.66	4.38	4.36	3.61*
		Female	3.86	4.32	3.43	2.44**
	21 months	Male	4.31	3.93	3.93	3.27
		Female	3.57	3.53	2.97*	3.46
Erythrocyte count (10^{12}/l)	12 months	Male	8.30	8.57	8.41	8.73*
		Female	8.30	8.07	8.42	7.88*
	21 months	Male	8.20	8.55	8.15	7.65**
		Female	7.87	7.26*	7.13	7.56
Haemoglobin (g/l)	12 months	Male	137	140	136	140
		Female	142	140	141	133**
	21 months	Male	144	154	145	139
		Female	143	135	131	141
Haematocrit (l/l)	12 months	Male	0.46	0.48	0.47	0.49
		Female	0.47	0.46	0.47	0.45*
	21 months	Male	0.428	0.432	0.426	0.410
		Female	0.409	0.390	0.393	0.392

From Bomhard & Ramm (1988)
* $P \leq 0.05$; ** $P \leq 0.01$

Incidences of periportal vacuolization of the liver were marginally increased in females. The incidences of periportal vacuolization in females were 1/50 for all treated dose groups, whereas an incidence of 1/50 was observed only at the high dose (180 ppm) in males (Table 10). In addition, incidences of centrilobular fine vacuolization were higher in males of the 60 and 180 ppm dose groups than in the control group. Further examination showed that the vacuoles in the liver were identified as lipids. In this study, there were no treatment-related effects in animals of the satellite and the main groups.

The LOAEL was 60 ppm (equal to 18 mg/kg bw per day) based on the increased incidence of centrilobular fine vacuolization in the liver of males. The NOAEL was 20 ppm (equal to 5.9 mg/kg bw per day). There was no evidence for carcinogenic potential, but the effects on the liver at the LOAEL and above were not very marked in intensity, posing a question as to whether a maximum tolerated dose (MTD) had been reached in this study.

The objective of the second study (Bomhard, 1991) was to examine the carcinogenic potential of tebuconazole at doses higher than those used in the first study. Groups of 50 male and 50 female NMRI mice were administered tebuconazole at a concentration of 0, 500 or 1500 ppm (equal to 0, 85 and 279 mg/kg bw per day in males and 0, 103 and 357 mg/kg bw per day in females, respectively) in their diet for 21 months. Groups of 10 male and 10 female animals (satellite groups) were analogously treated and sacrificed after a study duration of 12 months for haematological and clinical examinations.

Table 10. Incidences of periportal and centrilobular fine vacuolization of hepatocytes in the carcinogenicity study of tebuconazole in mice

Parameter	Incidence of vacuolization of hepatocytes[a]							
	Dietary concentration (ppm)							
	0		20		60		180	
	M	F	M	F	M	F	M	F
Focal periportal vacuolization, minimal	0/50	0/50	0/50	0/50	1/50	1/50	8/50	2/50
Periportal vacuolization	0/50	0/50	0/50	1/50	0/50	1/50	1/50	1/50
Focal centrilobular fine vacuolization, minimal	0/50	1/50	2/50	0/50	5/50	1/50	2/50	0/50
Centrilobular fine vacuolization, minimal	0/50	2/50	1/50	1/50	4/50	1/50	8/50	8/50
Centrilobular fine vacuolization, moderate	0/50	0/50	0/50	0/50	1/50	0/50	4/50	0/50

From Bomhard & Ramm (1988)
F, females; M, males
[a] Number of animals affected/total number of animals.

Table 11. Results of the combined chronic toxicity/carcinogenicity study of tebuconazole in mice (main groups)

Parameter	Dietary concentration (ppm)						Dose–response	
	0		500		1500			
	M	F	M	F	M	F	M	F
Number of animals examined	*50*	*50*	*50*	*50*	*50*	*50*	*n/a*	*n/a*
Mortality[a]	20/50	30/50	18/50	32/50	23/50	32/50	–	–
Body weight, week 91 (g)	46.5	41.3	44.5	39.3	46.6	44.1*	–	–
Feed consumption (g/kg bw per day)	146.6	188.8	169.8	206.1	186.0	237.7	+	+
Water consumption (g/kg bw per day)	208.3	314.0	213.2	297.7	204.1	279.4	–	–

From Bomhard (1991)
F, females; M, males; n/a, not applicable; * $P \leq 0.05$
[a] Number of animals that died/total number of animals.

Clinical signs, water intakes and mortality were unaffected in both treatment groups. The incidence of animals exhibiting increases in abdominal girth was elevated at the 1500 ppm level. Growth retardation was observed in males at 500 and 1500 ppm in a dose-related manner. At 1500 ppm, the differences in body weights were slightly greater than 10% at times, whereas they were around 6–7% at most at 500 ppm. Body weight was reduced in females at 1500 ppm, and deviations from the control group were less than 10%. Body weights at 21 months were comparable to those of the controls (Table 11).

The haematological examination did not provide any indication of damage to the blood in females at 500 ppm. Marginal effects on the haematocrit value and the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were observed in the males at 500 and 1500 ppm, but these changes were not considered toxicologically significant because they were minimal and no clear dose–response relationship was observed. The erythrocyte count, haemoglobin content, haematocrit value and thromboplastin time were generally reduced at 1500 ppm, whereas the thrombocyte and leukocyte counts were elevated, in some cases to a marked extent (Table 12).

Table 12. Major changes in clinical chemistry and haematological examinations in the carcinogenicity study of tebuconazole in mice

Clinical chemistry and haematology	Sacrifice time	Sex	Dietary concentration (ppm)		
			0	500	1500
ALAT (U/l)	12 months	Male	38.0	53.2*	236.3**
		Female	31.7	51.7*	272.5**
	21 months	Male	74.9	123.1**	480.8**
		Female	39.2	64.9*	419.4**
ASAT (U/l)	12 months	Male	31.9	37.5	121.3**
		Female	38.3	47.2*	144.0**
	21 months	Male	46.1	60.7	251.8**
		Female	36.9	59.0**	303.8**
Alkaline phosphatase (U/l)	12 months	Male	74	117**	181**
		Female	174	212	292
	21 months	Male	126	156	531**
		Female	182	328	517**
Cholesterol (mmol/l)	12 months	Male	3.71	1.99**	1.66**
		Female	2.84	1.48**	1.92*
	21 months	Male	3.88	1.57**	4.55
		Female	3.76	2.25**	3.59
Bilirubin (µmol/l)	12 months	Male	1.8	1.3**	1.3**
		Female	2.2	2.1	1.9
	21 months	Male	2.1	1.6**	5.0
		Female	2.7	2.1*	4.9
Inorganic phosphate (mmol/l)	12 months	Male	1.90	1.73*	2.14*
		Female	1.63	1.69	1.94**
	21 months	Male	1.60	1.75**	2.06**
		Female	1.62	1.64	1.93**
Leukocyte count (10^9/l)	12 months	Male	5.2	5.6	10.2**
		Female	3.9	3.7	10.7**
	21 months	Male	6.6	5.0*	9.8*
		Female	7.6	4.3	9.5
Erythrocyte count (10^{12}/l)	12 months	Male	8.90	9.11	8.51
		Female	8.40	8.99	7.49
	21 months	Male	9.13	8.25	7.95*
		Female	8.36	8.63	7.51
Haemoglobin (g/l)	12 months	Male	142	144	129**
		Female	139	148**	132
	21 months	Male	143	150	117**
		Female	132	131	125
Haematocrit (l/l)	12 months	Male	0.435	0.414	0.373**
		Female	0.422	0.432	0.381*
	21 months	Male	0.427	0.375**	0.375**
		Female	0.407	0.401	0.380

Table 12 (continued)

Clinical chemistry and haematology	Sacrifice time	Sex	Dietary concentration (ppm)		
			0	500	1500
MCHC (g/l erythrocytes)	12 months	Male	327	348**	347**
		Female	331	344	347*
	21 months	Male	334	402**	313**
		Female	324	328	328
MCH (pg)	12 months	Male	16.0	15.8	15.3
		Female	16.6	16.6	17.7
	21 months	Male	15.7	18.3**	14.6*
		Female	15.8	15.3	16.7
Thrombocyte count (10^9/l)	12 months	Male	1311	1323	1650**
		Female	1024	1274*	1284

From Bomhard (1991)
U, units; * $P \leq 0.05$; ** $P \leq 0.01$

The clinical laboratory tests, gross pathology, organ weights and histopathology afforded evidence for marked and dose-related hepatotoxicity in both treatment groups, with dose dependency (Tables 12 and 13). The main findings included a marked increase in the activities of ALT and AST, in some cases major enlargement of the liver, single-cell and focal necrosis, inflammation, bile duct hyperplasia and steatosis. The incidences of hepatocellular tumours (adenomas and carcinomas in males and carcinomas in females) were increased at 1500 ppm and were markedly above the range of spontaneous incidences observed in this mouse strain (Table 13). In addition, hepatocellular alteration and focal hyperplasia of hepatocytes, which are considered to be precancerous lesions, were also increased in males and females at 1500 ppm. The incidence of hepatocellular tumours was unaffected in both sexes at 500 ppm.

In addition, the incidences of histiocytic sarcoma at 0, 500 and 1500 ppm were 1/48, 2/49 and 3/48 in males and 1/47, 3/45 and 5/46 in females of the main groups, respectively. This tumour is not rare in aged mice. The review of historical data of nine studies (starting at 1982–1985) on NMRI mice from Bayer indicates that the incidence of this tumour in the 500 and 1500 ppm groups was incidental and not related to treatment. The histopathology of the interim necropsy of females at 1500 ppm showed an increase in the incidence of hyperkeratosis and acanthosis of the forestomach mucosa (Table 13). The incidence of these findings did not result in a treatment-related increase in the animals of the main groups. No evidence for carcinogenic effects of tebuconazole on other organs may be inferred from the incidence, type, location or distribution among the study groups of the neoplasms observed.

The rates of liver tumours in males and females were elevated to a highly significant extent at 1500 ppm and were markedly above the range of spontaneous incidences observed in this mouse strain. Especially in mice, liver tumours caused by a variety of chemical substances at hepatotoxic doses occur frequently, and it is thought that under these circumstances, elevated incidences of spontaneous, relatively frequent tumours in rodents have no relevance for humans if the exposure to humans lies in a non-toxic range. The relatively major effects, particularly those on the liver, represent unequivocal evidence that the MTD has been exceeded at both concentrations and underscore the correctness of dose selection in the first study (Bomhard & Ramm, 1988).

The LOAEL was 500 ppm (equal to 85 mg/kg bw per day), based on liver toxicity. The NOAEL for systemic toxicity was not established. The NOAEL for carcinogenicity effects was 500 ppm (equal to 85 mg/kg bw per day) based on the increased incidence of tumours seen at the LOAEL of 1500 ppm (equal to 279 mg/kg bw per day).

Table 13. Results of the combined chronic toxicity/carcinogenicity study in mice

Parameter	Incidence[a]						Dose–response	
	Dietary concentration (ppm)							
	0		500		1500			
	M	F	M	F	M	F	M	F
Liver								
Relative weight (mg/100 g)	5214	6060	6345**	6642	18 313**	21 141**	+	+
Absolute weight (mg)	2409	2524	2822**	2623	8522**	9405**	+	+
Gross pathology								
Enlarged	1/50	0/50	2/50	5/50	35/50***	32/50***	+	+
Irregular surface	1/50	3/50	0/50	3/50	30/50***	26/50***	+	+
Masses	6/50	1/50	3/50	1/50	13/50**	8/50*	+	+
Non-neoplastic changes								
Necrosis of single hepatocytes	3/47	0/47	11/48*	2/45	2/48	1/46	–	–
Focal hyperplasia of hepatocytes	6/47	1/47	2/48	0/45	23/48***	12/46***	+	+
Panacinar fine fatty vacuolation	0/47	1/47	14/48***	4/45	25/48***	19/46***	+	+
Centriacinar fatty vacuolation	1/47	3/47	1/48	13/45**	0/48	4/46	–	–
Periacinar hepatocytic hypertrophy	0/47	0/47	0/48	0/45	2/48	13/46***	–	+
Oval cell proliferation	0/47	0/47	0/48	0/45	23/48***	17/46***	+	+
Pigment-laden Kupffer cells	1/47	1/47	0/48	3/45	6/48	7/46*	+	+
Extramedullary haematopoiesis	0/47	5/47	2/48	1/45	7/48*	12/46	+	+
Hepatocellular alteration	0/47	0/47	2/48	0/45	3/48	7/46**	+	+
Focal hyperplasia of hepatocytes	6/47	1/47	2/48	0/45	23/48***	12/46***	+	+
Neoplastic changes								
Hepatocellular adenoma	3/47	0/47	2/48	0/45	17/48***	2/46	+[b]	–
Hepatocellular carcinoma	0/47	1/47	0/48	0/45	10/48***	12/46***	+[b]	+[b]
Stomach (main group)								
Hyperkeratosis and acanthosis	6/47	12/46	8/48	16/45	8/48	13/46	–	–
Stomach (interim sacrifice)								
Hyperkeratosis and acanthosis	1/10	2/10	2/10	6/10	6/10	8/10*	–	+
Adrenals								
Relative weight (mg/100 g)	24	28	21	33	23	34*	–	+
Absolute weight (mg)	11	12	10	13	11	15**	–	+

From Bomhard (1991)

F, female; M, male; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

[a] Number of animals affected/total number of animals.

[b] Not relevant for humans.

Rats

Groups of 50 male and 50 female Wistar rats were given tebuconazole at a concentration of 0, 100, 300 or 1000 ppm (equal to 0, 5.3, 15.9 and 55.0 mg/kg bw per day for males and 0, 7.4, 22.8 and 86.3 mg/kg bw per day for females, respectively) for 2 years in their diet (Bomhard & Ramm, 1988; Sander & Schilde, 1993 [review of the historical control data]). Ten similarly treated male and female animals were sacrificed after a study period of 12 months as satellite groups. The animals were inspected at least twice daily, and any clinical signs and special features were noted. Detailed

Table 14. Treatment-related effects on non-neoplastic lesions in the carcinogenicity study of tebuconazole in rats

Parameter	Incidence[a]							
	Dietary concentration (ppm)							
	0		100		300		1000	
	M	F	M	F	M	F	M	F
Liver								
Kupffer cell pigmentation	0/49	2/49	1/49	2/50	0/50	1/50	1/50	7/50
Spleen								
Increased haemosiderin	0/49	2/50	0/49	3/50	1/50	3/50	0/50	19/50
Adrenals								
Haemorrhagic degeneration in cortex	3/49	23/50	4/49	15/50	4/50	13/50	1/50	4/50

From Bomhard & Ramm (1988)
F, female; M, male
[a] Number of animals affected/total number of animals.

individual inspections took place once a week. Ophthalmological examinations were made at the start of the study, after 12 months and before the end of the study, covering groups of 10 males and 10 females in the control group and the 1000 ppm dose group. Individual body weights were recorded weekly for the first 13 weeks and once every 2 weeks thereafter. Feed and water intakes were determined groupwise once a week from the start of the study up to and including week 13 and every 2 weeks from week 15. Haematology, clinical chemistry and urinalysis were performed after 6, 12, 18 and 24 months from 10 animals per group. Animals that died spontaneously during the study or were moribund and sacrificed were dissected and their organs/tissues subjected to detailed gross pathological examination. After 12 and 24 months, all the survivors of the satellite groups and main groups, respectively, were sacrificed and autopsied, and their organs/tissues were subjected to detailed gross pathological and histopathological examinations.

The appearance, general behaviour and mortality were unaffected in the treated groups. In the 1000 ppm group, decreased water intake was noted in females, and growth of the males and females in this dose group was retarded. In the females at 1000 ppm, feed consumption was increased. The haematology, urinalysis and ophthalmological examination did not provide any indication of damage. In clinical chemistry, the females at 1000 ppm showed induction of microsomal enzyme systems. Absolute and relative weights of the liver, lungs and spleen were significantly increased in the females at 1000 ppm at interim sacrifice, but these increases were not observed at termination. Relative weights of the liver in the females at 300 ppm were also increased at the interim sacrifice. The absolute and relative weights of the adrenals were decreased in females in the 30 ppm and higher groups at termination. Gross pathological examination did not show any treatment-related changes. Histopathologically, treatment-related non-neoplastic lesions were detected in the liver, spleen and adrenals in females at 1000 ppm (Table 14). They were haemosiderin accumulation in the spleen (not confirmed by Berlin blue staining) and pigment deposits in the Kupffer star cells in the liver of females at 1000 ppm. The clearly reduced number of females with haemorrhagic degeneration of the adrenal cortex at 1000 ppm, which corresponded to the reduction of adrenal weights, was considered to be most likely a treatment-related functional effect, but not toxicologically significant.

With respect to neoplastic lesions, the incidence of C-cell adenomas in the thyroid showed very slight, but not statistically significant, increased tendency in all treated male rats compared with controls (Table 15). The incidence of C-cell hyperplasia, a precancerous lesion of C-cell adenoma,

Table 15. Thyroid C-cell tumours and C-cell hyperplasias in the carcinogenicity study of tebuconazole in rats

Parameter	Incidence[a]							
	Dietary concentration (ppm)							
	0		100		300		1000	
	M	F	M	F	M	F	M	F
C-cell hyperplasia	1/50	1/49	3/50	2/50	7/50	3/50	6/50	0/50
C-cell adenoma (b)	0/50	1/49	1/49	0/50	3/50	1/50	2/50	1/50
C-cell carcinoma (m)	0/50	0/49	1/49	0/50	0/50	0/50	1/50	0/50

From Bomhard & Ramm (1988)
(b), benign neoplasms; F, female; (m), malignant neoplasms; M, male
[a] Number of animals affected/total number of animals.

was also increased in the males in the two higher dose groups, but not significantly. There was, however, no clear dose–response relationship, and the incidences were within the range of spontaneously occurring thyroid C-cell tumours in old male Wistar rats (Bomhard, Karbe & Loeser, 1986). Furthermore, the histopathology data revealed no evidence of progression from adenoma to carcinoma. A review of historical data (Sander & Schilde, 1993) and data from the study provides strong evidence that the incidence and dose dependency of thyroid tumours observed in the study were not treatment related. In female rats, a higher frequency of endometrial adenocarcinoma was found in comparison with controls. These incidences were small and not dose related.

The LOAEL was 1000 ppm (equal to 55.0 mg/kg bw per day), based on body weight depression in both sexes and an increased incidence of pigment deposits in the Kupffer cells in the liver of females. The NOAEL was 300 ppm (equal to 15.9 mg/kg bw per day). Tebuconazole was not carcinogenic (Bomhard & Ramm, 1988).

2.4 Genotoxicity

Various in vitro and in vivo studies have been conducted on the genotoxicity of tebuconazole. The results are summarized in Table 16. No genotoxic activity was found in any study.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation study of reproductive toxicity, groups of 25 male and 25 female Wistar (Bor:WISW (SPF Cpb)) rats were given diets containing tebuconazole (purity 95.2%) at a concentration of 0, 100, 300 or 1000 ppm (equal to 0, 7.1, 21.6 and 72.3 mg/kg bw per day for males and 0, 9.1, 27.8 and 94.8 mg/kg bw per day for females, respectively). The rats were treated with active ingredient throughout the study, including mating period, gestation and pup lactation. Prepared diets were analysed for stability, homogeneity and concentrations before the start of the treatment and every 3 months thereafter; diets were stable and homogeneously distributed. All animals were examined twice daily for mortality and clinical signs and once daily during holidays and weekends. Body weights and feed consumption were monitored at weekly intervals. Parturition was observed and described. Litter size, sex distribution and malformations of pups were recorded. Sacrificed parental

Table 16. Results of studies of genotoxicity with tebuconazole

End-point	Test system	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation (Ames test)	*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537	20.0–12 500 µg/plate ± metabolic activation in DMSO[a]	97.0	Negative	Herbold (1983a)
	S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538	37.5–2400 µg/plate 39.5–450 µg/plate ± metabolic activation in DMSO[b]	96.6	Negative	Herbold (1983b)
	S. typhimurium strains TA98, TA100, TA1535 and TA1537 *Escherichia coli* WP2uvrA	15.6–500 µg/plate 31.3–1000 µg/plate without metabolic activation 156–5000 µg/plate with metabolic activation in DMSO	98.0	Negative	Ohta (1991d)
Forward mutation (CHO/HGPRT)	Chinese hamster ovary cell line HGPRT locus	80.0–100 µg/ml[c] without metabolic activation in DMSO 12.5–200 µg/ml with metabolic activation in DMSO	96.6	Negative	Lehn (1988)
Recombination repair capacity (rec-assay)	*Bacillus subtilis* H17 (rec^+) and M45 (rec^-)	0.3–20 µg/plate ± metabolic activation in DMSO	98.0	Negative	Ohta (1992)
Cytogenicity	Human lymphocytes	3.0–30.0 µg/ml without metabolic activation in DMSO 30.0–300 µg/ml with metabolic activation in DMSO	96.6	Negative	Herbold (1988a)
DNA polymerase repair capacity	*E. coli* (K12) p 3478 (pol^-) W 3110 (pol^+)	3.0–300.0 µg/ml without metabolic activation in DMSO 625–10 000 µg/ml with metabolic activation in DMSO	97.1	Negative	Herbold (1988b)
Unscheduled DNA synthesis	Rat hepatocytes	0.5–25.2 µg/ml	96.5	Negative	Cifone (1987)
Sister chromatid exchange	Chinese hamster ovary cells	4.0–30.0 µg/ml without metabolic activation in DMSO 15.0–120 µg/ml with metabolic activation in DMSO	96.5	Negative	Putman (1987)
In vivo					
Micronucleus formation	NMRI mouse bone marrow (5 males and 5 females per group)	200, 500 or 2000 mg/kg bw (single oral dose in 1% Cremophor)[d]	95.3	Negative	Herbold (1985)
Dominant lethal assay	NMRI male mice (50 males per group)	2000 mg/kg bw (single oral dose in 1% Cremophor)	93.5	Negative	Herbold (1986)

DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid

[a] Toxic at doses ≥ 500 µg/plate.

[b] Toxic at doses ≥ 60 µg/plate.

[c] Toxic at doses ≥ 75 µg/plate with and without metabolic activation.

[d] Doses reduced in second and third trials owing to inhibition of erythropoiesis by the test compound.

animals were necropsied. Organs or tissues with major macroscopic abnormalities were removed and processed for histopathology. Selected organs were weighed. Selected organs were also examined histopathologically.

At doses up to 1000 ppm, appearance, behaviour, general condition, mortality, fertility, insemination rate, gestation, duration of gestation, rate of stillbirths and male/female ratio were unaffected. No malformations were observed up to 1000 ppm. Mean body weights were consistently depressed in both male and female adult rats exposed to tebuconazole at 1000 ppm prior to mating, after mating, during lactation and following the lactation period in both the F_0 and F_{1B} parental generations. No statistically significant effects on feed consumption were observed in either parental generation prior to mating. However, there was a small, generally consistent depression in feed consumption observed in the 1000 ppm males or females of both the F_0 and F_{1B} parents over the entire measurement period (F_0 males: 10%; F_{1B}: males 8%, females 11%) compared with the respective controls. The fertility index ranged from 75% to 96% and fluctuated considerably, but changes in the index did not appear to be a dose-related effect in either the F_0 or F_{1B} generation. The insemination index, gestation index and mean gestation period were not different among the dose groups in either generation. There were statistically significant depressions in the viability index of the F_0 generation and the F_{1A} parents at 100 and 1000 ppm (90.3% and 88.1%, respectively, versus 98.5% in controls) and in the lactation index of F_{1A} or F_{1B} generation animals at 1000 ppm. However, in the F_{1B} generation, neither the F_{2A} nor F_{2B} litters were affected in a compound-related manner. At 1000 ppm, neonatal weights from birth through week 3 or 4 of lactation were consistently and statistically significantly depressed in both the F_0 and F_{1B} generations. Reductions in the mean litter size at birth, in the viability index (survival until day 5 after birth) and in the lactation index were seen at 1000 ppm. No treatment-related effects on any organs were noted on gross and microscopic examination. No treatment-related histopathological findings were observed. Absolute and relative liver weights were decreased in F_{1B} males but not females at the middle and high dose levels, respectively. Absolute and relative kidney weights were also somewhat lower at the highest dose tested compared with the controls.

The NOAEL for parental systemic toxicity and offspring toxicity is 300 ppm (equal to 21.6 mg/kg bw per day), based on reduced feed consumption, decreased body weights and decreased liver and kidney weights seen at the LOAEL of 1000 ppm (equal to 72.3 mg/kg bw per day). The NOAEL for reproductive toxicity is 1000 ppm, equal to 72.3 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity is 300 ppm (equal to 21.6 mg/kg bw per day), based on decreased pup body weights and decreased litter size seen at the LOAEL of 1000 ppm (equal to 72.3 mg/kg bw per day) (Eiben, 1987).

(b) Developmental toxicity

(i) Oral administration

Mice

In a developmental toxicity study, tebuconazole (purity 93.6%) was administered to groups of 25 presumed pregnant NMRI/ORIG Kisslegg mice by gavage at a dose level of 0, 10, 30 or 100 mg/kg bw per day from gestation day (GD) 6 to GD 15. The vehicle was 0.5% aqueous Cremophor EL emulsion (dose volume 5 ml/kg bw). The dams were inspected daily with respect to mortality, appearance and behaviour and were weighed daily. The dams were sacrificed on GD 18, followed by gross examination of all internal organs. The uteri and contents were examined, the numbers of implantations and live and dead fetuses were recorded, and the sex of all live fetuses was determined. The placenta was weighed; each fetus was weighed individually. Fetuses were examined for external and visceral abnormalities using a modified Wilson's technique and skeletal abnormalities after clearing and staining. In a supplementary study to examine maternal toxicity, groups of 10 mated and presumed pregnant mice were administered tebuconazole (purity 97.4%) by gavage at a dose of 0,

10, 20, 30 or 100 mg/kg bw per day from GD 6 to GD 15 and sacrificed on GD 16. The animals were observed for mortality and clinical signs, and one half of the mice were subjected to gross examination. The liver, kidneys, spleen and adrenal glands were weighed, and the livers were processed for microscopic examination. Blood was drawn from five anaesthetized mice per group for haematology and clinical chemistry evaluation.

No maternal deaths or treatment-related clinical signs or body weight gains were observed in the main or supplemental study. Feed consumption was not measured. In the supplemental study, haematocrit was significantly decreased at 30 and 100 mg/kg bw per day, and mean corpuscular volume (MCV) was significantly decreased at 20, 30 and 100 mg/kg bw per day; no clear dose-related trend was observed for either parameter. Serum enzyme activities (AST, ALT and alkaline phosphatase) were increased at most doses, but no clear dose-related trend was observed for these parameters. Liver triglycerides were elevated at 100 mg/kg bw per day. No clear trend was observed for the increase in absolute liver weight, but relative (to body weight) liver weight was increased at all doses, particularly at 100 mg/kg bw per day. Gross examination showed pale lobular livers in 100 mg/kg bw per day mice compared with none of five in controls. Microscopically, mild to severe liver cell vacuolation and moderate to severe lipidosis (ORO stain) were observed in five of five mice at 100 mg/kg bw per day (2/5 in control mice). It should be noted, though, that the livers (and the blood) were obtained from pregnant and non-pregnant animals, so the importance of inseminating the dams is not obvious. The livers used for the homogenates in the high-dose group were taken from pregnant mice only, whereas three of the livers examined histopathologically were from non-pregnant mice.

No treatment-related effect was observed on the number of live fetuses per dam, mean fetal weight, number of resorptions per dam, per cent postimplantation loss or sex ratio. The total number of resorptions was marginally increased at 100 mg/kg bw per day. There was a dose-dependent and statistically significant increase in the number of runts (fetuses weighing less than 1.3 g) per litter at 30 mg/kg bw per day (20/234; 8.6%) and 100 mg/kg bw per day (26/202; 13%) compared with the controls (5/236; 2.1%), but without a correlation to the mean fetal weight or stage of ossification at 30 mg/kg bw per day. Thus, the retarding effect at 30 mg/kg bw per day is considered only marginal. An increased incidence of common malformations was found at 100 mg/kg bw per day (13/202; 6.5%), which consisted most frequently of cleft palates and individual cases of micrognathia, rib fusion and spinal dysplasia; the incidence of malformations in the control group was 1/236 (0.4%). The incidence of cleft palate at this dose, 6/202 (3%), was also markedly higher than the mean incidence in historical controls (0.7%).

When the results of the main study and supplementary study are combined, the maternal toxicity LOAEL is 100 mg/kg bw per day, based on increased hepatic triglycerides, pale lobular liver and increased severity of hepatic vacuoles and lipidosis. The NOAEL for maternal toxicity is 30 mg/kg bw per day. The LOAEL for developmental toxicity is 30 mg/kg bw per day, based on a marginal retarding effect (an increased number of runts). The NOAEL for developmental toxicity is 10 mg/kg bw per day (Renhof, 1988b; Renhof & Karbe, 1988). The study authors concluded that the doses from 10 mg/kg bw per day must be regarded as exerting slight maternal toxicity, and doses from 30 mg/kg bw per day display clear maternal toxicity (Renhof & Karbe, 1988). The JMPR in 1994 (Annex 1, reference *71*) established the NOAEL for maternal toxicity as less than 10 mg/kg bw per day from the Renhof & Karbe (1988) study. The difference in maternal NOAELs (10 versus 30 mg/kg bw per day) between the previous JMPR and the present JMPR is probably due to the fact that increased activities of AST, ALT and alkaline phosphatase and increased liver weights at all doses were considered as adverse effects by the JMPR in 1994 (Annex 1, reference *71*).

Below is the summary of a combined report on developmental toxicity in mice (two main studies) and two satellite studies to evaluate maternal toxicity parameters.

Table 17. Liver toxicity parameters in tebuconazole-treated pregnant female mice

Clinical chemistry and liver homogenate parameters	Dose (mg/kg bw per day)			
	0	10	30	100
	(*n* = 10 dams)	(*n* = 10 dams)	(*n* = 10 dams)	(*n* = 10 dams)
Cytochrome P450 (nmol/g)	29.4	42.3	73.7**	116.4**
N-Demethylase activity (nmol/g per minute)	260.4	413.9	752.9**	975.4**
O-Demethylase activity (nmol/g per minute)	2.52	2.95	3.67	8.16**
Liver triglyceride (µmol/g)	8.8	7.0	11.9	14.8
AST (U/l)	2.74	2.60	2.12	3.17
ALT (U/l)	1.07	1.38	1.05	1.78
Alkaline phosphatase (U/l)	2.21	2.22	3.77*	3.37

From Becker & Biedermann (1995a)
U, units; * $P \leq 0.05$; ** $P \leq 0.01$

In a developmental toxicity study, 35 presumed pregnant NMRI KFM-HAN mice were administered tebuconazole (purity 95.8–96.8%) in 0.5% Cremophor EL by gavage at a dose of 0, 10, 30 or 100 mg/kg bw per day on GDs 6–15, inclusive. On GD 18, dams were sacrificed and subjected to gross necropsy, and all fetuses were examined externally. One half of the fetuses were examined viscerally, and the remaining fetuses were examined for skeletal malformations/variations. An additional 10 mated dams per group were included in a satellite study to assess the effects on haematology and clinical biochemistry parameters and pathological changes of target organs. In the satellite study, blood was collected on GD 16, and the dams were then euthanized and subjected to gross necropsy. Liver tissue samples were collected to determine cytochrome P450 content and *N*- and *O*-demethylase activities or triglyceride content. The spleens, kidneys, adrenals and livers from all females (both main and satellite groups) were weighed and examined histologically. Because of marginal developmental effects observed at all dose levels, a second study was conducted in which 30 mated dams per group received nominal doses of tebuconazole at 0, 1 or 3 mg/kg bw per day orally by gavage on GDs 6–15, inclusive. The evaluations of the groups were the same as those in the main study except that only liver and adrenals were weighed and examined histologically. A satellite study was also again conducted: an additional seven mated dams per group were added to assess the effects of tebuconazole on target organs. Satellite group animals were sacrificed on GD 16, and the liver and adrenals were weighed and examined histologically.

No treatment-related mortalities or clinical signs of toxicity were observed in the study. Feed consumption was marginally decreased in the 30 and 100 mg/kg bw per day dose groups during GDs 6–11. A marginal decrease in body weight gain was noted in the 100 mg/kg bw per day group during GDs 6–16. Effects on the liver were seen at 10 mg/kg bw per day and above. The dose of 10 mg/kg bw per day resulted in enzyme induction (cytochrome P450, *N*-demethylase) and increased vacuolization of the liver (Table 17); however, the liver enzyme induction at 10 mg/kg bw per day was not statistically significant. Higher doses of 30 and 100 mg/kg bw per day resulted in further signs of liver toxicity, such as increases in liver weight and lipid storage in livers. Liver toxicity was also evident from clinical chemistry and histopathology observations. *O*-Demethylase activity in the liver was increased at 30 mg/kg bw per day and above, as was the alkaline phosphatase level in the blood. Transaminases (AST and ALT) in the blood were increased at 100 mg/kg bw per day. In addition, the 100 mg/kg bw per day dose resulted in slight effects on the blood turnover, evident by an increase in reticulocytes combined with a shift from low fluorescent to high fluorescent reticulocytes and an increase in spleen weight.

At 30 mg/kg bw per day, a marginal but test article–related increase in postimplantation loss was evident compared with the control group. Treatment with 100 mg/kg bw per day resulted in statistically significant increases ($P < 0.05$ or 0.01) in postimplantation loss (35.3% versus 8.4% in controls) and the number of resorptions per dam (4.3 versus 1.0 in controls), including early resorptions (3.5 versus 0.8 in controls) and late resorptions (0.8 versus 0.2 in controls), and statistically significant decreases in mean fetal weights (1.1 g versus 1.2 g in controls) and the number of live fetuses per litter (8.1 versus 10.9 in controls). The combined incidence rate of litters containing fetuses with external, visceral and skeletal malformations was 1/21, 6/20 and 3/18 for the 0, 1 and 3 mg/kg bw per day groups, respectively, and 3/29, 7/28, 6/24 and 15/26 ($P < 0.01$) for the 0, 10, 30 and 100 mg/kg bw per day groups, respectively (historical control data 8/25 or 6/23). The incidences of exencephaly (partial acrania), cleft palate, open eyes, fused/bifurcated ribs, vertebral defects (includes dysplastic vertebral bodies and missing cervical and lumbar vertebral bodies, sacral vertebrae and coccygeal vertebrae) and retarded ossification of individual phalanges of the forelimbs and hindlimbs were statistically significantly increased ($P < 0.05$ or 0.01) in litters in the 100 mg/kg bw per day group. Increases in the incidence of exencephaly, acrania and skull malformations were also seen in the 10 and 30 mg/kg bw per day groups (but each finding was within the range of historical control data). There was also an apparently dose-related increase in the incidence of small growth on forepaw/toe, seen in 1 fetus (1 litter) in controls, 2 fetuses (2 litters) at 10 mg/kg bw per day, 5 fetuses (4 litters) at 30 mg/kg bw per day and 7 fetuses (4 litters) at 100 mg/kg bw per day, but no litters in the second study at 0, 1 or 3 mg/kg bw per day. This finding was observed at external examination only and was not confirmed at skeletal examination. Other malformations or variations were either not statistically significant or not treatment related at the lower doses; no increase in the incidence of malformations was seen in the second study at 1 or 3 mg/kg bw per day. Cleft palates occurred at similar incidences without a dose–response relationship and were within the range of current or historical control data at 1, 3, 10 and 30 mg/kg bw per day.

The maternal toxicity NOAEL is 30 mg/kg bw per day, based on increased hepatic enzyme induction (increased cytochrome P450 content and *N*-demethylase activity) and severity of vacuolization in liver cells at the LOAEL of 100 mg/kg bw per day. The developmental toxicity NOAEL is 10 mg/kg bw per day, based on a marginal increase in postimplantation loss and statistically significant increase of common malformations or variations at the LOAEL of 30 mg/kg bw per day (Becker & Biedermann, 1995a).

Suitability of mice for developmental toxicity study

The expert opinion of Christian (2005) contains a discussion of the evolution of the current teratology guidelines and a history of the use of mice in regulatory toxicology studies. Christian (2005) suggested that NMRI mice are not a suitable model for the evaluation of teratogenic effects because of lack of an adequate historical control database, high incidence of spontaneous malformations (cleft palate), high rate of spontaneous genetic defects and high reactivity to environmental stress. Christian (2005) concluded that "observations of malformations in the fetuses of NMRI mice should not be considered an appropriate criterion for calculation of a NOEL [no-observed-effect level]".

Similarly, the suitability of the mouse as a test species for developmental toxicity studies is addressed by Neubert (2000). He stated that "It should be noted that the mouse as a species has a strong tendency to display unspecific reactions in response to 'stress' (e.g. hunger, restraint, etc.). It is therefore not used routinely in prenatal toxicology, but only for special investigations."

Rats

In a range-finding study, groups of 25 female Wistar rats were treated by gavage with tebuconazole (purity 99.5%) at a dose of 0 or 100 mg/kg bw per day on GDs 6–15. The treatment had no effect on mortality rates, but body weight gain was retarded. The fertilization rate was not reduced by

treatment, but most litter parameters (e.g. number of implantations, litter size and losses) indicated an adverse effect. More runts and fetuses with malformations (micrognathia, hydronephrosis and hydroureter) were found in treated animals.

The NOAEL for maternal toxicity, embryotoxicity and teratogenicity was less than 100 mg/kg bw per day (Renhof, 1984).

In a second developmental toxicity study, tebuconazole (purity 93.4%) was administered to groups of 25 pregnant Wistar (Bor:WISW (SPF Cpb)) rats by gavage at a dose level of 0, 10, 30 or 100 mg/kg bw per day from GD 6 to GD 15. The vehicle was 0.5% aqueous suspension of Cremophor emulsion (dose volume 10 ml/kg bw). Stability and homogeneity were evaluated, but the results were not presented in the study report. On GD 20, caesarean sections were performed, followed by gross examination of all internal organs with a focus on the uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea. The uteri plus contents were weighed; uteri in non-pregnant females were stained to identify implantation sites. Fetuses were sexed, individually weighed and examined for gross abnormalities. One half of the fetuses in each litter were processed for examination of visceral and brain abnormalities using modified Wilson's technique, and the remaining half were cleared and stained for skeletal examination.

No treatment-related deaths occurred. No treatment-related clinical signs of toxicity were observed. Faecal alterations (e.g. loose stools) occurred at 100 mg/kg bw per day in 11 dams. At 30 and 100 mg/kg bw per day, dose-related reductions in weight gain were observed throughout the treatment period; at 100 mg/kg bw per day, retarded body weight gain was observed throughout gestation. Effects on litter parameters included an increased number of losses and a decrease in mean fetal weight at 100 mg/kg bw per day. At this dose, there were also more runts (below 3 g) and fetuses with malformations (mostly microphthalmia): the runt incidence was 36%, compared with 7% in controls, and the incidence of fetuses with malformations was 7%, compared with 2% in controls.

The NOAEL for maternal toxicity is 30 mg/kg bw per day, based on decreased body weight gain throughout the dosing period seen at the LOAEL of 100 mg/kg bw per day and above. The NOAEL for developmental toxicity is 30 mg/kg bw per day, based on decreased fetal weight, increased incidence of malformations, increased incidence of postimplantation losses and increased number of runts seen at the LOAEL of 100 mg/kg bw per day (Renhof, 1985a).

In a third developmental toxicity study, tebuconazole (purity 98.3%) was administered to groups of 25 pregnant Wistar/NAN (Kfm:WIST, Outbred, SPF) rats by gavage at a dose level of 0, 30, 60 or 120 mg/kg bw per day from GD 6 to GD 15. The vehicle was 0.5% aqueous suspension of Cremophor emulsion (dose volume 10 ml/kg bw). The dosing solutions were prepared daily. Determination of concentration, homogeneity and stability of the dosing solution was performed on one occasion during the treatment period. The dams were sacrificed on GD 21 (caesarean section) followed by gross examination of all internal organs with a focus on the uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea. The uteri plus contents were weighed; uteri in non-pregnant females were stained with ammonium sulfide solution to identify implantation sites. Fetuses were sexed, individually weighed and examined for gross abnormalities. One half of the fetuses in each litter were processed for examination of visceral and brain abnormalities using Wilson's technique, and the remaining half were cleared and stained for skeletal examination.

The dose of 30 mg/kg bw per day affected neither maternal nor fetal parameters. No compound-related mortality or clinical signs of toxicity were reported at any dose level. Although body weight gains for GDs 6–21 were slightly decreased (85% of controls) at the high dose, corrected or uncorrected body weight gains were not significantly different from those of controls at any dose level. Mean daily feed consumption during days 6–16 was significantly decreased with respect to controls at the middle dose (−7%) and at the high dose (−15%). There was a slight increase in daily

feed consumption (about 5%) during the post-dosing period. A significant decrease in corrected body weight (minus uterine weights) was reported for the high dose, and a dose-dependent and statistically significant increase in absolute and relative liver weights was reported at the high and middle doses. The statistically significant increase in liver weights at the middle dose (60 mg/kg bw per day) was minimal and was not considered biologically relevant by the Meeting. Reproductive parameters (numbers of corpora lutea and implantations) were not adversely affected. An increase in the numbers of early and late resorptions and a decrease in mean fetal weights (−10.6%) and in total live fetuses (19.4% below controls) were observed at the high dose. External examination of fetuses revealed a missing tail in one high-dose fetus and agnathia (lower jaw), microstomia and anophthalmia in another fetus of a different litter at the highest dose tested. No malformed or anomalous fetuses were observed in the control, 30 mg/kg bw per day or 60 mg/kg bw per day dose groups. Visceral examination of fetuses revealed findings of excess fluid in the thoracic cavity at the high dose (three pups in one litter, one pup in another litter) and at the low dose (one pup). Skeletal examination revealed increased incidences of supernumerary ribs, non-ossified cervical vertebrae and incompletely ossified sternebrae, indicating retardation of fetal development.

The maternal toxicity NOAEL is 60 mg/kg bw per day, based on increased absolute and relative liver weights and decreased body weight gains at the LOAEL of 120 mg/kg bw per day. The NOAEL for developmental toxicity is 60 mg/kg bw per day, based on higher incidence of resorptions, decreased litter size, reduced fetal body weight and marginally increased incidence of skeletal variations at 120 mg/kg bw per day (Becker, Vogel & Terrier, 1988a).

Rabbits

In a developmental toxicity study, tebuconazole (purity 93.4%) was administered to groups of 15 presumed pregnant Himalayan (CHBB:HM) rabbits by gavage at a dose level of 0, 3, 10 or 30 mg/kg bw per day in 0.5% aqueous Cremophor emulsion from GD 6 to GD 18. The dams were sacrificed on GD 29, followed by gross examination of all internal organs with a focus on the uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea. The uteri and contents were weighed; uteri in non-pregnant females were stained with ammonium sulfide solution to identify implantation sites. The livers were weighed. Fetuses were sexed, individually weighed and examined for gross abnormalities. The internal examination included the thorax, abdomen and pelvis and sex determination. The crania were examined for ossification, and the heads were fixed, serially sectioned and examined. The carcasses were cleared and stained for skeletal examination.

No treatment-related clinical signs of toxicity were observed. One dam of the control group died prior to sacrifice, but the death was not related to dosing. There was reduced body weight gain (74% of control, but not statistically significant) in dams of the highest dose group during the dosing period. At 30 mg/kg bw per day, a significant increase in resorptions per dam (0.8) was observed, which has to be assessed with regard to the unusually low number of resorptions per dam (0.2) in the control group. The historical control data reveal a range from 0.2 to 2.6 resorptions per dam. Also, the slightly increased postimplantation losses (11.3%) are clearly within the range of historical control data (2.6–38.8%). Therefore, these findings are not considered to be treatment-related effects.

The maternal toxicity NOAEL in the developmental toxicity study in rabbits is 10 mg/kg bw per day, based on reduced body weight gain during treatment at the LOAEL of 30 mg/kg bw per day. The developmental toxicity NOAEL is 30 mg/kg bw per day, the highest dose tested. A developmental toxicity LOAEL was not observed in this study (Renhof, 1985b).

In a second developmental toxicity study, tebuconazole (purity 98.2%) was administered to groups of 16 presumed pregnant Chinchilla (Kfm:CHIN, hybrids, SPF Quality) rabbits by gavage at a dose level of 0, 10, 30 or 100 mg/kg bw per day in 0.5% Cremophor EL (4 ml/kg bw) from GD 6 to GD 18. The dams were sacrificed on GD 28, followed by gross examination of all internal organs

with a focus on the uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea. The uteri and contents were weighed; uteri in non-pregnant females were stained with ammonium sulfide solution to identify implantation sites. The livers were weighed. Fetuses were sexed, individually weighed and examined for gross abnormalities. The internal examination included the thorax, abdomen and pelvis and sex determination. The crania were examined for ossification, and the heads were fixed, serially sectioned and examined. The carcasses were cleared and stained for skeletal examination.

In the high-dose group, maternal weight gain was decreased by 38% during the dosing period, 29% during the post-dosing period and 27% from GD 6 to GD 28. After correcting for gravid uterus weight, the high-dose group had a small weight loss (−0.3 g versus 1.4 g weight gain for controls), whereas the controls had a small weight gain. Feed consumption was decreased by 12% in high-dose does during dosing, but was similar to or slightly greater than that of controls during the post-dosing period. Absolute and relative liver weights were not affected by treatment, and no treatment-related gross lesions were observed in maternal animals. A statistically significant increase in postimplantation loss was observed at the high dose, as evidenced by increased fetal resorptions and decreased numbers of live fetuses per dam. Slightly decreased fetal body weight, which was correlated with a slightly decreased retarded ossification, was observed at the high dose; however, it lacked a clear dose–response relationship, and values were within historical and concurrent control ranges (35.1, 33.5, 35.0 and 33 g at 0, 10, 30 and 100 mg/kg bw per day). External examination revealed frank malformations (peromelia, palatoschisis, malrotation of hindlimb, agenesis of claws of the hind paw) in the high-dose group. Examination of the fetal heads by Wilson's technique revealed one fetus with hydrocephalus internus at the high dose. Skeletal examination of fetuses revealed abnormalities and delayed ossification in the high-dose group only. Of 90 fetuses born to dams treated at 100 mg/kg bw per day, eight (9%) were malformed, and five of these (6%) had peromelia. Peromelia was seen in none of a total of 346 fetuses in the other three groups combined. This finding was not observed in the other developmental studies in rabbits and probably should be assessed as retardation. The study suffers from some technical deficiencies, as there was no double staining, there was an insufficiently long observation time of fetal development and many indications of incomplete development also exist in controls. At 100 mg/kg bw per day, the percentage of fetuses with absent or incomplete phalangeal ossification was also increased.

The maternal toxicity NOAEL is 30 mg/kg bw per day, based on decreased body weight gains and decreased feed consumption during dosing at the LOAEL of 100 mg/kg bw per day. The developmental toxicity NOAEL is 30 mg/kg bw per day, based on increased postimplantation loss, frank malformations, hydrocephalus and increased external and skeletal abnormalities at the LOAEL of 100 mg/kg bw per day (Becker, Vogel & Terrier, 1988b).

In a third developmental toxicity study (main study), tebuconazole (purity 96.3–96.8%) was administered to groups of 16 presumed pregnant Chinchilla (Kfm:CHIN, hybrids, SPF Quality) rabbits by gavage at a dose level of 0, 10, 30 or 100 mg/kg bw per day in 0.5% Cremophor EL (dose volume 4 ml/kg bw) from GD 6 to GD 18. The dams were sacrificed on GD 28, followed by gross examination of all internal organs with a focus on the uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea. The uteri and contents were weighed; uteri in non-pregnant females were stained with ammonium sulfide solution to identify implantation sites. The livers were weighed. Fetuses were sexed, individually weighed and examined for gross abnormalities. The internal examination included the thorax, abdomen and pelvis and sex determination. The crania were examined for ossification, and the heads were fixed, serially sectioned and examined. The carcasses were cleared and stained for skeletal examination.

In a supplementary study, five female rabbits per dose group (0, 10, 30 or 100 mg/kg bw per day) were exposed to tebuconazole using the identical dosing regimen as for the third main study.

The purpose of this study was to assess the effects of tebuconazole on specific parameters of maternal toxicity in pregnant Chinchilla rabbits. Blood specimens were taken from the marginal ear vein on GDs 6, 12 and 19 postcoitum and subjected to full haematological and clinical chemistry analysis. The dams were sacrificed just after the last blood specimens had been taken, and the gravid uteri were removed by caesarean operation and weighed. All reproduction parameters were recorded (as above). The adrenals, kidneys, liver and spleen from all gravid dams were weighed separately and prepared for histological examinations. Two portions of 10 g each were taken from each liver before the preparation for determination of cytochrome P450, *N*-demethylase and *O*-demethylase activities and the triglyceride content.

No treatment-related clinical signs of toxicity or mortality were observed. At 100 mg/kg bw per day, feed intakes were decreased, and body weight loss occurred from GD 6 to GD 11, which was not recovered during the remaining gestation period. Overall body weight gain during the treatment period was also decreased by 17% compared with the control group. No treatment-related findings were noted during postmortem examination. The reproduction parameters affected in the high-dose group were a slightly increased mean postimplantation loss and a decrease in mean fetus weight (statistically significant), which was correlated with slightly retarded ossification and increased incidence of common malformations. The decrease in mean fetal body weight was 31.5, 32.0, 31.6 and 30.0 g at 0, 10, 30 and 100 mg/kg bw per day, respectively, with a historical control range of 29.2–35.9 g (Becker, 2006). At 30 mg/kg bw per day, three fetuses with external malformations were observed, which are related to the high spontaneous variability but not to a treatment-related effect. Abnormal findings at 100 mg/kg bw per day were noted in four fetuses during external and visceral examinations. Additional findings were evident in two of the four fetuses during examination of heads by Wilson's technique and in two of the four fetuses during skeletal examinations for abnormal findings. Slightly increased incidences of non-ossification and incomplete ossification, which were correlated with statistically significant reductions in mean fetal body weight (individual basis), were also noted in this group.

There were no treatment-related effects on the haematological or clinical chemistry parameters evaluated on GDs 6, 12 and 19. In addition, there was no observable effect at GD 19 on liver cytochrome 450, *N*-demethylase, *O*-demethylase or triglycerides. No treatment-related gross abnormality was noted upon macroscopic examination (GD 19). The absolute and relative organ weights of adrenals, kidneys, liver and spleen were unaffected by the treatment. Based on the first amendment to the study report by Becker & Biedermann (1995b), histopathological evaluation of the liver showed single-cell necrosis with increased incidences in test article–treated animals, whereas liver necrosis was evident in one female of the 30 mg/kg bw per day group. The number of animals investigated was too low to permit definitive interpretation of these findings. However, the liver is known to be the main target organ in the other species examined. Therefore, a treatment-related effect for these findings (from 10 mg/kg bw per day upwards) has to be assumed (Becker & Biedermann, 1995b).

The NOAEL for maternal toxicity is 30 mg/kg bw per day, based on decreased feed consumption and decreased body weight gains observed at the LOAEL of 100 mg/kg bw per day. The developmental toxicity NOAEL is 30 mg/kg bw per day, based on increased postimplantation loss, increased incidence of common malformations and increased external and skeletal abnormalities at the LOAEL of 100 mg/kg bw per day (Becker & Biedermann, 1995c).

In a fourth developmental toxicity study, tebuconazole (purity 98.5%) was administered to groups of 14 or 15 presumed pregnant Chinchilla (CHB-W) rabbits by gavage at a dose level of 0 mg/kg bw per day (14 rabbits) or 100 mg/kg bw per day (15 rabbits) in 0.5% Cremophor EL (dose volume 4 ml/kg bw) from GD 6 to GD 19. All rabbits were inspected twice daily. Body weights were recorded on day 0 and daily from GD 6 to GD 19. A caesarean section was performed on GD 19, 2 hours post-dosing. Blood was drawn from an extremity vein just prior to caesarean section.

All reproductive parameters were recorded. The liver, ovaries, adrenals and placentas of all pregnant females (surviving) were weighed. The right liver lobes and two placentas with fetuses from each of the pregnant females were fixed in buffered 4% formaldehyde. The adrenals and ovaries of three females from each group were also fixed in buffered 4% formaldehyde. Enzyme activities of the livers (7-ethoxycoumarin deethylase, 7-ethoxyresorufin deethylase, aldrin epoxidase, epoxide hydrolase, gluthathione-*S*-transferase, uridine diphosphate-glucuronosyltransferase, testosterone metabolism assay) were determined. Steroid concentrations (cortisol, corticosterone, 11-deoxycorticosterone and progesterone) were determined in adrenal tissue. Additionally, the 11β-hydroxylation of 11-deoxycorticosterone in adrenal mitochondria was measured. The blood samples were analysed for content of tebuconazole, as were fetal tissues.

Two tebuconazole-treated animals died during the study because of intubation errors. Dosing did not affect appearance or behaviour of the animals. Feed consumption was decreased (GDs 6–12). Body weight gain was decreased (GDs 6–10). Urination and amount of faeces were decreased. The necropsy findings did not reveal treatment-related effects. Absolute and relative weights of the liver, ovaries and adrenals and the absolute placental weight did not differ statistically between treated groups and the control group. Histopathological findings showed a distinct hypertrophy of adrenocortical cells from the zona fasciculata. In addition, centrilobular cytoplasmic change of the liver was observed in two females. The activity of 7-ethoxycoumarin deethylase was statistically significantly increased (55.1%). A statistically significant decrease (27.6%) in the activity of glutathione-*S*-transferase was evident at the 100 mg/kg bw per day level, which could be indicative of impaired liver function. A slightly increased 11β-hydroxylation of 11-deoxycorticosterone in the adrenal mitochondria and slightly increased concentrations of 11-deoxycorticosterone and corticosterone in the adrenal tissue occurred. No reproductive parameters were affected by the treatment, except for a slight, statistically significant decrease in fetal body weights. No external finding occurred in any of the fetuses of both study groups (control and treated groups). There was no indication of an accumulation of tebuconazole in the plasma of fetuses, and there was a good correlation between maternal plasma and fetal tissue levels for the individual females. No examination of the fetuses was performed.

Tebuconazole shows maternal toxicity. This study discusses the possibility that formerly (in two studies) recorded increased numbers of external, skeletal and visceral anomalies and variations could have been due to elevated levels of corticosteroids produced by the dams themselves as a response to stress. This study and its results cannot support this theory, as the increase in plasma levels is marginal in this test, with distinct hypertrophy of steroid-forming areas of the adrenals in two out of three histologically examined adrenals; however, the investigators did not examine the fetuses for malformations, so a definite conclusion cannot be made. Also, there were no statistically significant changes in the concentrations of selected steroids in maternal adrenal tissues and mitochondria. However, maternal toxicity has not been shown to be responsible for the teratogenic activity of tebuconazole via stress induction of elevated levels of corticosteroidal compounds.

The LOAEL is 100 mg/kg bw per day, based on marginally increased plasma levels and histopathological changes in adrenals. The NOAEL for maternal and developmental toxicity is less than or equal to 100 mg/kg bw per day. The study authors suggested a threshold value of 100 mg/kg bw per day for fetal malformations (Holzum, Schmidt & Hartmann, 1999).

(ii) Dermal application

Mice

In a dermal developmental toxicity study, 25 pregnant NMRI KFM-HAN (outbred, SPF Quality) mice received repeated dermal applications of tebuconazole (purity 98.1%) in aqueous 4% carboxymethylcellulose (dose volume 2.5 ml/kg bw) at a dose level of 0, 100, 300 or 1000 mg/kg bw per day from GD 6 through GD 15 inclusive. The application was covered with an occlusive bandage

and left for 6 hours and was then rinsed off with lukewarm water. The dams were inspected at least twice daily for mortality, clinical signs and changes in appearance and behaviour. Weights of the animals and feed consumption were recorded at regular intervals. At day 18 postcoitum, caesarean sections were performed, gross macroscopic examination of all internal and all external organs, with emphasis on the uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea, was performed, and the data were recorded. The fetuses were removed from the uterus, sexed, weighed individually and examined for gross external abnormalities. Half of the live fetuses were allocated to Wilson's slicing techniques for examination of the viscera and brain. The other half were placed in potassium hydroxide solution, stained with alizarin red S and examined for skeletal abnormalities, and all abnormalities were recorded. The uteri and contents of all uteri with live fetuses were weighed at necropsy on day 18 postcoitum to enable calculation of the corrected body weight gain. If no implantation sites were evident, the uterus was placed in an ammonium sulfide solution.

As no overt maternal toxicity was observed in this main study, an additional supplementary study, employing the same treatment regimen and the same strain of mice, was conducted to further assess the maternal toxicity of the compound. In the supplementary study, groups of 2 × 10 mated females (parts A and B) were used for each dose level. The tebuconazole used was 96.0% pure. All dams were sacrificed on day 16 postcoitum and necropsied, the liver and adrenals of group A animals were weighed, the pregnancy status of the animals was recorded, and sections of liver and adrenals were examined histopathologically (group A). Before sacrifice of the study group B dams, blood samples were collected from non-fasted animals. Following this, the females were sacrificed and necropsied. The pregnancy status was recorded, and the entire liver was taken for analysis of the cytochrome P450 content and the *N*-demethylase and *O*-demethylase activities. The blood samples were analysed for AST, ALT, glutamate dehydrogenase and alkaline phosphatase activities.

Treatment had no effect on clinical signs, mortality, local skin reactions, body weight, feed consumption or reproductive parameters (numbers of corpora lutea, implantations, resorptions and live fetuses). In the supplementary study, clinical biochemical examinations revealed increased ALT activity at 1000 mg/kg bw per day; there was also increased cytochrome P450 content and *N*-demethylase activity in liver tissue above 300 mg/kg bw per day and a marginal increase in *O*-demethylase activity. None of these parameters showed a dose–response relationship. There was a dose-related reduction in relative adrenal weights in all dose groups, which was statistically significant at 1000 mg/kg bw per day; absolute adrenal weights were also reduced in all treatment groups, but not in relation to dose. No effect was seen on liver weights, but histological examination revealed fatty changes in periportal areas in most mice at 300 mg/kg bw per day and in all mice at 1000 mg/kg bw per day. In general, no dose-related effects were observed in the fetuses, but slightly increased numbers (not statistically significant) of palatoschises and supernumerary ribs were found in the fetuses of the 1000 mg/kg bw dose group.

When results of the main study and supplementary study are combined, the NOAEL for maternal toxicity is 100 mg/kg bw per day based on increased activities of cytochrome P450 and *N*-demethylase in liver tissue and fatty changes in the periportal liver area seen at the LOAEL of 300 mg/kg bw per day. The developmental toxicity NOAEL is 1000 mg/kg per day, the highest dose tested (Becker et al., 1990). The JMPR in 1994 established a developmental toxicity NOAEL of 300 mg/kg bw per day, based on slightly increased external anomalies and skeletal anomalies seen at 1000 mg/kg bw per day (Annex 1, reference *71*). The present Meeting did not consider the slightly increased anomalies as adverse, and no clear dose–response relationship was observed.

Rats

In a dermal developmental toxicity study, tebuconazole (purity 97.4%) in aqueous 1% Cremophor EL was administered dermally to pregnant Wistar (Bor:WISW (SPF Cpb)) rats on GDs 6–15 at a nominal level of 0, 100, 300 or 1000 mg/kg bw per day. The test material was applied to a 25 cm^2

area of shaved skin (nominal doses of 0, 0.87, 2.6 and 8.7 mg/cm^2 per day) for 6 hours, then removed, followed by washing of the application site with lukewarm water. Dams were sacrificed on GD 20, and gross macroscopic observation of all organs was performed. At caesarean section, the uteri and their contents were weighed to obtain corrected maternal body weight gains and were examined to determine the number of implantations. The fetuses were sexed, weighed and examined for external malformations. Approximately 50% of the fetuses were examined for visceral malformations by Wilson's technique, and the rest were cleared in potassium hydroxide and stained with alizarin S for evaluation of skeletal malformations by Dawson's method.

No evidence of maternal toxicity (changes in body weights, corrected body weights, feed consumption, clinical signs, pathology, deaths, abortions, premature deliveries) were noted at any dose level. No developmental toxicity was noted at any dose level based upon indices of mean number of corpora lutea per dam, number of implantations per dam, number of live or dead fetuses per dam, number of resorptions per dam (early and late), mean fetal weights, sex ratios (per cent male), mean crown–rump length, mean number of runts per dam, and incidence of variations or malformations.

For maternal and developmental toxicity by the dermal route, the NOAEL is 1000 mg/kg bw per day, the highest dose tested. A LOAEL could not be established (Renhof, 1988a).

In a second dermal developmental toxicity study, tebuconazole (purity 97.4%) in aqueous 1% Cremophor EL was administered dermally to pregnant Wistar (Hanlbm: WIST (SPF)) rats on GDs 6–15 at a nominal level of 0 or 1000 mg/kg bw per day. The test material was applied to a 25 cm^2 area of shaved skin and covered with an occlusive dressing. Six hours after the application, the bandage was removed, and the skin was rinsed with lukewarm tap water. The animals were inspected at least twice daily for mortality, clinical signs, changed appearance and behaviour. The animals were weighed daily, and feed consumption was recorded at regular intervals. Dermal irritation was evaluated daily according to the Draize method. Dams were sacrificed on GD 21, and gross macroscopic observation of all organs was performed. At caesarean section, the uteri and their contents were weighed to obtain corrected maternal body weight gains and were examined to determine the number of implantations. The fetuses were sexed, weighed and examined for external malformations. Approximately 50% of the fetuses were examined for visceral malformations by Wilson's technique, and the rest were cleared in potassium hydroxide and stained with alizarin S for evaluation of skeletal malformations by Dawson's method.

Only the local skin irritation on the treated area was more common and more severe in females treated with the active substance than in control animals treated with vehicle only (statistically significant). No other effects on any of the parameters examined were seen in this study.

For maternal and developmental toxicity by the dermal route, the NOAEL is 1000 mg/kg bw per day, the highest dose tested. A LOAEL could not be established (Becker & Biedermann, 1995d).

2.6 Special studies

(a) Acute neurotoxicity

Rats

In an acute oral neurotoxicity study, tebuconazole (purity 96.2–97.3%) was administered by gavage to 12 male Fischer 344 rats at a dose of 0, 100, 500 or 1000 mg/kg bw and to 12 female Fischer 344 rats at a dose of 0, 100, 250 or 500 mg/kg bw. Because a NOAEL did not appear to be attained in this study, a second (supplemental) acute oral neurotoxicity study using the same batch of tebuconazole and doses of 0, 20 and 50 mg/kg bw administered to 12 Fischer 344 rats of each sex per group was performed. The test substance was suspended in 0.5% methylcellulose–0.4% Tween

80 in deionized water and administered at a dose volume of 10 ml/kg bw. Based on analytical results, actual doses were 0, 0, 21, 50, 103, 497 and 950 mg/kg bw for male rats and 0, 0, 21, 50, 103, 239 and 497 mg/kg bw for female rats. In the main study, functional observational battery (FOB) and motor activity tests were performed pretreatment, on the day of test material administration (day 0) and on days 7 and 14 post-treatment; in the supplemental study, an abbreviated FOB and motor activity tests were performed on day 0 only. At the completion of the main study (day 14), six rats of each sex in the control and high-dose groups were subjected to perfusion, and brain and nervous tissues were examined microscopically. No histopathological evaluations were performed in the supplemental study.

The high dose of 1000 mg/kg bw resulted in mortality of 6 of 12 male rats within 2 days of treatment. One male rat in the 500 mg/kg bw dose group also died. There was no treatment-related mortality in females of any dose group. Clinical signs of incoordination, decreased activity, and nasal and perianal stains were observed on day 0, primarily in the two highest dose groups (males, 500 and 1000 mg/kg bw; females, 250 and 500 mg/kg bw). In general, the incidence of clinical signs increased with dose. Compound-related signs were apparent in both sexes on the day of treatment (day 0) and resolved by day 3 following treatment. Effects on body weight were minimal; weights were 96–101% of control weights on day 14. There was no effect of treatment on brain weight, and there were no histopathological findings in the brain or tissues of the nervous system that could be attributed to treatment.

FOB tests on the day of treatment showed treatment-related effects on gait (incoordination, ataxia); activity, arousal and response to stimuli (all decreased); hindlimb grip strength (decreased); footsplay (decreased in females only); and body temperature (decreased) in one or both sexes in the two highest dose groups (males, 500 and 1000 mg/kg bw; and females, 250 and 500 mg/kg bw). Most of these parameters were statistically significantly different from control values ($P < 0.05$). Increased arousal in the open field and decreased footsplay were also noted in females at 100 mg/kg bw. All effects except decreased footsplay in 250 and 500 mg/kg bw females were resolved by post-test day 7. No changes in FOB parameters were observed in males in the 100 mg/kg bw dose group. No treatment-related FOB effects were observed in either sex in the 20 and 50 mg/kg bw dose groups. Relative to concurrent controls, motor and locomotor activities were increased on day 0 in males and females in the 100 mg/kg bw dose group and decreased in the higher dose groups (males, 500 and 1000 mg/kg bw; and females, 500 mg/kg bw; all $P < 0.05$). There was no statistically significant change in 250 mg/kg bw females. No adverse effect of treatment on motor activity was observed in either sex in the 20 and 50 mg/kg bw dose groups, although there was a slight (non-statistically significant) increase in 50 mg/kg bw males that was considered compound related. No effect of treatment was observed on motor or locomotor activity on days 7 and 14. There were no compound-related gross lesions in males or females that survived to terminal sacrifice. Compound-related microscopic lesions were not evident in 500 mg/kg bw females or in surviving 1000 mg/kg bw males.

The NOAEL is 50 mg/kg bw, based on increased motor activity in male and female rats and decreased footsplay in female rats at the LOAEL of 100 mg/kg bw (Sheets, Gilmore & Hamilton, 1997).

(b) Short-term study of neurotoxicity

Rats

In a 90-day dietary neurotoxicity study, tebuconazole (purity 96.7–98.2%) was administered to 12 Fischer 344 rats of each sex per dose at a dietary level of 0, 100, 400 or 1600 ppm. Based on analytical measurements, doses were 0.0, 7.6, 29.2 and 107 mg/kg bw per day for males and 0.0, 8.8, 34.0 and 122 mg/kg bw per day for females. The test diets were prepared in corn oil at 1% by weight of the diet, and a small amount of acetone was used in the preparation of the diet. FOB and motor activity tests were performed pretreatment and during weeks 4, 8 and 13. At the completion of the

study, six rats of each sex per dose group were subjected to perfusion; brain and nervous tissues were examined microscopically in high-dose and control groups only.

No deaths occurred, and there were no clinical signs attributable to treatment. Relative to controls, body weights of male and female rats in the group receiving 1600 ppm in the diet were statistically significantly reduced after 1 week, by 7% in males and 5% in females (both $P < 0.05$). Lower body weights continued throughout the remainder of the study, with reductions of 8% for males and 7% for females at study termination (both $P < 0.05$). Feed consumption was reduced throughout the study in this group, with average daily feed consumption for males and females of 94% and 92% relative to respective controls. Feed efficiency was reduced by 11% and 13% in high-dose males and high-dose females, respectively. Relative to controls, there were no treatment-related effects on body weight or feed consumption or feed efficiency in the other dietary groups. No treatment-related effects were observed in FOB tests or motor activity tests. There were no gross pathological or histopathological findings that could be attributed to treatment with tebuconazole.

The NOAEL in this subchronic neurotoxicity study in rats is 1600 ppm (equal to 107 mg/kg bw per day), the highest dose tested. A LOAEL was not achieved in this study. The study authors concluded that the present study established that treatment with tebuconazole through the diet produces no evidence of neurotoxicity at the highest dose tested. These results support the highest dietary concentration of 1600 ppm as an MTD, based on decreased feed consumption and weight gain, and the middle dose of 400 ppm as a NOEL in both sexes (Sheets, Gilmore & Hamilton, 1998).

(c) Developmental neurotoxicity

Rats

In a developmental neurotoxicity study, tebuconazole (purity 96.0–96.9%) in corn oil was administered via the diet to pregnant Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats (25 per dose) from GD 6 to lactation day (LD) 11 at a dose of 0, 100, 300 or 1000 ppm (equal to [GD/LD] 0/0, 8.8/16.3, 22.0/41.3 and 65.0/125.4 mg/kg bw per day). P dams were allowed to deliver naturally. On day 5 postpartum, litters were standardized to a maximum of 10 pups per litter. Pups were assigned to one of five subsets (20 pups of each sex per dose in each subset). Physical development landmarks were evaluated for all subsets (including surface righting, eye opening, pinna unfolding, acoustic startle response and pupil constriction); sexual maturation was evaluated in subsets 2–4. Subset 1 pups were sacrificed on postnatal day (PND) 12; brains were weighed for all subset 1 pups, and histopathological evaluations were performed on six pups of each sex in control and high-dose groups (morphometric analysis was performed on six pups of each sex in control, mid-dose and high-dose groups). Subset 2 pups were evaluated for learning and memory on days 23–25 (passive avoidance) and on days 59–63 (Water M-maze). Subset 3 pups were evaluated for motor activity (days 14, 18, 22 and 62) and for auditory startle habituation (days 23 and 63). Subset 4 pups received detailed weekly clinical evaluations. In addition, six animals of each sex per group in subset 4 were selected for neuropathological evaluations; brains were weighed, and the high-dose and control animals were evaluated histopathologically on day 83 (morphometric analysis was performed on six animals of each sex in control, mid-dose and high-dose groups). Subset 5 pups were sacrificed and necropsied on day 22.

At 1000 ppm, two P females died as a result of prolonged gestation. Body weights were slightly decreased ($P \leq 0.01$) in the P females during gestation (4–8%) and early lactation (6–12%). Body weight gains were decreased ($P \leq 0.01$ or 0.05) during GDs 6–9 (400%) and 6–21 (22%) and during LDs 1–12 (55–164%). When compared with concurrent controls, absolute (g/day per animal) feed consumption was reduced ($P \leq 0.05$ or 0.01) in the dams throughout gestation (9–23%) except during the GD 0–6 interval and during the LD intervals 4–7 (20%) and 7–12 (18%). Relative (g/kg bw per day) feed consumption was reduced ($P \leq 0.05$ or 0.01) starting on GD 6 (6–20%) and during

early lactation up to day 12 (8–12%). There was also an increase in alopecia in high-dose dams. The number of live fetuses per dam was decreased relative to concurrent controls (6%, $P \leq 0.01$), whereas the number of dead fetuses per dam was increased relative to concurrent controls (200%, $P \leq 0.01$). No treatment-related findings were observed in dams at 300 or 100 ppm.

At 1000 ppm, the stillborn index was increased (200%, $P \leq 0.01$), and the number of pup deaths (calculated by reviewers) was increased during days 1–5 (229%). Body weights were decreased ($P \leq 0.01$) in the males from PND 5 to PND 86 (7–23%) and in the females from PND 5 to PND 72 (5–24%). Pinna unfolding was delayed (19%, $P \leq 0.01$) relative to concurrent controls. There were decreases in several morphometric measurements of the brain, including decreased ($P \leq 0.01$) thickness of the cerebellum in the males and females on day 12 (10–14%) and on day 83 (7–9%) and an increased thickness of the germinal layer of the cerebellar cortex in the day 12 males (23%, $P \leq 0.01$). Absolute brain weights were decreased in the day 12 and day 83 animals (10–16%, $P \leq 0.01$ or 0.05). Relative (to body) brain weights were increased ($P \leq 0.01$ or 0.05) in the day 12 males and females (10–15%). There were also statistically significant changes in motor activity on days 14 (43% decrease in males [$P < 0.01$], 24% decrease in females [not significant]) and 22 (39% increase in males [$P < 0.05$], 19% increase in females [not significant]) and changes in auditory startle amplitude at both time points (decreased in both sexes on day 23 [14–33%], decreased in females [20%] and increased in males [71%] on day 63). At 300 ppm, there were also decreases in body weight (3–7%) and body weight gain (4–16%) (PNDs 5–23 and 72–86 in males, PNDs 5–51 in females). Pinna unfolding was delayed (16%). There were changes in auditory startle amplitude in both sexes: a dose-related decrease in females only on day 23 (26%), and a dose-related increase in males only on day 63 (18%). In addition, there was a decrease in absolute brain weight in both sexes (3–4%) on day 12 (statistically significant for females only) and in brain measurements (anterior/posterior cerebrum). At 100 ppm, there were decreases in body weight (3–7%) and body weight gain (5–13%) (PNDs 5–37 in males, PNDs 5–51 in females). There were decreases in motor activity (on days 14 and 18 in males [28–35%]) and changes in auditory startle amplitude (increased by 9% in day 14 females, increased by 16% in day 63 males, not significant). There was also a decrease in absolute brain weight in both sexes on day 12 (4%, statistically significant for both sexes) and in brain measurements (anterior/posterior cerebrum).

The LOAEL for maternal toxicity is 1000 ppm (equal to 65.0 mg/kg bw per day), based on decreased body weights, body weight gains and feed consumption, prolonged gestation with mortality and an increased number of dead fetuses. The NOAEL is 300 ppm (equal to 22.0 mg/kg bw per day). The LOAEL for offspring toxicity is 1000 ppm (equal to 65.0 mg/kg bw per day), based on decreased viability, decreases in body weights and absolute brain weights, brain measurements and evidence of developmental delay. The NOAEL is 300 ppm (equal to 22.0 mg/kg bw per day) (Parker, 2000).

A study was conducted by Moser et al. (2001) to evaluate adult neurological, immunological and reproductive parameters in rats. In this study, pregnant Tac:N(SD)fBR Sprague-Dawley rats were administered tebuconazole (purity 97.4%) in 0.7% methylcellulose by gavage at a dose of 0, 6, 20 or 60 mg/kg bw per day from GD 14 to PND 7; the pups were then dosed daily at the same levels from PND 7 to PND 42. Separate groups of rats were used for testing of immunological parameters, neurobehavioural testing using a screening battery of functional tests and cognitive evaluations. Other groups of rats were evaluated for reproductive development and function, whereas still others were sacrificed at the end of the dosing period for histological analyses of major organ systems, including neuropathological assessments.

Body weights of maternal animals were decreased at the highest dose tested during gestation. Pup viability and body weights were decreased in the highest dose group. There were no differences in the fertility indices in the exposed rats mated as adults. Developmental landmarks were mostly

unchanged by tebuconazole exposure. In the sheep red blood cell–immunized high-dose rats, spleen weights and cellularity were increased, and the ratio of cell types was altered compared with controls. There were, however, no biologically significant changes in the immune function of these rats. At necropsy on PND 46 or 152, kidney, liver and spleen weights were altered by tebuconazole treatment, but a dose–response relationship was not clear for most organs; only decreased kidney and increased liver weights were consistent in both sexes. Histological analyses were generally unremarkable outside of the brain. One month after the end of dosing, acquisition of learning the platform location in a water tank (i.e. Morris water maze) was impaired in the high-dose group; there were no differences in neuromuscular ability, motor activity or swim speed to account for this finding. Furthermore, there was no effect on recall of the position during a free-swim trial. Qualitative regional analysis of the brain indicated that the morphology was relatively normal across all regions with the exception of the neocortex and hippocampus. Neuropathological evaluations revealed pyknotic cells across hippocampal cell fields in animals of all tebuconazole treatment groups, with the highest incidence in the 20 and 60 mg/kg bw per day dose groups, coincident with cell loss within pyramidal cell layer of CA3-4 cell fields of the hippocampus and layer of the neocortex. Thus, perinatal exposure to tebuconazole produced neurobehavioural deficits and neuropathology in rats, but did not alter immunological or reproductive function.

The potential for developmental neurotoxicity produced by tebuconazole was investigated in two studies in rats: one by Bayer (Parker, 2000) and one by Moser et al. (2001). There were no neuropathological findings in the Bayer study (Parker, 2000), but Moser et al. (2001) reported that the neuropathological evaluations revealed pyknotic cells across hippocampal cell fields in animals of all tebuconazole treatment groups. An abbreviated neuropathology peer review was performed on slides of rat brain archived from the Moser et al. (2001) study. All available slides from rats in the high-dose and control groups were examined with knowledge as to treatment group. Although the slides were examined for the presence of any neuropathological alteration, particular emphasis was placed on scoring the numbers of dark neurons (referred to by Moser et al. [2001] as "pyknotic") in the hippocampus. Review of cresyl violet–stained sections from rats in the control and high-dose groups failed to confirm the presence of any treatment-related differences in the numbers of dark neurons within the hippocampus. Furthermore, the dark neurons present in the brain sections from this study are considered to be typical of those seen in association with handling artefact. Garman (2001) reported that "It is the opinion of this reviewing pathologist that it is inappropriate to refer to these dark neurons as 'pyknotic' and, furthermore, that these dark neurons are not indicative of any neuropathic process. This pathologist also found no evidence of any treatment-related neuron loss within the hippocampus".

These results from two studies in rats and neuropathology review were further analysed by Sheets (2002) to evaluate the potential of tebuconazole to produce neuropathology. Sheets (2002) concluded that, based on all of the available information, including that provided in the Moser et al. (2001) publication, Garman's (2001) review of the slides from that study and the absence of neuropathology in Bayer's developmental neurotoxicity study, there is no evidence that exposure to tebuconazole during development produces neuropathology at any dose level. The conclusion of the abbreviated neuropathology peer review regarding the Moser et al. (2001) study was that the dark neurons present in the brain sections were considered to be typical of those seen in association with handling artefact (Sheets, 2002). Subsequent to these findings by the neuropathology work group, Barone & Moser (2004) wrote a retraction "Letter to the Editor" about the findings in their original publication in 2001. Based on the above discussion, it is concluded that exposure to tebuconazole during development did not produce neuropathology at any dose level.

(d) Delayed neurotoxicity

No delayed toxicity studies were submitted.

(e) Combined toxicity

A study of acute oral toxicity was conducted to evaluate the potential potentiation effect of tebuconazole. An acute oral toxicity study in rats was conducted with tebuconazole plus triadimenol (combination) and tebuconazole plus dichlofluanid to evaluate the potential potentiation effect. Tebuconazole is a fungicide in the triazole class of fungicides, whereas triadimenol and dichlofluanid are fungicides belonging to the conazole class and phenylsulfamide class, respectively.

Groups of fasted adult male Bor:WISW (SPF Cpb) Wistar rats (five of each dose per group) were administered the test substance as a single dose by gavage formulated in Cremophor EL/demineralized water (2%). The dose volume was 10 ml/kg bw. The dosing regimen was as follows:

- Group 1 received tebuconazole (purity 94.7%) at a single dose of 5000 mg/kg bw.
- Group 2 received triadimenol (purity 97.1%) at a single dose of 710, 1000 or 1600 mg/kg bw.
- Group 3 received dichlofluanid (purity 99.4%) at a single dose of 5000 mg/kg bw.
- Group 4 received equitoxic doses (percentages based on the ratio of LD50 values) of a combination of tebuconazole (82.12%) and triadimenol (17.88%) at a single dose of 2000, 2240 or 3000 mg/kg bw.
- Group 5 received equitoxic doses of a combination of tebuconazole (50%) and dichlofluanid (50%) at a single dose of 5000 mg/kg bw.

The post-treatment observation period lasted 14 days. Clinical signs were recorded several times after dosing on day 1 and once a day thereafter. Body weight was recorded on the day of administration and on a weekly basis thereafter. Necropsy was performed at the end of the post-treatment period, and all animals were subjected to a gross pathological examination. Animals that died during the observation period were also subjected to a gross pathological examination.

The results of this study are summarized in Table 18.

Clinical signs such as bristled fur, pallor, apathy, reduced and later increased motility, spastic gait, staggering, cramps, slight convulsions, lateral recumbency, salivation, lacrimation, dyspnoea, diarrhoea and increased urine excretion were observed in these studies. Slight body weight loss was observed in these studies, but it was reversible until the end of the post-treatment observation period. Necropsy indicated changes in the lung (distended), liver (slight lobulation), spleen (pale), renal pelvis (dark red) and glandular stomach mucosa (reddened). No treatment-related macroscopic changes were observed in animals sacrificed at termination.

The results of these studies showed that equitoxic doses of tebuconazole and triadimenol administered orally to fasted rats resulted in a slightly potentiating effect. However, no potentiation was observed when equitoxic doses of tebuconazole and dichlofluanid were administered orally to fasted rats (Flucke, 1987).

(f) Cataract formation

Cats

Groups of four male and four female Forest of Dean cats received whole-body exposure to tebuconazole (purity 95.8%) in polyethylene glycol E 400 and ethanol at a mean analytical concentration of 61 or 309 mg/m^3 (99% of particles with an aerodynamic diameter of < 5 µm) for 6 hours per day for 4 weeks, followed by an observation period of 15 weeks. Control animals were exposed to aerosols of either the vehicle or about 20 mg/m^3 Scalex (KNJ 0953; positive controls for cataract).

Table 18. Acute toxicity and combined acute toxicity of tebuconazole

Dose (mg/kg bw)	Toxicological results[a]	Duration of clinical signs	Time of death	Mortality (%)
Group 1 tebuconazole				
5000	1/5/5	2 h – 12 days	6 days	20
LD_{50} > 5000 mg/kg bw				
Group 2 triadimenol				
710	1/5/5	30 min – 5 days	3 h	20
1000	2/5/5	20 min – 7 days	1–3 days	40
1600	4/5/5	40 min – 6 days	5 h – 2 days	80
LD_{50} 1089 mg/kg bw				
Group 3 dichlofluanid				
5000	0/5/5	7 h – 1 day	—	0
LD_{50} > 5000 mg/kg bw				
Group 4 tebuconazole (82.12%) + triadimenol (17.88%)				
2000	1/5/5	50 min – 8 days	4 days	20
2240	2/5/5	1 h – 8 days	1–6 days	40
3000	4/5/5	40 min –14 days	1–5 days	80
LD_{50} 3046 mg/kg bw (calculated); 2424 mg/kg bw (experimental) – slight potentiation				
Group 5 tebuconazole (50%) + dichlofluanid (50%)				
5000	2/5/5	3 h – 8 days	6 days	40
LD_{50} > 5000 mg/kg bw (calculated); approximately 5000 mg/kg bw (experimental) – no potentiation				

From Flucke (1987)

[a] Number of dead animals / number of animals with toxic signs / number of animals used.

During the weeks of exposure and observation period, body weights, clinical signs, mortality and ocular findings were recorded. At termination, gross pathological and histopathological examinations were performed.

Two low-dose males died during the study (death not attributed to test substance). At necropsy, it was found that the two male animals from the low-dose group, which died intercurrently, had thickening of the urinary bladder wall, mucous membrane inflammation, haemorrhage and haemorrhagic urine in the bladder. The treatment did not induce clinical symptoms and did not affect mortality rates or body weight gains. Cataracts due to lens fibre degeneration were found in all animals in the positive control group during the observation period of 4 months post-exposure. Exposure to tebuconazole did not result in cataract induction, but three females at 309 mg/m^3 and one positive control animal had yellow-tinged spots along the lens fissure. Examination of 42 untreated female cats aged 7–12 months showed that these ocular changes were not common spontaneous alterations; no such finding was seen in vehicle controls. The etiology of this finding and its toxicological relevance remain unclear. The 1994 JMPR established a NOAEL of 61 mg/m^3, equivalent to about 5 mg/kg bw per day, on the basis of ocular effects other than cataracts of unknown etiology at the highest concentration (Annex 1, reference *71*). The present Meeting affirmed the NOAEL of 61 mg/m^3 established by the JMPR in 1994 (Annex 1, reference *71*). The study authors established a NOAEL of 309 mg/m^3, the highest dose tested, for cataract development in cats (Märtins, Pauluhn & Kroetlinger, 1990).

Dogs

Groups of four female Beagle dogs were treated by head–nose exposure to tebuconazole (purity 97.1%) in polyethylene glycol and ethanol at a target aerosol concentration of 0, 150 or 800 mg/m^3 for 4 hours per day for 6 weeks and observed for 8 weeks. The analytical concentrations were 163 and

914 mg/m^3; about 90% of the particles had an aerodynamic diameter of less than 3 μm. No vehicle control group was included in the study. During the weeks of exposure and observation period, body weights, clinical signs, mortality, ocular findings, reflexes and feed intake were recorded, and lung function tests and blood examinations were performed. Measurements of the blood gases and the acid–base status were performed once near the end of exposure. At termination, organ weights, gross pathological and histopathological examinations were performed.

The treatment did not affect mortality rates. Most animals at 914 mg/m^3 began salivating immediately after exposure, and single animals also made tussive noises; these effects were reversed within 2 hours. In the reflex test, there were no treatment-related effects. A slight (statistically significant) drop in body temperature was recorded in both groups of exposed animals and was explained by the laboratory as being due to ethanol inhalation (central nervous system depression). Retardation of body weight gain was observed in both treated groups during the second half of the study, but with no dose–effect relationship. A lung function test revealed a marginal decrease in the mean minute volume in both treated groups and a slight reduction in the mean partial pressure of oxygen. The ophthalmic examinations showed no effects that could be related to treatment. Increased spleen weight observed at 914 mg/m^3 was the only change in organ weights. There were no deviations in other organ weights or other gross pathological changes at necropsy. No histopathological changes were recorded that could be related to inhalational treatment with tebuconazole.

The NOAEL was 163 mg/m^3, equivalent to 23 mg/kg bw per day, on the basis of clinical effects and questionable body weight effects seen at the LOAEL of 914 mg/m^3. The NOAEL for cataract induction was 914 mg/m^3, equivalent to 125 mg/kg bw per day (Märtins, 1991).

(g) Studies on metabolites

No toxicity studies were submitted; however, the registrant indicated that "Triazole fungicide metabolites were evaluated by the JMPR in 2008".

3. Observations in humans

In response to an inquiry, the Bayer company physician stated that "there are no known ill-effects on the health of the employees in the production of tebuconazole and production employees are subject to continuous medical supervision and are examined at least once annually" (Kollert, 1987). Bayer has monitored the health of personnel working in the production of tebuconazole since 1968. Routine occupational and medical examinations were conducted once a year, selected blood parameters were evaluated every 4 weeks, liver function tests were conducted every 2–3 years and thorax X-rays were performed every 3–6 years. Considering the occupational hygiene and use of personal protective equipment, the results of the monitoring programme indicate that no signs of changes in laboratory parameters, health impairments or permanent effects among employees have been recorded by the medical department (Faul & Krauthausen, 1995). Similarly, no ill-health effects have been reported in production employees during annual medical monitoring (including clinical chemistry, urinalysis, pulmonary functions, electrocardiogram) at the Kansas City, USA, plant since 1980 (Metz, Tice & Wey, 1996). In response to a separate inquiry, the company physician (J.D. Forbes) reported that "to date, with regard to our employees, we have had no reported overexposure situations nor have short-term or long-term health problems been identified which can be attributed to tebuconazole" during technical and formulation production (Wey & Forbes, 1997). No ill-health effects were reported in employees working in the production and formulation of tebuconazole in the Kansas City plant in the USA during annual routine checkups from 1991 to 2008. The medical examination included an annual checkup, clinical chemistry, blood counts, urinalysis, lung function testing, audiometry and vision testing. During the annual production period of 4–6 months per year, no worker accidents with tebuconazole occurred, and no consultations of the medical department due

to work or contact with tebuconazole were required (Steffens, 2009). Neumann & Hartmann (2009) reported that no relevant poisoning incidents were known to the company.

Comments

Biochemical aspects

In a toxicokinetic study, groups of male and female rats were given tebuconazole uniformly labelled with ^{14}C in either the phenyl ring or the 3,5-triazole ring as a single dose at 2 or 20 mg/kg bw or as 14 repeated doses of 2 mg/kg bw per day, followed by a single oral dose of radioactive tebuconazole at 2 mg/kg bw. Tebuconazole was rapidly absorbed from the gastrointestinal tract of rats and rapidly excreted from the body. Between 86% and 98% of the dose was excreted in the urine and faeces in 72 hours; most excretion occurred in the first 48 hours. Faecal excretion within 72 hours after administration was about 80% of the applied dose in males and about 65% in females; urinary excretion amounted to about 16% of the applied dose in males and about 33% in females. No significant differences in the absorption, distribution and excretion occurred following administration of a single oral low dose or high dose or repeated doses. Male rats with biliary fistulae excreted 90.7% of the dose with the bile, 7.4% in the urine and 1.5% in faeces within 48 hours, suggesting complete absorption of tebuconazole in intact rats. Only 0.3% of the radioactivity was detected in exhaled air within 72 hours following oral administration of tebuconazole. After 72 hours, less than 1% of the administered dose could be detected in the organs, tissues and the remaining carcass, indicating no potential for bioaccumulation. Highest residues were found in the liver and kidney. Tebuconazole was rapidly distributed (within 1 hour) in the body, as determined by whole-body autoradiography. The peak concentration of radioactivity in plasma was found at 0.33–1.7 hours. The terminal half-life of radiolabel was 31.9–52.5 hours.

Tebuconazole was extensively metabolized in the body following oral administration. Less than 0.7% of parent tebuconazole was detected in the excreta at 72 hours after administration. The metabolic pathway in rats also demonstrated sex-related differences. The main metabolites of tebuconazole in male rats were the oxidation products of one of the methyl groups of the tertiary butyl moiety (i.e. the alcohol and the carboxylic acid). Metabolism in female animals resulted preferentially in simple oxidation products (e.g. hydroxy and carboxy metabolites) and then conjugation to the glucuronide and sulfate, with only minor cleavage of the triazole moiety. In male animals, the primary oxidation products were further oxidized to triol and keto acid derivatives; in addition, cleavage of the triazole ring occurred, as indicated in trials with triazole-labelled compound. The free triazole accounted for about 5% of the administered dose in the urine of the males and 1.5% in that of females.

Toxicological data

Tebuconazole has low to moderate acute toxicity in mice and rats via the oral route. The oral LD_{50} of tebuconazole was 1700 and 4000 mg/kg bw in fasted female and male rats, respectively. The oral LD_{50} of tebuconazole in mice was 3023 and 1615 mg/kg bw in fasted female and male mice, respectively. The LD_{50} in rats treated dermally was greater than 2000 mg/kg bw. The LC_{50} in rats treated by inhalation (nose only) was greater than 0.82 mg/l. Tebuconazole was non-irritating to the eyes and skin of rabbits. Tebuconazole was not a skin sensitizer in guinea-pigs, as determined by the Magnusson & Kligman (maximization) test and the Buehler test.

In a non-GLP 28-day gavage study of toxicity in rats, decreases in haemoglobin concentration and haematocrit values were observed at 100 and 300 mg/kg bw per day. At 100 and 300 mg/kg bw per day, the absolute and relative weights of the liver and spleen were increased in both sexes, and the absolute weight of the kidney was increased in females. A reduced iron content was observed in

the spleen of females at 100 mg/kg bw per day. The NOAEL in the 28-day gavage study in rats was 30 mg/kg bw per day, on the basis of changes in haematological and clinical chemistry parameters and organ weights at 100 mg/kg bw per day. In a 90-day dietary toxicity study in rats, reduced body weight gain was observed at 400 ppm in females during the first 6 weeks. Histopathological examination revealed an increased incidence of intraplasmatic vacuoles in the cells of the zona fasciculata of the adrenals (probably lipid accumulation) in some females at 400 ppm and in all females at 1600 ppm. The NOAEL was 100 ppm (equal to 10.8 mg/kg bw per day), based on a reduction in body weights in females at 400 ppm (equal to 46.5 mg/kg bw per day).

The NOAEL in a 90-day dietary study of toxicity in dogs was 200 ppm (equal to 8.5 mg/kg bw per day), based on decreased body weight gain and feed consumption at 1000 ppm (equal to 41 mg/kg bw per day). Two 1-year dietary studies of toxicity were conducted in dogs with tebuconazole. The overall NOAEL was 100 ppm (equal to 2.9 mg/kg bw per day), based on intracytoplasmic vacuoles in cells of the zona fasciculata of the adrenals and slight hypertrophy accompanied by an increased incidence of large fatty vacuoles seen at 150 ppm (equal to 4.4 mg/kg bw per day) and above.

The carcinogenic potential of tebuconazole was studied in mice and rats. Two carcinogenicity studies were conducted in mice. In the first study, the NOAEL was 20 ppm (equal to 5.9 mg/kg bw per day), based on the increased incidence of centrilobular fine vacuolization in the liver of males at 60 ppm (equal to 18 mg/kg bw per day). There was no evidence of any carcinogenic potential, but the effects on the liver at the LOAEL and above were not very marked in intensity, posing a question as to whether an MTD had been reached in this study. Therefore, a second carcinogenicity study was conducted at higher doses. In the second study, no NOAEL was identified. The LOAEL was 500 ppm (equal to 85 mg/kg bw per day), based on liver toxicity. The incidence of liver tumours in male and female mice was significantly elevated at 1500 ppm (equal to 279 mg/kg bw per day) and was markedly above the range of spontaneous incidences observed in this mouse strain.

In the carcinogenicity study in rats, the NOAEL was 300 ppm (equal to 15.9 mg/kg bw per day), based on body weight depression in both sexes and an increased incidence of pigment deposits in the Kupffer cells in the liver of females at 1000 ppm (equal to 55 mg/kg bw per day). No treatment-related tumours were observed.

Tebuconazole was not genotoxic in an adequate range of in vitro and in vivo genotoxicity tests.

The Meeting concluded that tebuconazole is unlikely to be genotoxic.

In view of the absence of genotoxic potential, the absence of carcinogenicity in rats and no carcinogenicity in mice relevant to human dietary exposure levels, the Meeting concluded that tebuconazole is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, the reproductive parameters were not affected at doses up to 1000 ppm (equal to 72.3 mg/kg bw per day), the highest dose tested. The NOAEL for parental systemic toxicity and offspring toxicity was 300 ppm (equal to 21.6 mg/kg bw per day), based on reduced feed consumption and decreased body weights in parental animals and pups seen at 1000 ppm (equal to 72.3 mg/kg bw per day).

Several developmental toxicity studies in mice, rats and rabbits using gavage administration were submitted. The overall NOAEL for maternal toxicity in the oral gavage studies in mice, rats and rabbits was 30 mg/kg bw per day, mainly based on decreases in body weights and body weight gains (during the early treatment period) at 100 mg/kg bw per day. Marginal effects in studies in mice (haematological effects) and rats (reduced body weight gains) were not considered as adverse. Selected liver parameters (enzymes, weights and clinical chemistry) were evaluated in developmental toxicity studies in mice and rats. Changes in the liver parameters in these studies were considered an adaptive response and not considered as adverse. In one study in mice, there was an increase in the number of small fetuses (runts) at doses of 30 mg/kg bw per day and above. These small fetuses,

defined on the basis of low body weights, were considered unlikely to be due to a single exposure or a small number of exposures. The NOAEL for developmental toxicity in mice was 10 mg/kg bw per day. In other studies in mice, rats and rabbits, developmental effects included increased resorptions, a decreased number of live fetuses, decreased fetal weights, incomplete ossification and visceral and skeletal anomalies. In addition, postimplantation loss was observed in mice. These developmental effects were observed consistently at doses above 30 mg/kg bw per day and in the presence of maternal toxicity in all studies. The overall NOAEL for developmental toxicity was 30 mg/kg bw per day in rats and rabbits.

The Meeting concluded that tebuconazole caused developmental toxicity and teratogenic effects at doses that were maternally toxic in rats and rabbits.

In a study of acute neurotoxicity in rats with tebuconazole, the NOAEL was 50 mg/kg bw based on increased motor activity in male and female rats and decreased footsplay in female rats at 100 mg/kg bw. In a 90-day study of neurotoxicity in rats, no systemic or neurotoxic effects were seen at doses up to 1600 ppm (equal to 107 mg/kg bw per day), the highest dose tested. In a developmental neurotoxicity study in rats with dietary administration, the maternal NOAEL was 300 ppm (equal to 22 mg/kg bw per day), based on decreased body weights, body weight gains and feed consumption, prolonged gestation with mortality and an increased number of dead fetuses at 1000 ppm (equal to 65 mg/kg bw per day). The offspring toxicity NOAEL was 300 ppm (equal to 22 mg/kg bw per day), based on decreased pup viability, decreases in body weights and absolute brain weights, brain measurements and evidence of developmental delays seen at 1000 ppm (equal to 65 mg/kg bw per day), the highest dose tested. Tebuconazole did not produce neurobehavioural or neuropathological changes.

Workers did not report any adverse effects while handling tebuconazole in a production facility. The workers were monitored by routine physical examination and clinical chemistry measurements.

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

Toxicological evaluation

The Meeting reaffirmed the ADI of 0–0.03 mg/kg bw on the basis of an overall NOAEL of 2.9 mg/kg bw per day in two 1-year dietary toxicity studies in dogs, based on histopathological alterations in the adrenals seen at the LOAEL of 4.4 mg/kg bw per day, and using a safety factor of 100.

The Meeting established an acute reference dose (ARfD) of 0.3 mg/kg bw on the basis of a maternal and developmental toxicity NOAEL of 30 mg/kg bw per day in studies of developmental toxicity in rats and rabbits based on maternal toxicity manifested as decreases in body weight gains in the early treatment period and visceral and skeletal anomalies seen at higher doses. The increased incidence of small fetuses, defined on the basis of low body weights, was considered unlikely to be due to a single exposure or a small number of exposures. The ARfD is supported by the NOAEL of 30 mg/kg bw per day observed in a 28-day oral (gavage) toxicity study in rats based on changes in haematological parameters seen at the LOAEL of 100 mg/kg bw per day, which might be produced by a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Twenty-one-month studies of toxicity and carcinogenicity[a,b]	Toxicity	20 ppm, equal to 5.9 mg/kg bw per day	60 ppm, equal to 18 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 85 mg/kg bw per day	1500 ppm, equal to 279 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
	Developmental toxicity[c]	Maternal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	10 mg/kg bw per day	30 mg/kg bw per day
Rat	Twenty-eight-day study of toxicity[c]	Toxicity	30 mg/kg bw per day	100 mg/kg bw per day
	Two-year studies of toxicity and carcinogenicity[b]	Toxicity	300 ppm, equal to 15.9 mg/kg bw per day	1000 ppm, equal to 55 mg/kg bw per day
		Carcinogenicity	1000 ppm, equal to 55 mg/kg bw per day[d]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	300 ppm, equal to 21.6 mg/kg bw per day	1000 ppm, equal to 72.3 mg/kg bw per day[d]
		Offspring toxicity	300 ppm, equal to 21.6 mg/kg bw per day	1000 ppm, equal to 72.3 mg/kg bw per day[d]
		Reproductive toxicity	1000 ppm, equal to 72.3 mg/kg bw per day[d]	—
	Developmental toxicity[c]	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[c]	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
Dog	Two 1-year studies of toxicity[a,b]	Toxicity	100 ppm, equal to 2.9 mg/kg bw per day	150 ppm, equal to 4.4 mg/kg bw per day

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

Estimate of acceptable daily intake for humans

0–0.03 mg/kg bw

Estimate of acute reference dose

0.3 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 tebuconazole

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Complete and rapid
Dermal absorption	Not available
Distribution	Extensive
Potential for accumulation	None
Rate and extent of excretion	Rapid and extensive
Metabolism in animals	Extensive; metabolic pathways include hydrolysis, oxidation and conjugation

Toxicologically significant compounds in animals, plants and the environment	Tebuconazole and 1,2,4-triazole
Acute toxicity	
Rat, LD_{50}, oral	1700 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 0.82 mg/l, dust (4 h exposure, nose only)
Rabbit, dermal irritation	Non-irritating
Rabbit, ocular irritation	Non-irritating
Guinea-pig, dermal sensitization	Not a sensitizer (Magnusson & Kligman and Buehler tests)
Short-term studies of toxicity	
Target/critical effect	Adrenals/hypertrophy of zona fasciculata cells (dogs)
	Liver, blood system and adrenals (rats)
Lowest relevant oral NOAEL	2.9 mg/kg bw per day (overall from two 1-year toxicity studies in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (rabbits)
Lowest relevant inhalation NOAEC	0.0156 mg/l
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver toxicity (mice and rats)
Lowest relevant NOAEL	5.9 mg/kg bw per day (carcinogenicity study in mice)
Carcinogenicity	Not carcinogenic in rats, but hepatocarcinogenic in mice; unlikely to pose a carcinogenic risk at human dietary exposure levels
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects
Lowest relevant reproductive NOAEL	1000 ppm, equal to 72.3 mg/kg bw per day, highest dose tested (rats)
Developmental target/critical effect	Developmental toxicity, including teratogenicity, only at maternally toxic doses in rats and rabbits
Lowest relevant developmental NOAEL	30 mg/kg bw per day (rats, rabbits); 10 mg/kg bw per day (mice; runts)
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity	Increased motor activity in rats
Subchronic neurotoxicity	No neurotoxicity in rats
Developmental neurotoxicity	No neurodevelopmental toxicity in rats
Other toxicological studies	
	None
Medical data	
	No adverse effects reported

Summary

	Value	Study	Safety factor
ADI	0–0.03 mg/kg bw	Two 1-year toxicity studies in dogs	100
ARfD	0.3 mg/kg bw	Developmental toxicity studies in rats and rabbits, supported by a 28-day study of toxicity in rats (gavage)	100

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