BOSCALID

First draft prepared by
D.B. McGregor[1] and Roland Solecki[2]

[1] Toxicity Evaluation Consultants, Aberdour, Scotland; and
[2] Federal Institute for Risk Assessment, Berlin, Germany

Explanation 47
Evaluation for acceptable daily intake 48
1. Biochemical aspects 48
1.1 Absorption, distribution and excretion 48
2. Toxicological studies 70
2.1 Acute toxicity 70
2.2 Short-term studies of toxicity 72
2.3 Long-term studies of toxicity and carcinogenicity 78
2.4 Genotoxicity 83
2.5 Reproductive toxicity 85
(a) Multigeneration studies 85
(b) Developmental toxicity 87
2.6 Special studies 90
(a) Neurotoxicity 90
(b) Liver and thyroid effects 92
(c) Immunotoxicity 93
3. Observations in humans 94
Comments 94
Toxicological evaluation 96
References 98

Explanation

Boscalid is the provisionally approved International Organization of Standardization (ISO) name for 2-chloro-*N*-(4'-chlorobiphenyl-2-yl)nicotinamide (International Union of Pure and Applied Chemistry, IUPAC) or 2-chloro-*N*-(4'-chloro[1,1'-biphenyl]-2-yl)-3-pyridinecarboxamide (Chemical Abstracts Service, CAS). It is an anilide fungicide that inhibits mitochondrial respiration, thereby inhibiting spore germination, germ-tube elongation, mycelial growth, and sporulation of pathogenic fungi on the leaf surface, and is used against a broad spectrum of diseases in a wide range of crops. Boscalid was reviewed for the first time by the present Meeting at the request of the Codex Committee on Pesticide residues (CCPR). All critical studies complied with good laboratory practice (GLP).

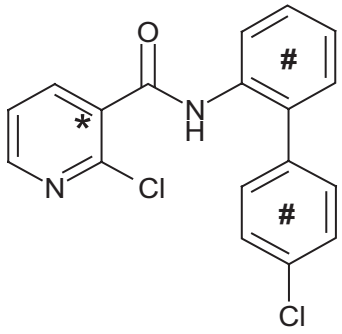
Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Studies of metabolism in rats treated orally were conducted with [diphenyl-ring-U-^{14}C]boscalid (batch Nos 641-1018 and 641-1101; purity, > 98%, radiochemical purity, > 95%), [pyridin-ring-3-^{14}C] boscalid (batch No. 640-1026; purity, > 97%; radiochemical purity, > 95%) and nonradiolabelled boscalid (batch No. 01174-236; purity > 99%). The positions of the radiolabel are shown in Figure 1.

Figure 1. Structure and position of ^{14}C-labelled boscalid



= [diphenyl-U-^{14}C]boscalid
* = [pyridine-3-^{14}C]boscalid

The absorption, distribution, elimination and kinetics of ^{14}C-labelled boscalid were investigated in male and female Wistar rats given doses of 50 and 500 mg/kg bw. The experiments were performed with boscalid labelled in the diphenyl ring. An additional experiment investigating the balance and excretion pattern was performed with boscalid labelled in the pyridine ring.

Tissue distribution was determined using groups of 12 male and 12 female rats for each dose. For all other experiments in vivo, groups of four male and four female rats were used.

The stability, homogeneity and correctness of the test substance preparation were analytically verified.

The results of these experiments are summarized in Table 1. There was no radioactivity in the expired air after the administration of the diphenyl- or pyridine-ring label at a dose of 500 mg/kg bw. Within 168 h after administration of a single oral dose at 500 mg/kg bw (diphenyl label), 3% of the administered radioactivity was excreted in the urine of males and females and 91% (males) and 97% (females) was found in the faeces. Radioactivity remaining in tissues and organs 168 h after dosing was less than 1 µg eq/g at a dose of 500 mg/kg bw, except in the gastrointestinal tract.

Virtually the same excretion pattern was observed as with the diphenyl label after the administration of the pyridine label as a single oral dose at 500 mg/kg bw and after repeated oral administration of diphenyl-labelled boscalid (14 doses of unlabelled boscalid at 500 mg/kg bw, one dose of labelled boscalid at 500 mg/kg bw).

Within 168 h after a single oral dose of diphenyl-labelled boscalid at 50 mg/kg bw, 16% of the administered radioactivity was excreted in the urine of males and females and 85% (males) and 79% (females) was found in the faeces. Radioactivity remaining in tissues and organs 168 h after dosing was less than 0.3 µg eq/g at a dose of 50 mg/kg bw, except in the gastrointestinal tract. The overall recovery of radioactivity was in the range of 93.9–102.8% in all experiments.

Within 48 h after administration of [^{14}C]boscalid at a dose of 500 mg/kg bw, 11% (males) and 12% (females) of the administered radioactivity was excreted via bile, while after administration of 50 mg/kg bw of [^{14}C]boscalid, 39% (males) and 40% (females) of the administered radioactivity was excreted via bile. These percentages are considerably lower than the 70–90% dose recovery in faeces. The difference indicates that boscalid is not completely absorbed from the gastrointestinal tract.

Table 1. Excretion balance (as a percentage of administered radioactivity) at 168 h after oral dosing and biliary excretion after 48 h

	Excretion balance (percentage of administered radiobel)			
	Dose (mg/kg bw)			
	500	50	500	500
	Diphenyl-labelled	Diphenyl-labelled	Diphenyl-labelled	Pyridine-labelled
	Single dose[a,b]	Single dose[a,b]	Repeated doses	Single dose
Males				
Urine 0–168 h	2.73	16.38	2.64	5.21
Cage wash	0.41	0.26	0.11	0.10
Faeces 0–168 h	90.69	84.86	94.88	89.61
Carcass + organs	0.05	0.04	0.11	0.04
Total	93.87	101.54	97.74	94.96
Bile 0–48 h	10.69	39.29	—	—
Females				
Urine 0–168 h	2.88 (5.67)	15.72 (25.74)	3.99	3.80
Cage wash	0.04 (0.12)	1.03 (0.03)	0.18	0.30
Faeces 0–168 h	97.35 (87.26)	79.27 (80.50)	98.47	92.15
Carcass + organs	0.02 (0.05)	0.04 (0.07)	1.10	0.02
Total	100.29 (93.10)	96.06 (106.34)	102.75	96.27
Bile 0–48 h	11.93	39.92	—	—

[a] Experiments with females were repeated for the lowest and highest doses because of evidence that some faecal samples had been mislabelled. These data were not used in the calculation of kinetic parameters.
[b] Numbers in parenthese are results for the repeated experiments.

If it is assumed that the amount of radioactivity excreted in bile and urine represents the amount of [^{14}C]boscalid that is bioavailable, then bioavailability at the lowest dose (50 mg/kg bw) is approximately 50%.

At both doses, the changes in radioactivity concentration with time were similar in blood cells and in plasma. During the first 24 h after dosing, lower concentrations of radioactivity were found in blood cells than in plasma, while at later times this relationship was reversed in the groups at the highest dose (Table 2).

Table 2. Radioactivity in blood cells and plasma of rats given a single dose of [^{14}C]boscalid (diphenyl label)

Tissue	Concentration of radioactive residues (µg eq/g tissue)											
	Dose (mg/kg bw)											
	50						500					
	Males			Females			Males			Females		
	Time-point (h)											
	8	24	35	8	24	35	8	24	35	8	24	35
Blood cells	0.64	0.12	NA	0.78	0.13	NA	1.74	0.31	0.22	1.35	0.43	0.13
Plasma	1.14	0.14	NA	1.52	0.17	NA	3.52	0.40	0.09	2.84	0.51	0.17

From Leibold et al. (2000) and Leibold (2002)
NA, not available.

In rats given a single oral dose of [^{14}C]boscalid at 500 mg/kg bw, the plasma concentration–time curve showed two maxima. The first plasma maximum was reached 0.5–1 h after dosing with concentrations of 2.61 µg eq/g in males and 3.52 µg eq/g in females. The second plasma maximum occurred after 8 h, when plasma concentrations were 4.46 and 3.77 µg eq/g in males and females, respectively. At later times, plasma concentrations declined to 0.01 µg eq/g in males and 0.03 µg eq/g in females at 120 h after dosing. The initial half life (t½) was calculated to be 8.0 h in males and 9.1 h in females. The terminal t½ was 20.2 h in males and 27.4 h in females. The area under the concentration–time curve (AUC) was 68.4 µg eq × h/g in males and 75.5 µg eq × h/g in females.

In rats given a single oral dose of 50 mg/kg bw of [^{14}C]boscalid, the plasma concentration–time curve also showed two maxima. The early peak was reached after 0.5 h when plasma concentrations were 0.99 µg eq/g in males and 1.40 µg eq/g in females. The later peak occurred after 8 h, when plasma concentrations were 1.54 and 1.58 µg eq/g in males and females, respectively. After this second maximum, there was a biphasic decline in plasma concentrations to 0.01 µg eq/g in both males and females at 120 h after dosing. The initial t½ values were 7.2 h and 8.2 h in males and females, respectively. Terminal t½ values were 41.7 and 30.1 h in male and female rats, respectively. The AUC values were 21.2 µg eq × h/g in males and 24.4 µg eq × h/g in females. Increasing the dose by a factor of about 10 thus resulted in an increase of the AUC values by a factor of approximately 3 in both sexes. These kinetic data are summarized in Table 3.

Table 3. Pharmacokinetic parameters of radioactivity in plasma after single oral doses of [^{14}C]boscalid (diphenyl label)

Parameter	Dose (mg/kg bw)			
	50		500	
	Males	Females	Males	Females
First C_{max} (µg eq/g)	0.99	1.40	2.61	3.52
First T_{max} (h)	1.0	0.5	0.5–1.0	0.5–1.0
Second C_{max} (µg eq/g)	1.54	1.58	4.46	3.77
Second T_{max} (h)	8	8	8	8
Initial t½ (h)	7.2	8.2	8.0	9.1
Terminal t½ (h)	41.7	30.1	20.2	27.4
AUC (µg eq × h/g)	21.2	24.4	68.4	75.5

From Leibold et al. (2000) and Leibold (2002)
AUC, area under the curve of concentration–time.

After a single oral dose of [^{14}C]boscalid at 500 mg/kg bw, tissue radioactivity concentrations were measured 8, 18, 24 and 35 h after dosing. At the lowest dose of 50 mg/kg bw, the corresponding radioactivity measurements were made 8, 17, 21 and 24 h after dosing. In general, tissue radioactivity concentrations in both sexes were in the same range at the respective time-points and doses. The pattern of distribution and elimination in various organs and tissues was also similar. Tissue radioactivity concentrations declined with time and at a similar rate to the plasma concentrations. Throughout the time-course of the experiments, the highest concentrations of radioactivity were found in the gastrointestinal tract and liver. Concentrations of radioactivity were lowest in the brain. No evidence for a cumulative potential could be deduced from the available data (Leibold et al., 2000; Leibold, 2002).

Studies of metabolism in rats treated by application to the skin were conducted using [diphenyl-ring-U-^{14}C]boscalid (batch No. 641-2017; radiochemical purity, > 98% and non-radiolabelled boscalid (batch No. 01183-190; purity, 99.3%).

The absorption, distribution and excretion of radioactivity was studied in male Wistar rats given a single dermal administration of [^{14}C]boscalid mixed with non-labelled boscalid and taken up in water at nominal doses of 0.01, 0.10 and 1.00 mg/cm^2 corresponding to 0.1, 1.0 and 10 mg/rat or about 0.4, 4 and 40 mg/kg bw. Groups of four animals were exposed according to the regimen described in Table 4.

Table 4. Design of an experiment in rats given [^{14}C]boscalid as a single dermal application

Duration of exposure (h)	1	4	10	24	10[a]	10
Rats killed after (h)	1	4	10	24	24[a]	72

From Leibold & Hoffmann (2001)

[a] Only at the lowest dose.

Twenty-four hours before dosing, an intrascapular area (about 10 cm^2) of each rat was clipped free of hair and washed with acetone. A silicone ring was glued to the skin and the test substance preparation (about 10 µl/cm^2) was applied within the ring from a syringe that was weighed before and after application. A nylon mesh was then glued to the surface of the silicone ring and a porous bandage used to encircle the trunk of the rat. The rats (four per group) were dosed and then placed in metabolism cages in order to collect excreta for up to 72 h.

After the required exposure period, the protective cover was removed and the exposed skin was washed with a mild soap solution. At the end of the various collection periods, rats were killed and the following specimens/tissues were assayed for remaining radioactivity: excreta, blood cells, plasma, liver, kidneys, carcass, treated skin (application site) and non-treated areas (surrounding skin). For balance estimates, the cage wash, skin wash and the protective cover (including the silicone ring) were also assayed for radioactivity. The results are given in Table 5.

The radioactivity absorbed was excreted mainly in the faeces. Due to the very low skin penetration, concentrations of radioactivity in organs and tissues analysed were also very low. Low amounts of the radioactivity (0.15–3.76%) remained in the skin at the application site at the end of exposure.

Table 5 Radioactivity absorbed (percentage of administered dose and total amount) by rats given [^{14}C]boscalid as a single dermal application

Exposure time (h)	Sacrifice time (h)	Dose (mg/cm^2)					
		1.00		0.1		0.01	
		% absorbed	mg/animal	% absorbed	mg/animal	% absorbed	mg/animal
1	1	0.17	0.0184	0.37	0.0046	0.52	0.0005
4	4	0.33	0.0250	0.25	0.0032	2.02	0.0025

10	10	0.42	0.0462	0.63	0.0080	8.07	0.0102
10	24[a]	—	—	—	—	6.26	0.0070
10	72	1.48	0.1364	2.07	0.0262	5.72	0.0065
24	24	0.41	0.0391	2.63	0.0343	10.93	0.0143

From Leibold & Hoffmann (2001)
[a] Only at the lowest dose.

The dosing regime most similar to a worst-case situation during work in which the operator did not wash his hands, was an exposure of 10 h. The proportions of applied radioactivity that were absorbed through the skin during this period of exposure at a dose of 0.01, 0.1 and 1.0 mg/cm^2, respectively, were 8.07%, 0.63% and 0.42%. A group of rats receiving the lowest dose was killed 24 h after the start of the 10 h exposure. In this group, the proportion absorbed was 6.26%. Thus, an average (worst-case) dermal penetration of 8% can be assumed (Leibold & Hoffmann, 2001).

The penetration of [diphenyl-ring-U-^{14}C]boscalid (batch No. 641-2017; radiochemical purity, > 98% and non-radiolabelled boscalid (batch No. 01183-190; purity, 99.3%) through isolated rat and human epidermal preparations in vitro was determined after 24 h exposure to boscalid at a concentration of 10, 100 or 1000 µg/cm^2. On the day before application of the dose, the integrity of the epidermal membranes and their suitability for use in the study was assessed by measuring the penetration of tritiated water applied to the membranes and comparison of the results with exclusion criteria. After this membrane integrity check and before dose application, the receptor chamber was refilled with ethanol : water (1 : 1 v/v). The receptor fluid was chosen on the basis that boscalid is soluble in ethanol.

The dose formulation was applied to the upper surface of the epidermal membranes using a positive displacement pipette. The amount of solution applied to each membrane was calculated by weight difference of the positive-displacement pipette before and after dose administration. Duplicate 0.1 ml samples of receptor fluid were taken immediately before dosing and at 0.5, 1, 2, 4, 6, 10 and 24 h after addition of the formulation. An equal volume of fresh receptor fluid was added to the receptor chamber after each sampling occasion, excluding the final sample time, in order to maintain a constant volume of receptor fluid in the receptor chamber of the diffusion cell.

Twenty-four hours after the last receptor fluid sample was taken, the receptor fluid was removed from the receptor chambers of all cells and retained. Any residual formulation was washed from the surface of the skin with a soap solution without organic solvent and rinsed with deionized water. The washings were retained for analysis. The membrane preparations were removed from the cells and homogenized. All washings from the dismantled cell were retained. Radioactivity was measured in the receptor fluid, skin section, skin washings and apparatus washings. The percentage of the applied dose in each of the samples and the rate of penetration through the epidermal membranes (µg equivalents/cm^2 per h) were calculated. The stability, homogeneity and correctness of the test substance preparation were analytically verified.

Recoveries at 24 h were 99%, 96% and 106% for the rat epidermal membranes and 98%, 96% and 98% for the human epidermal membranes exposed to boscalid at 1, 10 and 100 mg/ml, respectively. The permeability coefficients (the ratio of the rate of penetration in µg equivalents/cm^2 per h to the difference in test compound concentration across the membrane × 10^{-5} cm/h) were 51.6,

Table 6. Transmembrane migration of boscalid in rat and human epidermis in vitro

Time (h)	Mean cumulative absorption of [^{14}C]boscalid (μg/cm^2 skin)					
	Administered dose (μg/cm^2)					
	10		100		1000	
	Rat	Human	Rat	Human	Rat	Human
0.5	0.264	0.002	0.302	0.009	NA	NA
1	0.462	0.014	0.657	0.032	7.703	1.404
2	0.691	0.029	1.178	0.108	11.47	1.929
4	0.977	0.046	1.667	0.157	13.03	2.026
6	1.143	0.061	1.890	0.204	15.63	2.143
10	1.366	0.103	2.593	0.304	20.64	4.295
24	1.906	0.264	3.650	1.320	27.87	5.283
Lag time (h)	0.055	0.185	0.110	0.227	0.219	0.115
Mean rate of penetration (μg/cm^2 per h)	0.302	0.015	0.685	0.050	3.813	0.498
Permeability coefficient (× 10^{-5} cm/h)	51.6	2.572	7.188	0.53	6.571	0.858

From Thornley & Bryson (2001)
NA, not applicable.

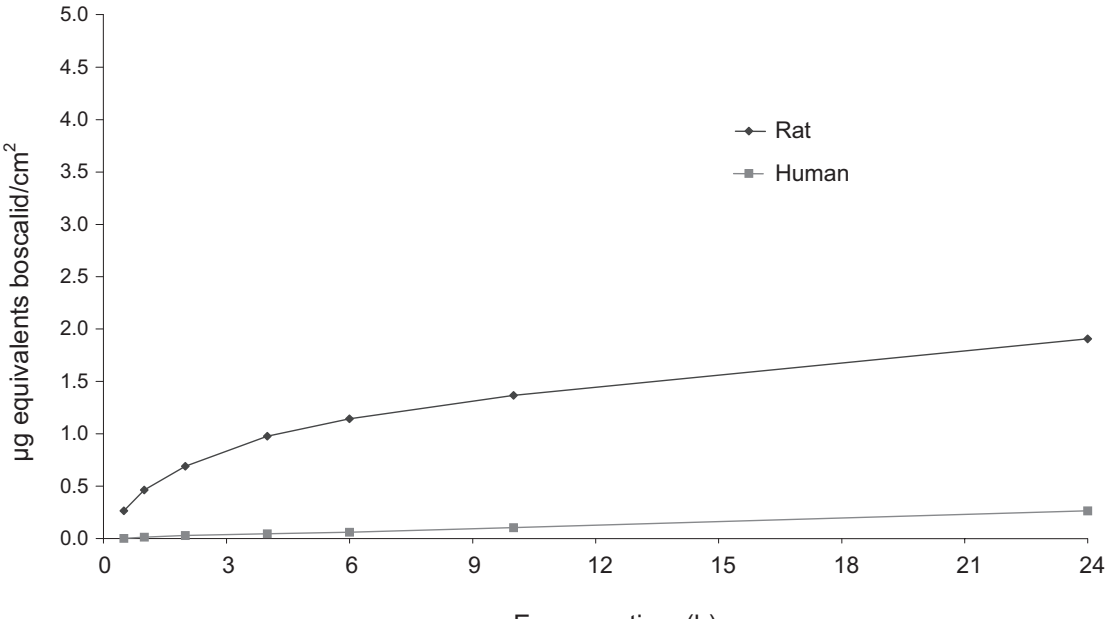
7.2 and 6.6 for rat epidermal membranes and 2.6, 0.53 and 0.86 for human epidermal membranes exposed at 10, 100 and 1000 μg/cm^2, respectively (Table 6).

Transfer of radioactivity across rat epidermal membranes was rapid during the early sampling times of the experiment, with a lag time at all doses of less than 14 min. Thereafter, the amount of radioactivity transferred decreased with time, the transfer rate between 10 h and 24 h being less than 14% of the initial rate. At the end of the study, the radioactivity transferred was 33%, 4% and 3% of the 10, 100 and 1000 μg/cm^2 doses, respectively. At the lowest, intermediate and highest doses, respectively, 31%, 73% and 96% of the applied radioactivity was recovered in the skin washings and was therefore unabsorbed. For a 10- and a 100-times increase in the concentration of the formulation applied to rat membranes, a corresponding 2.18- and 12.63-times increase in the initial rate of absorption was apparent.

Radioactivity was absorbed through human epidermal membranes within 14 min of application, regardless of the concentration of formulation. As with the rat, there was evidence to suggest an initial high rate of absorption within the first 4 h for some human epidermal membranes. The rate of absorption increased 3.3-fold and 33.2-fold for a 10- and 100-times increase in the concentration of boscalid.

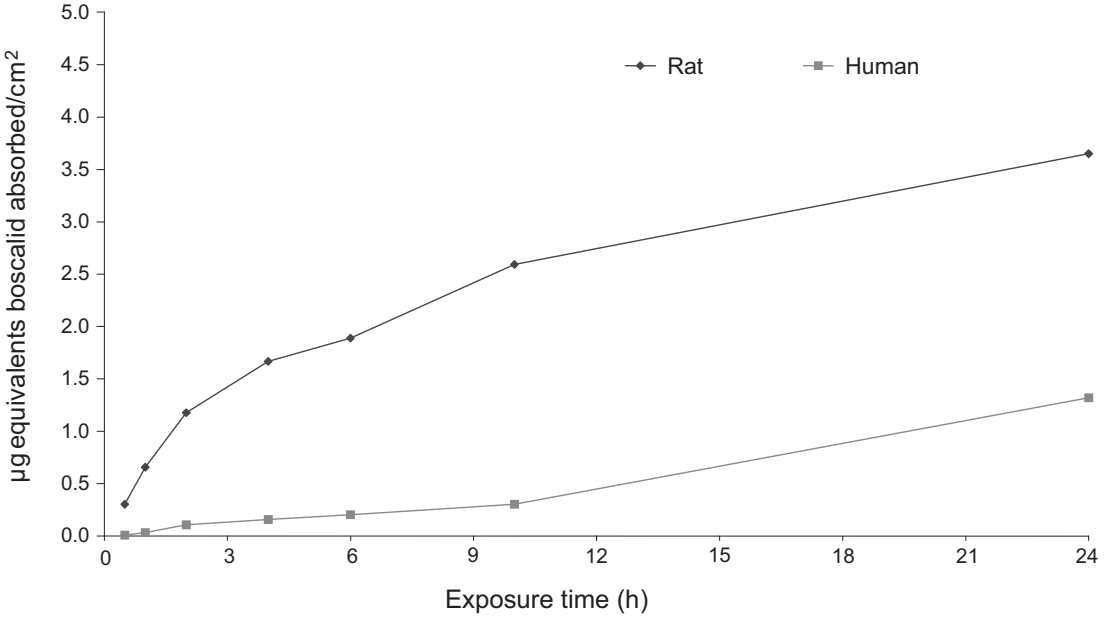
The time-courses of transfer across rat and human epidermal membranes at each concentration of boscalid are shown in Figures 2–4.

Figure 2. Transfer of radioactivity across rat and human epidermal membranes after a single application of [^{14}C]boscalid at a nominal dose of 10 µg/cm^2 (1 mg/ml)



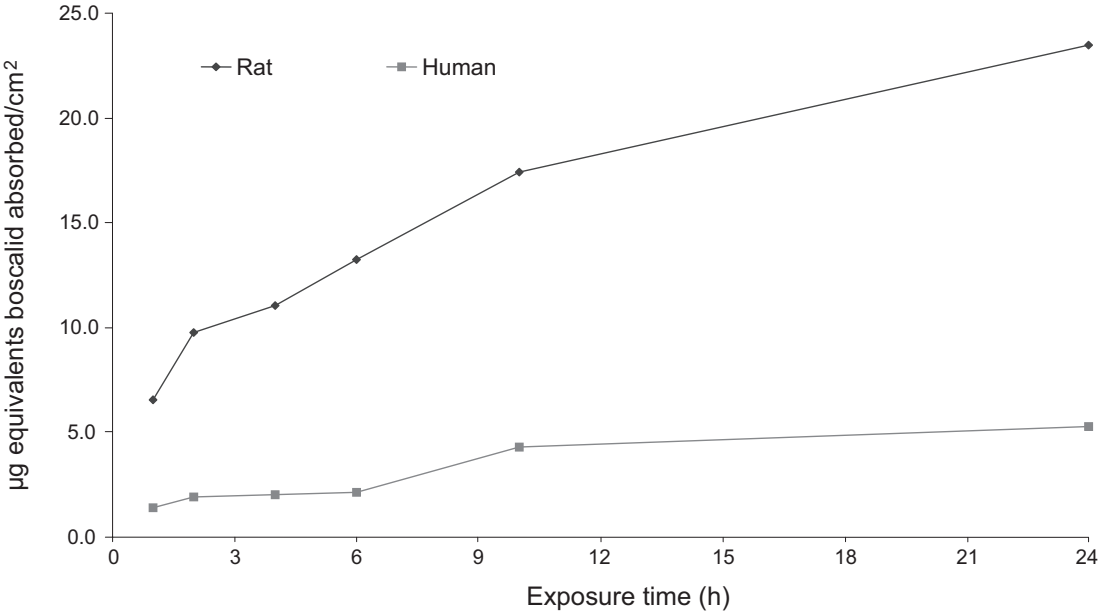
From Thornley & Bryson (2001)

Figure 3. Transfer of radioactivity through rat and human epidermal membranes after a single application of [^{14}C]boscalid at a nominal dose of 100 µg/cm^2 (10 mg/ml)



From Thornley & Bryson (2001)

Figure 4.Transfer of radioactivity through rat and human epidermal membranes after a singlapplication of [^{14}C]boscalid at a nominal dose of 1000 µg/cm^2 (100 mg/ml)



From Thornley & Bryson (2001)

The differences in the skin penetration between human and rat skin at the different doses (based on the rates of penetration) are shown in Table 7. Based on comparison of penetration rates or permeability coefficients, it appears that penetration rate through rat epidermis is at least a 7.7-fold that through human epidermis. The recovery of radiolabel was determined at the end of the experiment and summarized in Table 8 (Thornley & Bryson, 2001).

Table 7. Difference in penetration of boscalid through rat and human skin

Species	Mean rate of penetration (µg/cm^2 per h)		
	Dose administered (µg/cm^2)		
	10	100	1000
Rat epidermis	0.302	0.685	3.813
Human epidermis	0.015	0.050	0.498
Fold difference	20.1	13.7	7.7

From Thornley & Bryson (2001)

Table 8. Study of dermal penetration in vitro: recovery of radioactivity through rat and human skin

Parameter	Recovery of ^{14}C radiolabel (% of applied dose) at 24 h								
	Dose applied								
	0.01 mg/cm² (1 mg/ml)			0.1 mg/cm² (10 mg/ml)			1.00 mg/cm² (100 mg/ml)		
	Rat	Human	Ratio	Rat	Human	Ratio	Rat	Human	Ratio
Receptor fluid	32.54	4.545	7.2	3.954	1.416	2.8	3.482	0.641	5.4
Receptor cell wash	1.809	0.485	3.7	0.368	2.596	0.1	1.150	0.509	2.3
Total penetrated	*34.35*	*5.03*	*6.8*	*4.322*	*4.012*	*1.1*	*4.632*	*1.15*	*4.0*

Skin	33.07	61.59	0.5	17.94	17.29	1.0	4.366	1.086	4.0
Total penetrated + skin	*67.42*	*66.62*	*1.0*	*22.26*	*21.3*	*1.0*	*8.998*	*2.236*	*4.0*
Surface washings	31.28	31.16	1.0	73.06	73.45	1.0	96.33	95.30	1.0
Donor cell wash	0.635	0.309	2.1	1.014	1.806	0.6	0.376	0.299	1.3
Total	*99.34*	*98.09*	*1.0*	*96.33*	*96.39*	*1.0*	*105.70*	*97.84*	*1.1*

From Thornley & Bryson (2001)

The total amount of radiolabel that fully penetrated the skin membrane ("absorbed radiolabel") was recovered in the receptor fluid and in the wash of the receptor cell. At the lowest and highest concentrations, the amount of absorbed radiolabel was approximately sevenfold and fourfold higher in specimens from rats than from humans, while at the intermediate concentration, the absorption ratio was approximately 1. When the amount of radioactivity remaining associated with the skin was also taken into account, no differences in the "potentially absorbable" percentages of administered radiolabel were observed between species in the groups at the lowest and intermediate doses, while a fourfold higher recovery was established in the group at the highest dose.

Therefore, it can be concluded that absorption through rat epidermal membranes in vitro was the same as through human epidermal membranes at the lowest and intermediate doses (0.01 and 0.1 mg/cm²) and approximately fourfold that at the highest concentration (1.00 mg/cm²). The results obtained for the lowest and intermediate concentrations were considered to be the most relevant, because these concentrations fall in the range of the expected operator exposure. Thus, based on the results of the study of dermal penetration in vitro, is the Meeting concluded that there is no notable difference between rats and humans in the extent of bioavailability after dermal exposure to boscalid.

Metabolism

Studies of metabolic transformation (Grosshans & Knoell, 2001) were conducted in rats given [diphenyl-ring-U-^{14}C]boscalid (batch No. 641-2018; radiochemical purity, > 99%, specific activity, 5.23 MBq/mg) or [pyridine-3-^{14}C]boscalid (batch No. 640-1026; radiochemical purity, > 97%, specific activity, 5.81 MBq/mg) or non-radiolabelled boscalid (batch No. 01174-236; purity, > 99.4%). Male and female Wistar rats were orally dosed with [^{14}C]boscalid at a nominal dose of 50 and 500 mg/kg bw.

Patterns of radioactive metabolites in excreta (urine, faeces, bile), plasma, and tissues (liver and kidney) were analysed by high-performance liquid chromatography (HPLC). Metabolites were identified by mass spectroscopy (LC-MS and LC-MS/MS) and, in some cases, nuclear magnetic resonance analysis of isolated fractions. Metabolite patterns of samples generated in this study were compared with those obtained in kinetic studies in which rats were given single oral high (500 mg/kg) and low (50 mg/kg) doses and repeated high doses (14 × non-radiolabelled, 1 × radiolabelled; 500 mg/kg). The structures of the identified metabolites recovered from rat excreta, plasma and tissues are given in Table 19.

After administration of an oral dose of [^{14}C]boscalid to male and female rats, a large number of metabolites were detected in urine. Predominant metabolites were a 4-hydroxyl diphenyl ring metabolite (named M510F01 in the tables) and its glucuronic acid conjugate (named M510F02 in the tables). The proportion of the 4-hydroxy-diphenyl metabolite excreted via urine ranged from 0.5% to 3% in the group at 500 mg/kg bw and from 10% to 16% in the group at 50 mg/kg bw, while the proportion of its glucuronyl conjugate was in the range of 0.1–4% of the dose. Two more

metabolites found in the urine at up to 2% of the administered dose were formed by conjugation of glucuronide (M510F48) and cysteine (M510F05) at the expense of chlorine in the pyridine moiety. Minor metabolites identified in urine are identified in Figure 5 and Table 19 as M510F03, M510F04, M510F12, M510F20, and M510F42. Traces of parent substance could be detected. Metabolites found in the group receiving pyridine-labelled boscalid included traces of 2-chloronicotinic acid (M510F47), while 4-chloro-2-aminobiphenyl was not detected in the group receiving diphenyl-labelled boscalid. Individual metabolite recoveries from urine and faeces after dosing with diphenyl-labelled boscalid and pyridyl-labelled boscalid are described in Tables 9–14.

In all dose groups and independent of sex and label, the parent substance boscalid was the major component and represented from 57% to 85% of the administered dose in the groups at the highest dose and from 30% to 41% in the groups at the lowest dose. The 4'-hydroxyl diphenyl metabolite (M510F01) and the 2-sulfydryl metabolite (M510F06) derived from the cysteine conjugate of the 2-chloro-pyridine moiety were identified to be predominant metabolites in all dose groups. The metabolites M510F20 and M510F63 were mainly found in the groups at the lowest dose. As minor metabolites, M510F05, M510F11 and M510F48 were also found in the faeces.

Major metabolites in bile were the glucuronyl conjugate of the 4'-hydroxyl diphenyl metabolite (M510F02) and M510F05 in which cysteine is conjugated with the 2-chloro-pyridine moiety. As minor components, the 4'-hydroxyl diphenyl metabolite (M510F01), and the metabolites M510F57 and M510F58 (in which there has been introduction of a hydroxyl group and cysteine into the diphenyl moiety) were identified. In addition, traces of M510F50 (hydroxyl group on the pyridine moiety) were found. The percentages of these metabolites found in bile are given in Table 15.

Table 9. Summary of metabolites identified in urine and faeces of rats given [diphenyl U-^{14}C]boscalid at a dose of 500 mg/kg bw

Metabolite	Total excretion (% of administered dose)			
	Urine (0–48 h)		Faeces (0–48 h)	
	Male	Female	Male	Female
M510F02	1.73	1.60	—	—
M510F42	0.10	0.03	—	—
M510F03	—	0.08	—	—
M510F48	0.37	0.39	1.26	1.05
M510F04	—	0.13	—	—
M510F05	0.34	0.34	0.64	0.70
M510F01	0.57	2.23	4.32	9.06
M510F20	0.02	0.02	0.80	0.57
Boscalid	—	—	80.46	64.96
M510F06	—	—	6.10	10.29

From Grosshans & Knoell (2001)

Table 10. Summary of metabolites identified in urine and faeces of rats given [pyridine 3-^{14}C]boscalid at a dose of 500 mg/kg bw

Metabolite	Total excretion (% of administered dose)			
	Urine (0–48 h)		Faeces (0–48 h)	
	Male	Female	Male	Female
M510F47	0.07	0.06	—	—
M510F02	2.74	2.14	—	—
M510F42	0.13	0.04	—	—
M510F03	—	0.13	—	—
M510F48	0.44	0.33	6.50	0.38
M510F04	—	0.10	—	—
M510F05	0.40	0.37	4.87	1.52
M510F01	0.51	1.02	8.18	10.54
M510F20	—	0.01	1.05	0.73
Boscalid	—	—	75.63	56.96
M510F06	—	—	1.04	12.24

From Grosshans & Knoell (2001)

Table 11. Summary of metabolites identified in urine and faeces of rats given [diphenyl U-^{14}C]boscalid at a dose of 50 mg/kg bw

Metabolite	Total excretion (% of administered dose)			
	Urine (0–48 h)		Faeces (0–48 h)	
	Male	Female	Male	Female
M510F02	2.95	4.33	—	—
M510F42	0.18	0.25	—	—
M510F48	1.10	2.28	—	2.84
M510F04	0.08	0.22	—	—
M510F05	0.48	0.59	—	1.85
M510F01	9.58	15.79	21.81	18.99
M510F11	—	—	2.31	0.53
M510F20	0.57	0.46	6.21	3.79
Boscalid	—	0.06	41.00	30.45
M510F06	—	—	4.88	7.57
M510F63	—	—	0.60	4.01

From Grosshans & Knoell (2001)

Table 12. Summary of metabolites identified in urine and faeces of rats given boscalid as 15 daily oral doses at 500 mg/kg bw (14 doses non-radiolabelled, last dose with radiolabelled [diphenyl U-^{14}C]boscalid)

Metabolite	Total excretion (% of administered dose)			
	Urine (0–48 h)		Faeces (0–48 h)	
	Male	Female	Male	Female
M510F02	0.22	1.03	—	—
M510F03	—	0.07	—	—
M510F42	0.26	—	—	—
M510F48	0.02	0.26	—	—
M510F04	—	0.02	—	—
M510F05	0.04	0.08	—	—
M510F01	1.34	1.94	2.46	12.60
M510F11	—	—	0.14	—
M510F12	0.06	—	—	—
M510F20	0.14	0.04	—	0.51
Boscalid	0.11	0.05	85.15	75.82
M510F06	—	—	2.60	1.41
M510F63	—	—	0.14	—

From Grosshans & Knoell (2001)

Table 13. Summary of identified metabolites in urine and faeces of rats given [diphenyl U-^{14}C]boscalid at a dose of 500 mg/kg bw[a]

Metabolite	Total excretion (% of administered dose)			
	Urine (0–48 h)		Faeces (0–48 h)	
	Male	Female	Male	Female
M510F02	0.69	2.41	—	—
M510F42	0.22	0.08	—	0.20
M510F03	—	—	—	—
M510F48	0.03	0.47	0.42	0.63
M510F04	—	0.04	—	—
M510F05	0.09	0.07	—	—
M510F01	1.04	1.52	4.10	5.50
M510F11	—	—	1.33	0.58
M510F20	0.05	0.10	—	—
Boscalid	0.16	0.04	80.37	68.26
M510F06	—	—	7.00	3.00
M510F63	—	—	0.32	1.35

From Grosshans & Knoell (2001)

[a] Dose groups from the kinetic study (see Table 1)

Table 14. Summary of identified metabolites in urine and faeces of rats given [pyridine 3-^{14}C]boscalid at a dose of 500 mg/kg bw[a]

Metabolite	Total excretion (% of administered dose)			
	Urine (0–48 h)		Faeces (0–48 h)	
	Male	Female	Male	Female
M510F47	0.10	0.07	—	—
M510F02	0.08	1.64	—	—
M510F42	0.48	0.05	—	—
M510F48	0.04	0.26	—	—
M510F04	0.01	0.01	—	—
M510F05	0.17	0.03	—	—
M510F01	2.93	0.94	4.84	4.35
M510F12	0.34	0.04	—	—
M510F20	0.26	0.06	—	—
Boscalid	0.07	0.02	72.91	70.16
M510F06	—	—	7.59	3.81
M510F63	—	—	0.46	0.25

From Grosshans & Knoell (2001)

[a] Dose groups from the kinetic study (see Table 1)

Table 15. Summary of metabolites identified in bile of female rats given [diphenyl U-^{14}C]boscalid as a single oral dose at 50 mg/kg bw or 500 mg/kg bw

Metabolite	Total excretion (% of administered dose)	
	Dose (mg/kg bw)	
	50	500
M510F02	19.27	4.78
M510F57	1.32	0.41
M510F58	0.27	0.09
M510F03	1.48	0.21
M510F22/F23	—	0.10
M510F05	14.24	3.59
M510F01	1.71	0.28

From Grosshans & Knoell (2001)

All metabolites were present in the liver at less than 0.3% of the administered dose. The metabolites M510F01, M510F02, M510F05, and M510F06 already identified in urine and faeces were also found in the liver. In addition, the metabolite M510F46 (introduction of a hydroxyl group and a glutathione group in the diphenyl ring moiety), the metabolite M510F45 (introduction of a glutathione group in the diphenyl ring moiety), and the metabolite M510F43 (exchange of chlorine for glutathione in the pyridine moiety) were identified (Table 16).

Table 16. Summary of metabolites identified in the liver of rats given [pyridine 3-^{14}C]boscalid as a single oral dose at 50 mg/kg bw or 500 mg/kg bw

Metabolite	Concentration of metabolites (µg equivalents per g)			
	Dose (mg/kg bw)			
	50		500	
	Male	Female	Male	Female
M510F47	—	—	0.21	—
M510F46	1.63	1.11	2.63	4.22
M510F02	1.88	3.08	19.43	16.42
M510F42	0.17	—	—	—
M510F45	0.66	0.41	4.04	8.25
M510F43	0.92	2.16	13.54	12.34
M510F05	0.13	0.29	2.08	1.13
M510F01	0.84	0.73	2.19	2.50
Boscalid	0.12	0.21	1.01	1.10
M510F06	0.33	0.43	2.92	3.66

From Grosshans & Knoell (2001)

The kidney revealed the metabolites M510F01, M510F02, M510F03, M510F05, M510F06, M510F48 and parent already identified in excreta. The proportion of these metabolites was in the range of <0.01% to 0.06% of the administered dose (Table 17).

Table 17. Summary of metabolites identified in kidney of rats given [pyridine 3-^{14}C]boscalid as a single oral dose at 50 mg/kg bw or 500 mg/kg bw

Metabolite	Concentration of metabolites (µg equivalents per g)			
	Dose (mg/kg bw)			
	50		500	
	Male	Female	Male	Female
M510F02	1.72	1.18	5.60	5.95
M510F42	0.20	—	—	—
M510F03	0.17	—	2.83	—
M510F48	0.30	0.80	3.00	3.23
M510F05	0.27	3.04	2.61	28.85
M510F01	0.48	0.58	2.37	3.98
Boscalid	0.68	1.69	6.92	10.48
M510F06	0.10	0.14	2.68	1.62

From Grosshans & Knoell (2001)

In plasma, M510F01, M510F02, M510F06, M510F48 and parent were detected at or less than 0.01% of the dose (Table 18).

Table 18. Summary of metabolites identified in blood plasma of rats given [pyridine 3-^{14}C]boscalid as a single oral dose at 50 mg/kg bw or 500 mg/kg bw

Metabolite	Concentration of metabolites (µg equivalents per g)			
	Dose (mg/kg bw)			
	50		500	
	Male	Female	Male	Female
M510F02	0.39	0.54	1.62	2.86
M510F48	0.12	0.07	0.91	0.52
M510F01	0.07	0.09	0.22	0.26
BAS 510F	0.26	0.29	1.74	1.85
M510F06	0.10	0.19	1.38	1.86

From Grosshans & Knoell (2001)

Metabolic pathway

After oral administration of [^{14}C]boscalid, the unchanged parent compound was predominantly found in faeces and in trace amounts in the urine, liver, and in plasma. Overall, comparison of the sexes, the different labels, and the different doses showed no remarkable differences in the metabolite patterns. The absorbed boscalid was intensively metabolized following two principal routes and a secondary route.

One principal reaction was oxidation at the 4-position of the phenyl ring, followed mainly by conjugation with glucuronic acid and to a smaller extent by sulfate. The other principal reaction was substitution of the chlorine of the 2-chloropyridine moiety by conjugation with glutathione. The glutathione moiety was then cleaved to the cysteine conjugate followed by further cleavage to the SH-compound and subsequent *S*-methylation, *S*-glucuronidation or oxidation to a sulfate.

The discovery of 2-chloronicotinic acid in urine (using the pyridine label) indicates hydrolysis of the amide bond between the pyridine ring system and the diphenyl ring system. However, the other cleavage product, chloro-aminobiphenyl, could not be detected using the diphenyl label and cleavage of the parent compound at the amide bond appeared to be negligible.

To a smaller extent, the introduction of glutathione could also occur in the diphenyl ring system followed by cleavage steps down to the SH-compound and subsequent *S*-methylation. Combinations of these reactions led to the large number of observed metabolites. The proposed metabolic pathway is shown in Figure 5. The structures of the identified metabolites are shown in Table 19 (Grosshans & Knoell, 2001).

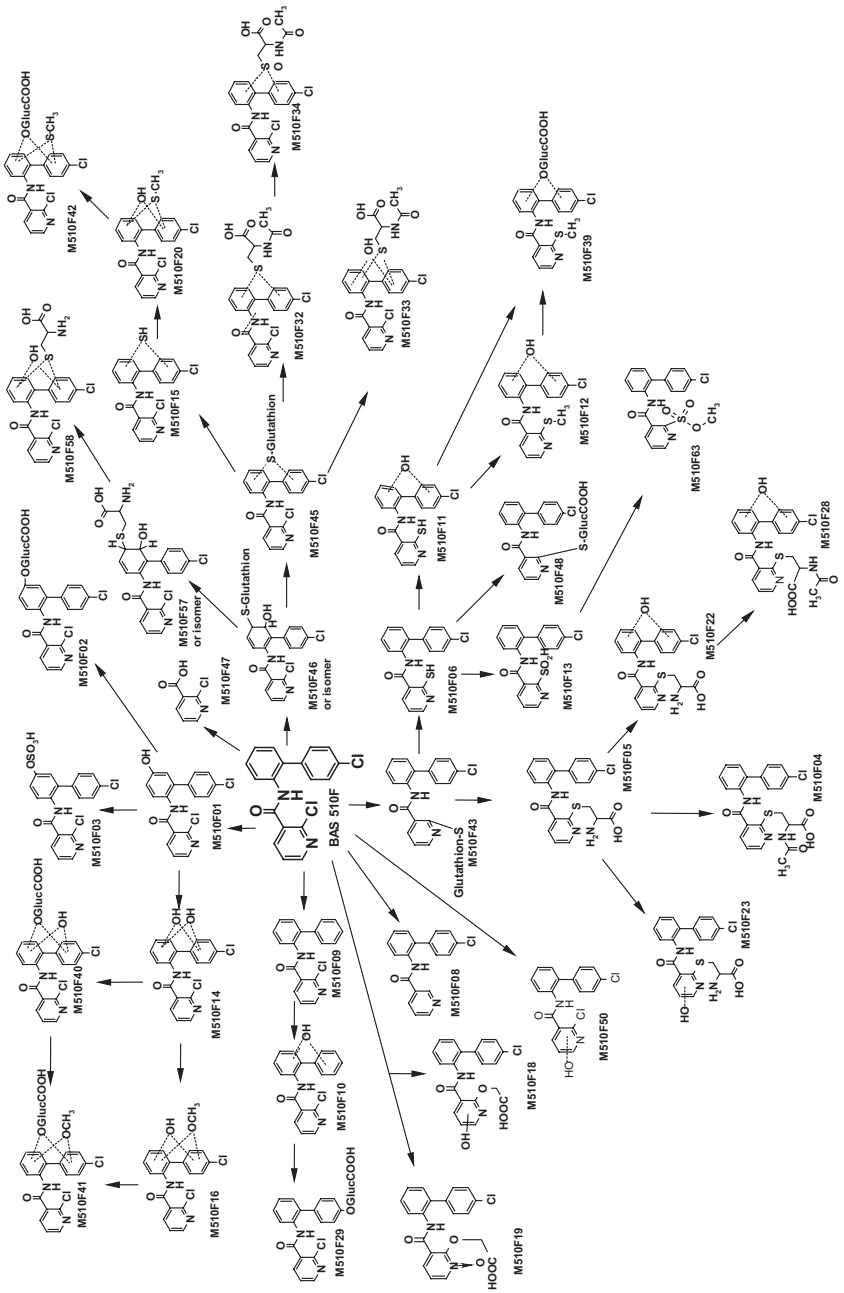
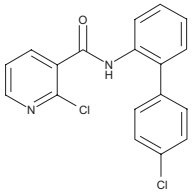
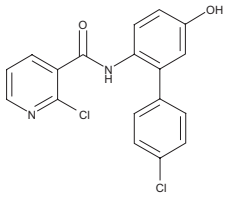
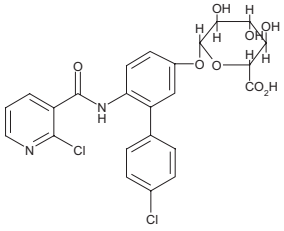
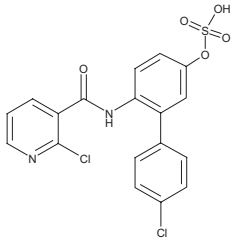
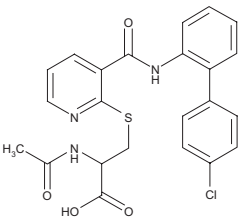
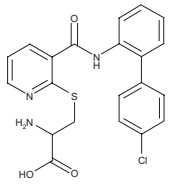
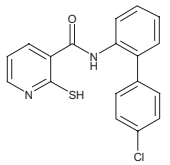
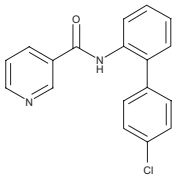
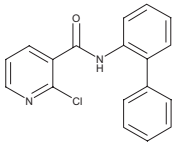
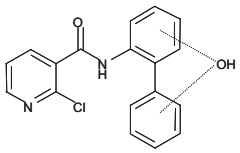
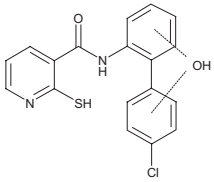
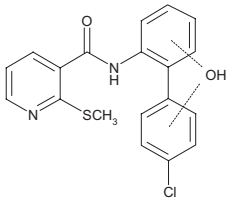
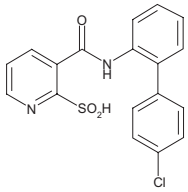
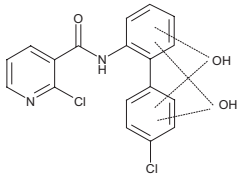
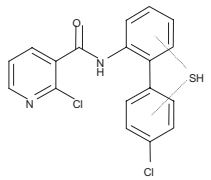
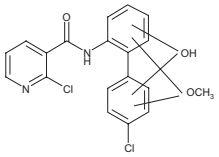
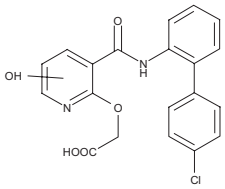
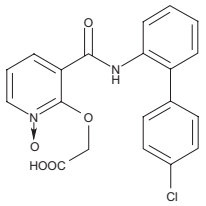
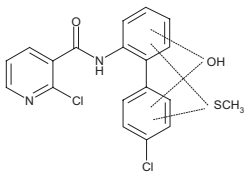
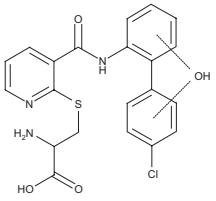


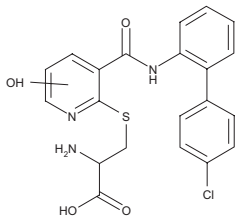
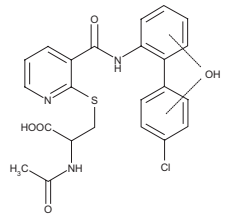
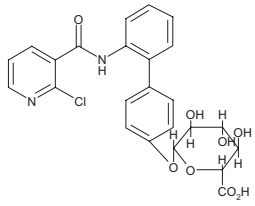
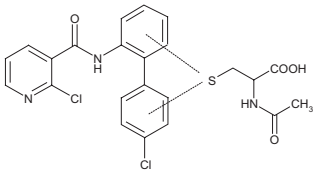
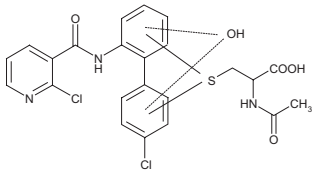
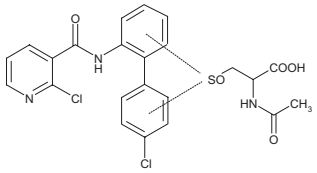
Figure 5. Metabolic pathway of boscalid in rats

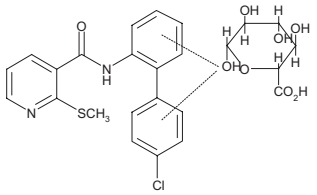
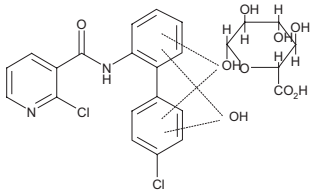
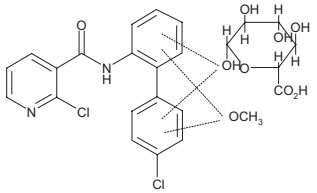
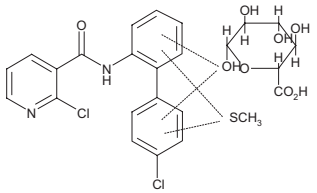
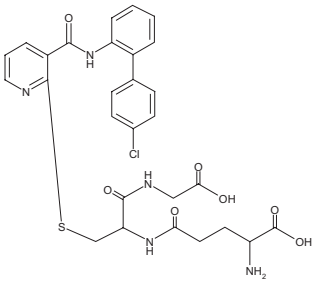
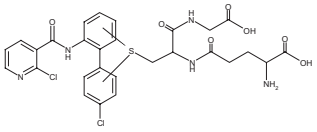
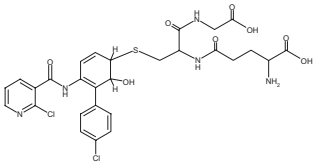
Table 19. Structures of identified metabolites in excreta, plasma and tissues of rats

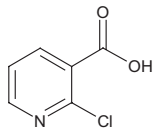
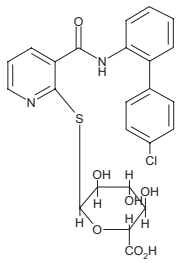
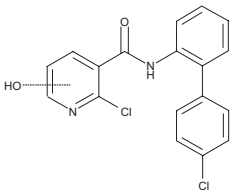
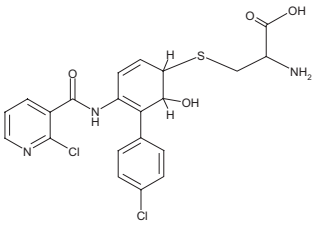
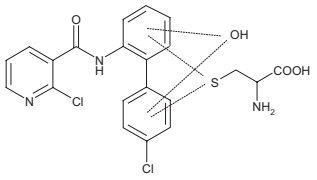
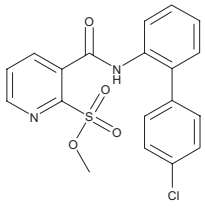
Metabolite	Structure
BAS 510F	
M510F01	
M510F02	
M510F03	
M510F04	
M510F05	

Metabolite	Structure
M510F06	
M510F08	
M519F09	
M510F10	
M510F11	
M510F12	
M510F13	

Metabolite	Structure
M510F14	
M510F15	
M510F16	
M510F18	
M510F19	
M510F20	
M510F22	

Metabolite	Structure
M510F23	
M510F28	
M510F29	
M510F32	
M510F33	
M510F34	

Metabolite	Structure
M510F39	
M510F40	
M510F41	
M510F42	
M510F43	
M510F45	
M510F46 or isomer	

Metabolite	Structure
M510F47	
M510F48	
M510F50	
M510F57 or isomer	
M510F58	
M510F63	

From Grosshans & Knoell (2001)

2. Toxicological studies

2.1 Acute toxicity

The acute oral toxicity of boscalid (batch No. N 26, purity 95.3% was evaluated in groups of five male and five female fasted Wistar rats given boscalid as a single dose at 2000 or 5000 mg/kg bw by gavage in 0.5% aqueous Tylose CB 30.000, using dose volumes of 10 and 20 ml/kg bw respectively. The rats were then observed for up to 14 days. The stability of boscalid and the homogeneity and concentrations of the dosing solutions were confirmed by analysis.

There was no mortality in either males or females. Signs of toxicity noted at 5000 mg/kg bw included impaired general condition, dyspnoea, excitation, erythema and piloerection in males and females. These signs were observed on day 1 in two males and in one female. All rats appeared normal within 2 days after dosing. Body-weight development was normal. There were no macroscopic pathological findings in rats killed at the end of the observation period (Wiemann & Hellwig, 1998c; Wiemann, 2000e). The oral LD_{50} was > 5000 mg/kg bw in male and female rats.

The acute percutaneous (dermal) toxicity of boscalid (batch No. N 26, purity, 95.3%) was investigated in five male and five female Wistar rats given the test material at a dose of 2000 mg/kg bw as a 0.5% aqueous Tylose CB 30.000 preparation under a semi-occlusive dressing for 24 h. The application area was about 50 cm^2. The stability of boscalid, its homogeneity and concentration in the vehicle was confirmed by analysis.

No mortality occurred during the 14 days of observation after application.

No clinical signs of toxicity were observed and body-weight development was normal. One day after application, a well-defined erythema was observed in a single female on the first day after application. Body-weight development appeared to be normal. There were no macroscopic pathological findings in rats killed at the end of the observation period (Wiemann & Hellwig, 1998b; Wiemann, 2000a). The dermal LD_{50} for boscalid in male and female rats was > 2000 mg/kg bw.

A test for the acute toxicity of boscalid (batch No. N 26, purity 95.3%) was conducted in a group of five male and five female Wistar rats exposed by inhalation via a head/nose inhalation system for 4 h to boscalid at a mean analysed atmospheric concentration of 6700 µg/l. The observation time was 14 days. Boscalid was demonstrated to be stable and homogeneously distributed in the exposure atmospheres. Particle size analysis of the boscalid sample used revealed a mass median aerodynamic diameter (MMAD) of 3.4 µm, which is within the respirable range. No mortalities occurred in the test group. Clinical observations included attempted escape behaviour, irregular respiration and respiratory sounds, as well as urine-smeared fur, piloerection and squatting posture. No unusual clinical observations were made from day 3 after exposure onward. Body-weight development was not adversely affected by the test substance exposure. No macroscopic pathological findings were noted in exposed animals at the end of the study. The inhalation LC_{50} of boscalid in male and female rats was > 6700 mg/m^3 (Gamer & Hoffmann, 1998).

Boscalid (batch No. N 26, purity, 95.3%) was evaluated for acute dermal irritation potential in two male and four female New Zealand White rabbits. Boscalid (0.5 g) was applied to the intact skin for 4 h on a 2.5 cm × 2.5 cm test patch under a semi-occlusive dressing. After the patches were removed, the treated area was rinsed with Lutrol and Lutrol/water (1:1). The skin irritation was scored at 1, 24, 48 and 72 h after removal of the test material. The stability of the test substance over the study period was confirmed. Results from the individual animals are shown in Table 20.

Table 20. Skin irritation scores (erythema/oedema) in rabbits exposed to boscalid

Rabbit No.	Time after patch removal (h)				Mean
	1	24	48	72	
1	1/0	0/0	0/0	0/0	0/0
2	1/0	0/0	0/0	0/0	0/0
3	1/0	1/0	0/0	0/0	0.3/0
4	2/0	1/0	0/0	0/0	0.3/0
5	1/0	1/0	0/0	0/0	0.3/0
6	0/0	0/0	0/0	0/0	0/0

From Wiemann & Hellwig (1998d) and Wiemann (2000b)

The average score (24–72 h) for dermal irritation was 0.2 for erythema and 0.0 for oedema. Skin findings were reversible within 48 h in all rabbits (Wiemann, & Hellwig, 1998d; Wiemann, 2000b). The Meeting concluded that boscalid was not irritant to the skin of rabbits.

Boscalid (batch No. N 26, purity, 95.3%) was evaluated for acute eye irritation potential in two male and four female New Zealand White rabbits. Approximately 0.1 ml of undiluted boscalid was applied to the conjunctival sac of an eye of each rabbit. The test substance was washed from the eyes after 24 h. Examination of the eyes was carried out 1, 24, 48 and 72 h after the application of the test substance. The homogeneity of the test substance and its stability over the study period were confirmed. Eye irritation scores for the individual rabbits are shown in Table 21.

Table 21. Eye irritation in rabbits exposed to boscalid

Rabbit No.	Corneal opacity	Iris	Conjunctival redness	Conjunctival swelling
1	0	0	0.3	0
2	0	0	0.7	0
3	0	0	0.3	0
4	0	0	0.3	0
5	0	0	0.3	0
6	0	0	0.3	0

From Wiemann & Hellwig (1998e) and Wiemann (2000c)

The mean eye irritation scores were 0.0 for corneal and iridial effects, 0.4 for conjunctival redness and 0.0 for swelling. All effects were reversible within 72 h after application (Wiemann & Hellwig, 1998e; Wiemann, 2000c). The Meeting concluded that boscalid was not irritant to the eyes of rabbits.

The potential of boscalid (batch No. N 26, purity, 95.3%) to produce delayed contact hypersensitivity in Pirbright White (Dunkin-Hartley) guinea-pigs was tested using the Magnusson-Kligman maximization test. In a preliminary test, it was established that a concentration of 5% boscalid in 1% Tylose CB 30.000 was non-irritant, while 10% boscalid in the same vehicle was irritant. The stability of boscalid over the study period and in the vehicle was confirmed by analysis. Homogeneity of the preparation was ensured by stirring.

Twenty guinea-pigs were used in the test group and 10 in the control group. The first phase of induction was conducted by intracutaneous injections (two of each preparation per guinea-pig) of 5% boscalid in 1% Tylose CB 30.000, the same preparation with Freund adjuvant or Freund adjuvant with

1% Tylose CB 30.000. The injection sites were examined after 24 h. One week after the intradermal induction, the second phase of induction was conducted, consisting of two percutaneous applications separated by an interval of 24 h of 2 × 2 cm filter paper squares containing 25% boscalid under occlusive dressings. The challenge was performed 2 weeks after the dermal induction, by applying 5% boscalid on filter paper under occlusive dressing for 24 h. Skin reactions were scored at 24 h and 48 h after patch removal. After the intradermal application of 5% boscalid in 1% aqueous Tylose CB 30.000, there was a well defined erythema and moderate edema in all guinea-pigs.

Separate tests using α-hexylcinnamaldehyde as a positive control had been conducted twice per year in the laboratory concerned to demonstrate the continuing ability of the test procedures to detect sensitizing compounds.

One guinea-pig in the group receiving boscalid and one guinea-pig in the vehicle control group died from pneumonia unrelated to treatment. The numbers of guinea-pigs with skin reactions after the challenge are summarized in Table 22. A few animals (4 out of 19) in the group receiving boscalid showed skin reactions at challenge, while vehicle alone caused no skin irritation (Wiemann & Hellwig, 1998a; Wiemann, 2000d).

Table 22. Maximization test in guinea-pigs exposed to boscalid: results of challenge

Group	No. of positive reactions/No. of guinea-pigs tested		
	24 h	48 h	Total
Control group	0/10	0/10	0/10
Test group	3/19	4/19	4/19

From Wiemann & Hellwig (1998a) and Wiemann (2000d)

The evaluation criteria used were those of the EEC Directive 93/21 for the 18th Amendment of the Directive 67/548 EEC (Publication No. L 110A, May 4th, 1993). This requires a minimum of 30% of the test animals to show skin reactions for a classification as a sensitizer. On the basis of these criteria, the results indicated that boscalid is non-sensitizing, since only 4 out of 19 guinea-pigs (21%) showed a very slight skin reaction at challenge.

2.2 Short-term studies of toxicity

Mice

Groups of 10 male and 10 female C57BL mice were given diets containing boscalid (batch No. N 26, purity, 95.3%) at a concentration of 0, 150, 1000, 4000 or 8000 ppm, equal to 0, 29, 197, 788 and 1518 mg/kg bw per day in males and 0, 42, 277, 1184 and 2209 mg/kg bw per day in females, for about 3 months. The stability of boscalid in the diet was verified and the homogeneity of the dietary mixtures was verified before the start of the study. Analyses for correct concentrations were performed before the study start and at week 8.

Food consumption and body weight were determined once per week. The state of health was checked twice per day. Blood samples were taken from all mice for haematology and blood chemistry examination at the end of the dosing period. All mice were subjected to complete gross examinations, and weights of selected organs were determined. Histopathological examinations were conducted on all organs from the control group and the group at the highest dose and on lung, liver, kidneys and all gross lesions from all dose groups.

There were no mortalities in the study and boscalid did not cause clinical signs of toxicity in any of the dose groups. There were no boscalid-related effects on body weight, food consumption

or haematology at any dose. Blood chemistry examination showed a dose-dependent, statistically significant decrease in serum cholesterol of 12%, 26% and 28%, respectively, in male mice at 1000 ppm and greater. The toxicological significance of the reduced cholesterol is not clear, since reduction of cholesterol is not associated with a pathognomonic effect, i.e. one that is indicative of a specific condition. In addition, there were statistically significant reductions in serum total protein, albumin and globulin in male mice at 4000 and 8000 ppm. Slightly increased alanine aminotransferase activities were found in serum of female mice at 4000 and 8000 ppm, but not in male mice. The reduced protein values in males and the increased values of alanine aminotransferase activities in females might indicate that liver function was impaired at higher doses.

Liver weights were significantly increased in male mice at 1000, 4000 and 8000 ppm by approximately 12%, 11% and 27%, respectively. In female mice, liver weights were significantly increased in all groups except at 1000 ppm (where the increase was not statistically significant) by approximately 8%, 7%, 12% and 22%, respectively. Liver weights relative to body weights were also increased in male and female mice at a dietary concentration of 1000 ppm and greater. The Meeting noted that the liver weights of the male and female mice of the control group were lower than those in the lowest range for control groups in the historical database, which included eight studies. Including the present study, the historical mean for males was 1208.3 mg (range, 1096.8–1443.0 mg, the first value being the value for controls in the present study). Similarly, for females the historical mean liver weight for controls was 969.7 mg (range, 886.9–1053 mg, the first value being the value for controls in the present study). There were no significant changes in weights of other organs that could be attributed to treatment.

Histopathology did not reveal any treatment-related effects in any organ except for the liver. Steatosis of varying intensity (grade 2, slight, to grade 4, marked) was recorded in all control and treated mice. This fatty change was, mostly, diffusely distributed, but with a centrilobular pronouncement. No treatment-related gain in fatty infiltration was observed in female mice, but in male mice grade 4 was recorded with dose-related incidences of 0 out of 10, 0 out of 10, 0 out of 10, 3 out of 10 and 5 out of 10, respectively.

The target organ in this study in mice was the liver, with a sex-related difference in response to treatment with boscalid. Although hypocholesterolaemia does not appear to have toxicological significance, the observation of reductions in serum concentrations of several important chemicals synthesized by the liver probably should not be ignored. The observation of increased lipid accumulation in males in the groups receiving the two higher doses would also suggest hepatic malfunction. Therefore, the Meeting considered that the liver weight changes observed at 1000 ppm and greater in both sexes were indications of an adverse effect, although this effect was more pronounced in male mice and was not accompanied by histological change until a dietary concentration of 4000 ppm. The no-observed-adverse-effect level (NOAEL) in mice given diets containing boscalid for 3 months was 150 ppm, equal to 29 mg/kg bw per day in males and 42 mg/kg bw per day in females, on the basis of increased liver weight at 1000 ppm, equal to 197 mg/kg bw in male mice and 277 mg/kg bw in female mice (Mellert et al., 2000a).

Rats

Groups of 10 male and 10 female Wistar rats were given diets containing boscalid (batch No. N 26, purity, 95.3%) at a dietary concentration of 0, 100, 500, 2000, 5000 or 15 000 ppm, equal to 0, 7, 34, 137, 347 and 1055 mg/kg bw per day in males and 0, 8, 40, 159, 395 and 1225 mg/kg bw per day in females, for about 3 months. The stability of boscalid in the diet was verified and the homogeneity of the dietary mixtures was verified before the start of the study. Analyses for correct concentration were performed before the start of the study and at week 8.

Food consumption and body weight were determined once per week. State of health was checked twice daily. Ophthalmological examinations were carried out before the start and towards

the end of dosing. Blood samples were taken from all rats for haematology and blood chemistry examination at the end of the dosing period. Urine was analysed at the end of the exposure period. All rats were subjected to complete gross examinations, and weights of selected organs were determined. Histopathological examinations were conducted on all organs from animals in the control group and in the group at the highest dose and on lung, thyroid, liver, kidneys and all gross lesions from all dosed groups.

There were no mortalities in the study and boscalid did not cause clinical signs of toxicity in any of the dosed groups. There were no boscalid-related effects on body weight or food consumption at any dose.

Erythrocyte counts and erythrocyte volume fraction values were increased in male rats at a dietary concentration of 2000 ppm and greater, and haemoglobin concentrations were increased in male rats at 5000 and 15 000 ppm. No corresponding haematological changes were observed in female rats, but prothrombin time was significantly reduced in females at 15 000 ppm.

Blood chemistry examination showed dose-dependent, statistically significant increases in gamma-glutamyltransferase activity in male rats at 2000, 5000 and 15 000 ppm and in female rats at 5000 ppm and greater. In addition, alkaline phosphatase activity was decreased in female rats at 500 ppm and greater, having significantly increased at 100 ppm. A decrease in the activity of this enzyme is generally not considered as adverse. No other enzyme activities were affected by treatment. Concentration of bilirubin decreased with increasing dose in male rats at 2000 ppm group and greater, but not in females, and the concentrations of triglycerides were reduced in males and females at 15 000 ppm. Other blood chemistry changes were increases (in contrast with mice in the previous study) in total protein and albumin in males at 5000 and 15 000 ppm and in total protein, albumin, globulin and cholesterol in females at 15 000 ppm.

There were no test-substance related effects seen in urine analysis and ophthalmoscopy.

Organ-weight measurement revealed increased weight of the liver and the thyroids in male and female rats. Liver weights were significantly increased in the group at 15 000 ppm by approximately 19% in male rats and 23% in female rats. Female rat liver weight was also increased in the group at 5000 ppm by approximately 9%. Thyroid weights were significantly increased in groups of male rats at 2000 and 15 000 ppm (but not at 5000 ppm) by approximately 21% and 34% and in groups of female rats at 5000 and 15 000 ppm by approximately 17% and 31%. Liver weights relative to body weights were also increased in male and female rats in groups at 5000 and 15 000 ppm and relative thyroid weights were increased in males at 2000 and 15 000 ppm and in females at 5000 and 15 000 ppm.

Centrilobular (zone 3) hypertrophy was recorded in 8 out of 10 male and 2 out of 10 female rats at 5000 ppm and in 10 out of 10 male and 7 out of 10 female rats at 15 000 ppm. It was also noted that in those cases where centrilobular hypertrophy occurred in male rats, fat accumulation was observed more commonly in the periphery of the lobules than in mid-zonal (zone 2) areas. All other hepatic lesions were unrelated to treatment.

In thyroids, follicular cell hypertrophy and diffuse hyperplasia of grade 1 (minimal) or 2 (slight) were recorded in some male rats of all groups. The incidences of grades 1 and 2 hypertrophy and hyperplasia were 1 out of 10, 2 out of 10, 3 out of 10, 7 out of 10, 7 out of 10 and 8 out of 10 in the groups at 0, 100, 500, 2000, 5000 and 15 000 ppm, respectively. There were no similar records of hypertrophy or hyperplasia in female rats, in spite of the organ-weight changes that had been observed. All other thyroid lesions were unrelated to treatment. Also, no lesions recorded in other organs were treatment-related. Many of the dose-related hepatic changes that were found are indicative of an adaptive response to a chemical that is an inducer of enzymes. This hypothesis was examined in another study that demonstrated a proliferation of smooth endoplasmic reticulum (see below). Such changes are fully reversible as long as degenerative or necrotic changes do not also occur in the liver. Degenerative changes were not described in this study. In the thyroid, hypertrophy and hyperplasia

incidences were increased in a dose-related fashion. However, since these observations can, and did, occur in rats in the control group, it is necessary to take note of the frequency of their occurrence.

Taking these factors into consideration, the Meeting concluded that the NOAEL in male rats given diets containing boscalid for 3 months was 500 ppm, equal to 34 mg/kg bw per day, on the basis of altered clinical-chemical changes (increases in gamma-glutamyltransferase activity) and increased incidences of thyroid hypertrophy and hyperplasia in male rats at 2000 ppm, equal to 137 mg/kg bw in male rats and this dose, equal to 159 mg/kg bw could be considered as a NOAEL in female rats (Mellert et al., 2000b).

Groups of 10 male and 10 female Wistar rats were given boscalid (batch No. N 46; purity, 96.3%) at a dose of 0 (vehicle control; 0.5% aqueous carboxymethylcellulose and 0.5% Cremophor EL solution), 100, 250 or 1000 mg/kg bw per day by the dermal route under semi-occlusive dressing, for 6 h per day, 5 days per week, for 4 weeks.

Food consumption and body weight were determined weekly. The rats were examined for signs of toxicity or mortality at least once a day. Additionally, clinical examinations were carried out before daily treatment. Detailed clinical examinations in an open field were conducted before the start of the dosing period and weekly thereafter. Ophthalmological examinations were carried out before the start and towards the end of the dosing period. Urine analysis, blood chemistry and haematological examinations were carried out at the end of the dosing period. All rats were subjected to gross pathological assessment, followed by histopathological examinations. The stability of boscalid in the vehicle was verified and the homogeneity of the dose preparations was verified. Analyses for correct concentration were performed.

One female rat receiving the highest dose died on day 13 of the dosing period. The cause of death appeared to be septicaemia. The post-mortem findings indicating cause were localized in the uterus (pyometra) and urinary tract (papillary necrosis and severe suppurative pyelonephritis of the kidney, dilation and inflammation of the urinary bladder and ureter). These changes were unrelated to boscalid administration.

There were no signs of skin irritation at any dose.

The only finding in this study was a statistically significant reduction in total bilirubin in the serum of male rats at 1000 mg/kg bw (3.02 µmol/l in controls vs 2.64 µmol/l; $p < 0.05$). The 13% reduction in serum bilirubin concentration could be related to the induction of hepatic metabolic enzymes. A similar reduction was observed in the 3-month dietary study of boscalid. Also, boscalid has been shown to increase the activity of various microsomal enzymes (see below). Therefore, these changes (in the absence of any other pathologically relevant observations) are considered to be signs of an adaptation rather than toxicologically relevant adverse effects. There were no other test-substance related changes at any dose. The NOAEL in rats exposed dermally for 4 weeks was 1000 mg/kg bw per day, the highest dose tested (Mellert et al., 2000c).

Dogs

Groups of five male and five female pure-bred beagle dogs were given diets containing boscalid (batch No. N 37, purity, 94.4%) at a dietary concentration of 0, 250, 2500 or 25 000 ppm, equal to 0, 7.6, 78.1 and 728.9 mg/kg bw for males and 0, 8.1, 81.7 and 824.8 mg/kg bw for females, for 3 months.

Measurements were made of food consumption daily and body weights weekly. The dogs were examined at least once each working day for any signs of toxicity and a check for any moribund or dead animals was made twice a day, Mondays to Fridays, and once per day at weekends and on public holidays. Blood chemistry and haematological examinations as well as urine analyses were carried out once before and on two occasions during the administration period. Ophthalmological

examinations were carried out 8 days before the beginning of the administration period and on study day 91. All dogs were subjected to gross pathological assessment, followed by histopathological examinations.

The stability of boscalid in the diet was verified. The homogeneity of the mixtures and the correctness of the concentrations were demonstrated.

There was no boscalid-related mortality or clinical signs of toxicity at any dose. The faeces in all males and females at 25 000 ppm and transiently in three males and three females at 2500 ppm were soft and light-brown discoloured.

Slight body-weight losses and retarded body-weight gains were observed during the initial phases of treatment at 25 000 ppm in both sexes. Thereafter, body-weight gain was reduced in females only. At the end of the study, females in the control group had gained 1 kg of body weight while females at 25 000 ppm had gained only 0.2 kg. At the end of the study, in males at the highest dose there was no difference in body-weight gain compared with controls. Food consumption in males at the highest dose was 96% of the total food provided vs 100% in the controls. Food efficiency in males and particularly in females at 25 000 ppm was reduced.

Ophthalmoscopy revealed no pathological changes in any dose group. Blood chemistry and haematological examinations did show some test-substance related changes. In females in the group at 25 000 ppm there was a significant, 11% reduction in erythrocyte counts and a significant, 11% reduction in haemoglobin concentration at the end of the study. Midway through the study (week 6), these parameters were also lower in dogs in the group at 25 000 ppm, but not significantly so. No other haematological changes were recorded in either sex.

With regard to blood chemistry, alkaline phosphatase activities were significantly increased by approximately threefold in males at 25 000 ppm at weeks 6 and 13. In females, alkaline phosphatase activities were significantly increased at 2500 and 25 000 ppm by almost twofold and threefold, respectively, but only at week 13. The activities of alanine aminotranferase and aspartate aminotransferase enzymes were reduced at various times in the groups at 2500 and 25 000 ppm, but reductions in these activities is not normally considered to be an adverse response. Alkaline phosphatase activity is a sensitive indicator of hepatic enzyme induction in dogs and so the observed increases, in the absence of increases in other hepatic enzymes in serum, can be interpreted as being the result of enzyme induction rather than hepatotoxicity (Keller-Rupp, 1988). In reaching this conclusion, the Meeting assumed that liver was the source of this enzyme.

Triglyceride concentrations were significantly increased after 6 weeks of treatment at 25 000 ppm by approximately 2.1-fold in males and 1.8-fold in females. After 13 weeks, triglyceride concentrations were significantly increased in males at 250 ppm and greater and in females at 2500 ppm and 25 000 ppm. The increases were dose-related and reached approximately twofold in both males and females at 25 000 ppm. Although the concentration of triglycerides at 13 weeks was significantly higher in males at 250 ppm, the difference (30%) was no greater than in females (30%) and could probably be viewed as an lowest-observed-adverse-effect level (LOAEL). However, additional considerations included the fact that: (1) the control group concentrations appeared to be particularly low in this study; and (2) apart from the group values for male and females at 25 000 ppm for both sexes and for males at 2500 ppm at 6 weeks, all triglyceride concentrations were within the range for historical controls (historical data were supplied in the report for the performing laboratory for February 1995–January 1998: male beagles, 3-month studies: mean, 0.38; range, 0.24–0.45, n = 10; and female beagles, 3-month studies: mean, 0.45; range, 0.38–0.51, n = 9). On the other hand, assuming that there had been proper randomization, low concentrations of triglyceride should be a characteristic of all these dogs and the dose-related concentration increases were monotonic throughout the dose range in males and females at weeks 6 and 13. Furthermore, as in the great majority of studies in dogs, the group sizes are small and therefore totally reliance on statistically significant differences in pair-wise comparisons cannot be made.

The mean liver weights were significantly increased in the groups at 2500 and 25 000 ppm by approximately 18% and 42%, respectively, in males and by approximately 19% and 49%, respectively, in females. Liver weights relative to body weights were also significantly increased in females in these two groups and in males at 25 000 ppm. Although there were no significant increases in absolute thyroid weights, there was a trend, particularly in females, and thyroid weight relative to body weight was significantly increased in females at 25 000 ppm by approximately 43%.

No test-substance related gross or histopathological changes were observed at any dose, therefore, with the exception of the reduction in erythrocytes and haemoglobin concentration, virtually all other changes were indicative of enzyme induction rather than frank toxicity.

The NOAEL for beagle dogs was 250 ppm, equal to 7.6 mg/kg bw per day in males and 8.1 mg/kg bw per day in females, on the basis of increased serum triglyceride concentrations and effects on organ weight of liver in males and females at 2500 ppm, equal to78.1 mg/kg bw per day in males and 81.7 mg/kg bw per day in females (Schilling et al., 2000).

Groups of five male and five female pure-bred beagle dogs were given diets containing boscalid (batch No. N 37, purity, 94.4%) at a concentration of 0, 200, 800, 2000 or 20 000 ppm, equal to 0, 5.5, 21.8, 57.4 and 544.0 mg/kg bw for males and 0, 5.8, 22.1, 58.3 and 592.9 mg/kg bw for females, for about 12 months.

Measurements were made of food consumption daily and body weights weekly. The dogs were examined at least once each working day for any signs of toxicity and a check for any moribund or dead animals was made twice a day, Mondays to Fridays, and once a day at weekends and on public holidays. Blood chemistry and haematological examinations as well as urine analyses were carried out once before and after approximately 3, 6 and 12 months. Ophthalmological examinations were carried out 7 days before the beginning of the administration period and on study day 359. All dogs were subjected to gross pathological assessment, followed by histopathological examinations.

The stability of boscalid in the diet was verified. The homogeneity of the mixtures and the correctness of the concentrations were demonstrated.

There was no mortality during the study. Clinical signs of toxicity consisted of vomiting during the later part of the study in a single female at 20 000 ppm. The faeces in all males and females at 20 000 ppm were soft and light-brown discoloured.

Body weights of females in the group at 20 000 ppm initially decreased and their body-weight gain was reduced for the rest of the study period, so that the terminal mean body weights were 13.5 kg in the controls and 12.1 kg in the group at 20 000 ppm. Body-weight gain was also reduced in females at 2000 ppm, to 12.0 kg at the end of the study.

There were no treatment-related changes in the haematological parameters measured. Blood chemistry analysis showed that there were increases in alkaline phosphatase activity in male dogs at 2000 and 20 000 ppm throughout the study, although at termination the higher mean value for the males at 2000 ppm was not significantly different from that of the controls. Alkaline phosphatase activity was also increased in females in the group at 20 000 ppm throughout the study. Of the other serum enzymes measured, alanine aminotransferase activity was reduced in males at 2000 and 20 000 ppm throughout the study and alanine aminotranferase and aspartate aminotransferase activities were reduced in females at 20 000 ppm at 3 months. Decreases in the activity of these aminotransferases are not normally considered to be adverse responses.

There were significant increases in serum total protein, globulin, cholesterol and triglyceride concentrations and a decrease in chloride concentration in females at 20 000 ppm at 3 months; total protein concentrations were also increased in females at this dose at 6 months. There were no significant or dose-related increases in these parameters at 12 months. In male dogs, serum triglyceride

concentrations were increased at 20 000 ppm throughout the study and chloride concentrations were reduced at 3 months in the same group. No treatment-related changes were observed in urine.

Mean liver weights were significantly increased in female dogs at 20 000 ppm by 42% and, although the difference was not statistically significant, the liver weight in male dogs in the group at 20 000 ppm was 30% higher than that in the controls. Thyroid weights were significantly increased in males in the groups at 2000 and 20 000 ppm by approximately 39% and 54%, respectively, and were non-significantly higher in females at 20 000 ppm by approximately 42%. The weights of liver and thyroid relative to body weight were also increased in these groups of male and female dogs. The histopathological changes recorded were incidental or spontaneous and unrelated to treatment.

The NOAEL was 800 ppm, equal to 21.8 mg/kg bw per day in males and 22.1 mg/kg bw per day in females, on the basis of body-weight reductions in females and thyroid-weight increases in male and female dogs at 2000 ppm, equal to 57.4 mg/kg bw per day in males and 58.3 mg/kg bw per day in females (Wiemann et al., 2000).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female C57BL mice were given diets containing boscalid (batch No. N 37; purity, 94.4%) at a concentration of 0, 80, 400, 2000 or 8000 ppm, equal to 0, 13, 65, 331 and 1345 mg/kg bw in males and 0, 18, 90, 443 and 1804 mg/kg bw in females for 18 months.

Food consumption and body weight were determined once per week during the first 13 weeks and at 4-week intervals thereafter. A check of the general state of health of the mice was made at least daily. Additionally, the mice were examined in detail and palpated once per week. Blood smears were prepared after 12 months and 18 months, and from all mice killed in extremis. After 18 months of treatment, the mice were subjected to gross pathological assessment, measurement of organ weights and histopathology.

The stability and homogeneity of the test substance in the diet, and the correct concentrations were confirmed by analysis. There was no increase in mortality or clinical signs of toxicity in this study that could be attributed to treatment. Food consumption was not adversely affected but body weight was reduced in groups of male mice at a dietary concentration of 400 ppm and greater and in females at 8000 ppm. Although there was a monotonic reduction in mean body weight throughout the dose range for male mice, the maximum reduction was only approximately 9%.

Blood smears did not indicate any test-substance related effects.

Liver weights were increased in males at 8000 ppm by 16% and in females at 2000 and 8000 ppm by 8% and 10%, respectively. Liver weights relative to body weight also increased in males at 400 ppm and greater and in females at 2000 and 8000 ppm. Organ weights of adrenals and relative testes weights were also increased in treated groups, but these differences were either a consequence of extremely low adrenal weights in the control group (all adrenal weights being within the range for historical controls) or, in the case of testicular weights, failed to show a clear dose–response relationship. In neither case was the weight change accompanied by histological aberrations.

Histopathological investigations demonstrated an increased incidence of peripheral hypertrophy of hepatocytes in male mice, from 0 out of 50 in all groups up to and including the group at 2000 ppm to 29 out of 50 in the group at 8000 ppm, and in female mice from 0 out of 50 in all groups up to and including the group at 400 ppm to 10 out of 50 in the group at 2000 ppm and 45 out of 50 in the group at 8000 ppm. Fatty infiltration was common in all groups of males and females. In males the distribution was almost entirely centrilobular, and the more prevalent severity gradings (grades 3 and 4) were similar in all groups. In addition, low incidences of grade 5

(the highest score possible) were observed in the groups at 2000 and 8000 ppm. In females, fatty infiltration was predominantly diffuse, but shifted towards a centrilobular distribution at higher doses; however, the incidences of grades 3 and 4 severity (there was none at grade 5, the highest possible score) for centrilobular and diffuse fatty infiltration were similar in all groups.

There was no evidence for any treatment-related increased incidence of benign or malignant neoplasms in any organs of male or female mice. The Meeting concluded that boscalid is not carcinogenic in mice. The NOAEL in this 18-month study of toxicity and carcinogenicity in mice was 80 ppm, equal to 13 mg/kg bw per day in males, on the basis of reduced body-weight gain in male mice at 400 ppm, equal to 65 mg/kg bw per day. The NOAEL in female mice was 400 ppm, equal to 90 mg/kg bw per day, on the basis of liver-weight increases and hepatic peripheral hypertrophy at 2000 ppm, equal to 443 mg/kg bw per day (Mellert et al., 2001d).

Rats

Groups of 20 male and 20 female Wistar rats were given boscalid (batch No. N 37; purity, 94.4%) at a concentration of 0, 100, 500 or 2500 ppm, equal to 0, 4.4, 21.9 and 110.0 mg/kg bw per day in males and 0, 5.9, 30.0 and 150.3 mg/kg bw per day in females for 24 months. The study had begun with a dose at 15 000 ppm, equal to 739.0 mg/kg bw in males and 1000.4 mg/kg bw in females, but this was discontinued after approximately 17 months of treatment.

Food consumption and body weight were determined once per week during the first 13 weeks and at 4-week intervals thereafter. The rats were examined for signs of toxicity or mortality at least once per day; moreover, comprehensive clinical examinations and palpations of the rats were performed once per week. Ophthalmological examinations were carried out before the start and towards the end of dosing for rats in the control group and for rats at the highest dose. Blood samples were taken from all rats for haematology and blood chemistry examination after about 3, 6, 12, 13, 18 and 24 months of treatment. Urine samples were collected for analysis at about these same times. All rats were subjected to complete gross examinations, and weights of selected organs were determined. Histopathological examinations were conducted on all organs from rats at 0 and 2500 ppm and on all gross lesions, thyroids, lungs, liver and kidneys from rats at 100 and 500 ppm.

The stability of the test substance, the homogeneous distribution, stability and correct concentration of the test substance in the diet were confirmed by analysis.

The group at 15 000 ppm was discontinued after approximately 17 months because of severe effects on body weight that were expected to progress. However, this was clearly a decision taken late in the study. In comparison with the group at 0 ppm, the body-weight deficit was 8.5% in males and 14.4% in females at this stage of the study. Mortality was not affected (there being a single death in each of the groups of males and females at this dose, similar mortalities occurred in males at 100 and 2500 ppm and in females at 500 ppm at 17 months) and the blood chemistry and haematology observations were similar to those for the group at 2500 ppm (some observations being clearly adverse while several others were not).

In the remaining groups, mortality at 24 months was unaffected by treatment. Survival percentages in the groups at 0, 100, 500 and 2500 ppm, respectively, were 75%, 80%, 70% and 70% in males and 65%, 85%, 65% and 95% in females. No boscalid-related clinical observations were made and there were no significant effects on body-weight gain. There were sporadic increases in feed consumption in groups of females at 500 and 2500 ppm, but these were assessed as being incidental and not related to treatment.

No boscalid-related ophthalmic effects were observed. Decreases were observed in erythrocyte volume fraction, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) in female rats at 2500 ppm, but there were no changes in reticulocyte counts for either sex. Prothrombin

times were slightly, but significantly reduced at 3 months in females at 500 and 2500 ppm (28.8 s vs 27.6 and 26.9 s, respectively).

Serum concentrations of cholesterol were increased in male rats at 2500 ppm at 12 months and females at 3, 12, 13 and 18 months. In addition, cholesterol concentrations were increased in females at 500 ppm, at a single sampling time of 13 months (which happened to be about the same time as there was a significant increase in gamma-glutamyltransferase activity in males at this dose). Serum concentrations of bilirubin were decreased in males at 2500 ppm at almost every blood sampling time and at 500 ppm at 12 and 18 months. Reduced serum concentrations of bilirubin were found in female rats at 100 ppm and greater at 3 and 13 months and females at 2500 ppm at 24 months.

There were treatment-related changes in the activities of several serum enzymes in the study. Serum gamma-glutamyltransferase activities were increased in male rats at 2500 ppm at all blood sampling times, at 500 ppm at 3, 6 and 12 months and at 100 ppm at 3 months. The activity of this enzyme was also increased in females at 2500 ppm at 3 and 12 months. For example, the mean activities of gamma-glutamyltransferase in male rats at 12 months were (± standard deviation):14.7 ± 7, 15 ± 9, 20 ± 7** and 34 ± 20** units in the groups at 0, 100, 500 and 2500 ppm groups, respectively (** $p < 0.02$). Reduced activity was observed in serum alkaline phosphatase in male rats at 2500 ppm at all blood sampling times and at 500 ppm at 3 and 6 months. Alkaline phosphatase activity was also reduced in female rats at 2500 ppm at 3, 6, 12, 13 and 18 months and at 500 ppm at 3 months. Alanine aminotransferase activity was reduced in males at 2500 ppm at 6 and 18 months, in females of the same group at 3, 6, 12 and 13 months and at 100 and 500 ppm at 3 and 6 months. Asparate aminotransferase activity was also reduced in female rats at 2500 ppm, but only at 6 months. Although statistically significant, many of these changes were small and reductions in the activities of aminotransferases are not associated with toxicity. There were no treatment-related changes in the urine parameters examined.

The only significant changes in absolute organ weights were an approximately 31% increase in thyroid weight in male rats at 2500 ppm and an approximately 3% increase in brain weight in females at 100 ppm. The latter was clearly not treatment-related. Liver weight relative to body weight was increased by approximately 11% in females at 2500 ppm, but not in males. At autopsy, the number of male rats with enlarged thyroids or with a focus in the thyroids was slightly higher in the group at 2500 ppm. There were also a number of rats with cystic degeneration of the testes. These occurred in all groups, at incidences of 2 or 3 out of 20, except for the group at 2500 ppm, where there were 9 out of 20.

Microscopic examination revealed slightly increased incidences of diffuse hypertrophy and of focal hyperplasia of the thyroid follicular cells. A few thyroid follicular cell adenomas were also found (Table 23). In liver, there were increased incidences of centrilobular hypertrophy in males at 2500 ppm (0 ppm, 0 out of 20; 2500 ppm, 6 out of 20) and female rats (0 ppm, 0 out of 20; 2500 ppm, 16 out of 20). The incidences of eosinophilic foci were increased in males at 500 and 2500 ppm (3 out of 20, 4 out of 20, 7 out of 20 and 9 out of 20 in the groups at 0, 100, 500 and 2500 ppm, respectively), but not in females (1 out of 20, 1 out of 20, 2 out of 20 and 2 out of 20). In the testes, there was an increased incidence in cystic degeneration in the group at 2500 ppm.

Table 23. Incidences of thyroid follicular cell lesions in rats fed diets containing boscalid for 24 months

Lesion	Dietary concentration (ppm)							
	Males				Females			
	0	100	500	2500	0	100	500	2500
No. of rats in group	20	20	20	20	20	20	20	20

Diffuse hypertrophy	3	1	3	6	0	1	0	4
Focal hyperplasia	1	1	2	4	0	0	0	2
Adenoma	0	0	2	1	0	0	1	0

From Mellert et al. (2001b)

The NOAEL was 100 ppm, equal to 4.4 mg/kg bw per day in males and 5.9 mg/kg bw in females, on the basis of increased serum gamma-glutamyltransferase activity in males at 500 ppm, equal to 21.9 mg/kg bw per day in males and 30.0 mg/kg bw per day in females (Mellert et al., 2001b).

Groups of 50 male and 50 female Wistar rats were given diets containing boscalid (batch No. N 37, purity, 94.4%) at a concentration of 0, 100, 500, 2500 or 15 000 ppm, equal to 0, 4.6, 23.0, 116.1 and 768.8 mg/kg bw per day in males and 0, 6.0, 29.7, 155.6 and 1024.4 mg/kg bw per day in females for 24 months. The group at 15 000 ppm was discontinued after approximately 17 months of treatment. The conduct of this experiment overlapped the 24-month study of toxicity described above. The studies should be looked upon as largely independent of each other.

Food consumption and body weight were determined once per week during the first 13 weeks and at 4-week intervals thereafter. The rats were examined for signs of toxicity or mortality at least once per day; moreover, comprehensive clinical examinations and palpations of the rats were performed once per week. Blood smears were taken from the groups at 0 and 2500 ppm at the end of the study. All rats were subjected to complete gross examinations, and weights of selected organs were determined. Histopathological examinations were conducted on all organs from rats at 0 and 2500 ppm and on all gross lesions, thyroids, lungs, liver and kidneys from rats at 100 and 500 ppm.

The stability of the test substance, the homogeneous distribution, stability and correct concentration of the test substance in the diet were confirmed by analysis.

The group at 15 000 ppm was discontinued after approximately 17 months of treatment. In this group, there had been one death of a male rat by 14 months and this had increased to four deaths when the group was withdrawn at 17.5 months. However, there had been no deaths in females up to that time. Furthermore, male rats in the group at 15 000 ppm were gaining weight at a rate similar to the group at 0 ppm up to the time of withdrawal, although female rats at 15 000 ppm were gaining weight less rapidly than the other groups of female rats. They did not appear to have been subjected to pathological examination. The remainder of this description refers only to the groups at 0, 100, 500 and 2500 ppm.

Body weight at the end of the study was significantly reduced by 16.9% in females at 2500 ppm, but there was no similar effect in males. Food consumption was not affected by treatment. Examination of the blood smears at terminationdid not reveal any treatment-related changes in the leukocyte differential count or erythrocyte morphology.

Organ-weight changes included significant reductions in absolute kidney weight of 4.8% and 7.5% in female rats in groups at 500 and 2500 ppm, respectively. In males, there were increases in the absolute weights of the testes by approximately 20% in the group at 500 ppm only and increases in the absolute weights of the thyroids by approximately 18% in the group at 2500 ppm. In this same group, thyroid weight relative to body weight was increased by approximately 17%. At autopsy, enlarged thyroids were noted in 2 out of 50 and 1 out of 50 male rats at 500 and 2500 ppm, respectively. All other gross lesions were either single occurrences or were approximately equally distributed between the groups, including the control group.

Microscopic examination revealed an increased incidence of diffuse hypertrophy of the thyroid follicular cells in male rats at 2500 ppm (Table 22) and focal hyperplasia of the thyroid follicular cells

in males and females rats at 2500 ppm. Diffuse follicular cell hypertrophy can be viewed as indicative of increased function induced by thyroid-stimulating hormone (TSH), while the focal hyperplasia indicated continued stimulation of the thyroid to which the pre-existing cell numbers were unable to respond further. A few thyroid follicular cell adenomas were also found (Table 24). The incidence in males at 2500 ppm is considered to be almost significant (p = 0.06) according to Fisher's (one-sided) exact test. The numerical increase of thyroid follicular cell adenomas in males and females at 2500 ppm is considered to be a test-substance related effect based on the additional non-neoplastic changes in this organ. Using the United States Environment Protection Agency (EPA) benchmark dose software version 1.3.2 to fit the data to a number of statistical models, the benchmark dose lower 95% confidence limit (BMDL) was 76 mg/kg bw per day

Table 24. Thyroid follicular cell lesions in rats fed diets containing boscalid for 24 months

Lesion	Dietary concentration (ppm)							
	Males				Females			
	0	100	500	2500	0	100	500	2500
No. of rats	50	50	50	50	50	50	50	50
Diffuse hypertrophy	2	5	6	22	2	0	0	4
Focal hyperplasia	1	1	1	9	2	2	1	7
Adenoma	0	0	1	4	0	1	0	3

From Mellert et al. (2001a)

In liver, there were increased incidences of centrilobular hypertrophy in male rats in the groups at 500 and 2500 ppm (0 ppm, 0 out of 50; 100 ppm, 0 out of 50; 500 ppm, 2 out of 50; and 2500 ppm, 27 out of 50) and at 2500 ppm in female rats (0 ppm, 0 out of 50; 100 ppm, 0 out of 50; 500 ppm, 0 out of 50; and 2500 ppm, 11 out of 50). Eosinophilic foci were also recorded in the liver of male rats in all groups, but with slightly higher incidences at 500 and 2500 ppm, (8 out of 50 and 9 out of 50, respectively) compared with 3 out of 50 and 4 out of 50 at 0 and 100 ppm, respectively. No eosinophilic foci were recorded for livers of females in any group. The observed changes in the liver suggested an increased functional activity of this organ and, as will be described later, they may be the primary cause of the neoplastic response in the thyroid.

The only other proliferative response noted that appeared to be related to treatment with boscalid was a diffuse hyperplasia of the papillary transitional cells in the urinary bladder. This hyperplasia was recorded in all groups of male rats (with incidences of either 6 or 7 out of 50), but the incidence was highest (1 out of 50) in the group at 2500 ppm. The hyperplasia was accompanied by inflammation in the urinary bladder.

The NOAEL was 100 ppm, equal to 4.4 mg/kg bw per day, on the basis of increased gamma-glutamyltransferase activity and hepatic centrilobular hypertrophy and eosinophilic foci in males and increased cholesterol in females at 500 ppm, equal to 23.0 mg/kg bw per day in males and 29.7 mg/kg bw in females (Mellert et al., 2001a).

Boscalid has a tumorigenic effect in rats that is not considered to be relevant for humans, because: very high exposure levels would be required to produce such a response; the effect has a low potency, as indicated by the marginal, non-statistically significant increase in (thyroid follicular cell) adenomas and the absence of any carcinomas in rats; and the fact that rats are known to be particularly sensitive to this type of tumour compared with human beings.

Because no significant increase in the incidence of thyroid tumours was observed and all the tumours reported were benign, the Meeting considered that was not necessary to state a formal

mode of (carcinogenic) action. Nevertheless, data were available that supported the proposal that the thyroid effects are unlikely to occur in human beings. These data are summarized here.

The elements of a proposed mode of action would be that perturbation of the hypothalamus–pituitary–thyroid homeostasis beyond normal bounds leads to thyroid follicular cell hyperplasia stimulated by excess TSH. Evidence for the endocrinological changes is provided in the section on *Liver and thyroid effects*, below, where experiments are described in which rats were fed diets containing boscalid at a concentration of 15 000 ppm for 2 weeks. This evidence indicated that boscalid induces proliferation of hepatocellular smooth endoplasmic reticulum, cytochrome P450 enzymes and all of the three glucuronyltransferase enzymes examined. It is a reasonable prediction that glucuronyltransferase enzymes involved in the metabolism (conjugation) of thyroxin (T4) and triiodothyronine (T3) would also be induced, thereby accelerating their excretion. The experiment provides evidence for reductions in circulating T3 and T4. The feedback response of the hypothalamus–pituitary system to this reduction would be synthesis and release of larger quantities of TSH. Increased serum TSH was observed in the experiment. The expected consequential hyperplasia in the thyroid was observed in the long-term experiment in rats described above. All these events could occur in human beings in whom, however, the homeostatic control mechanisms are far more robust than in rats (Capen et al., 1999). Thus, the high doses required in rats to produce even a marginal increase in thyroid follicular cell adenomas would be expected to be much higher in human beings. Although many of the necessary steps for thyroid follicular cell adenoma induction by this hormonal mechanism have been demonstrated, weaknesses in the scheme are that the endocrinological data are derived from a single experiment and the single dose of boscalid investigated was well in excess of the dose used to demonstrate thyroid follicular cell hyperplasia and benign neoplasia.

2.4 Genotoxicity

Boscalid was tested for genotoxicity in a range of assays in vitro and in vivo (Table 25). There was no evidence for induction of gene mutation induction in *Salmonella typhimurium* or *Escherichi coli* (Engelhardt & Hoffman, 1998) in the standard plate assay or in a preincubation assay in vitro. The dose range used extended into a range that was bacteriotoxic for this compound. No significant response was observed in a single study of mutations at the *Hprt* locus in Chinese hamster ovary (CHO) cells in vitro, after exposure for 4 h to boscalid at concentrations up to 100 µg/ml or 1050 µg/ml in the absence or presence of an exogenous metabolic activation system, respectively (Engelhardt & Hoffmann, 2000a). Thus, boscalid did not induce gene mutations in bacterial cells or cultured mammalian cells.

In a single study on the induction of chromosomal aberrations in cultures of V79 cells, no significant increases in the proportion of abnormal cells were observed after exposure for 4 h in the presence of an exogenous activation mixture or 18 h and 28 h in its absence with boscalid at concentrations of up to 500 or 125 µg/ml, respectively (Engelhardt & Hoffmann, 1999a).

No genotoxic activity was observed in a study on the induction of unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes exposed for 18–20 h to boscalid at concentrations of up to 50 µg/ml. At a concentration of ≥ 50 µg/ml, boscalid precipitated in the culture medium and at 100 µg/ml was toxic. Treatment with 2-acetylaminofluorene at 1 µg/ml induced a substantial response under the same culture conditions (Engelhardt & Hoffmann, 2000b).

In a test for clastogenicity and aneugenicity, induction of micronucleus formation was examined in bone-marrow cells of NMRI mice. Groups of five male mice were dosed by intraperitoneal

End-point	Test object	Dose[a](LED/HID)	Purity (%)	Result	Reference
In vitro					
Gene mutation	*S. typhimurium* strains TA100, TA1535, TA1537, TA98; *E.coli* WP2*uvrA*, standard plate and pre-incubation protocols	2750 µg/plate (toxic)	95.3	Negative ± S9	Engelhardt & Hoffmann (1998)
Gene mutation	Chinese hamster ovary (CHO) cells, *Hprt* locus	500 µg/ml −S9 (precipitation at 31 µg/ml), 4 h 1000 µg/ml +S9, 4 h	94.4	Negative ± S9	Engelhardt & Hoffmann (2000a)
Chromosomal aberration	Chinese hamster lung V79 cells	500 µg/ml −S9 (precipitation at 31 µg/ml), 4 h500 µg/ml +S9 4 h; 125 µg/ml +S9, 28 h	94.4	Negative ± S9	Engelhardt & Hoffmann (1999a)
Unscheduled DNA synthesis	Liver cells from male Wistar rats	50 µg/ml, 18–20 h	94.4	Negative	Engelhardt & Hoffmann (2000a)
Micronucleus formation	Male & female NMRI mouse bone-marrow cells, 2 h after dosing	2000 mg/kg bw, intraperitoneal injection × 2	94.4	Negative	Engelhardt & Hoffmann (1999b)

[a] LED, lowest effective dose; HID, highest ineffective dose; S9, 9000 × *g* supernatant from livers of male Sprague-Dawley rats

injection with boscalid at a dose of 0, 500, 1000 or 2000 mg/kg bw on two occasions separated by 24 h. The highest dose was selected on the basis of a preliminary limit test in which two intraperitoneal injections at 2000 mg/kg bw were administered at an interval of 24 h to male and female mice. Clinical signs recorded were squatting posture, piloerection and a general poor condition that was similar in males and females. For this reason, the test for genotoxicity was conducted with a single sex (males) only. In addition, two groups of five mice were dosed with the positive-control substances cyclophosphamide (for clastogenicity) or vincristine (for aneugenicity) by intraperitoneal injection on a single occasion. All mice were killed 24 h after dosing. Target concentrations of boscalid were verified analytically. No increases in micronucleated polychromatic erythrocytes were observed in any group dosed with boscalid. Large increases in the incidence of micronuclei were observed in both of the positive-control groups (Engelhardt & Hoffmann, 1999b).

The Meeting concluded that boscalid is unlikely to be genotoxic.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of 25 male and 25 female sexually immature Wistar rats were fed diets containing boscalid (batch No. N 37, purity, 94.4%) at a concentration of 0, 100, 1000 or 10 000 ppm, equal to 0, 10.1, 101.2 and 1034.5 mg/kg bw per day for males and 10.7, 106.8 and 1062.0 mg/kg bw per day for females averaged over the pre-mating period of exposure of the F_0 parental rats in a two-generation study of reproduction. After 10 weeks, the rats were mated and allowed to rear the ensuing F_1 litters to weaning.

F_0 females received the same dietary concentrations during gestation and lactation, which were equal to 0, 8.7, 88.7 and 907.4 mg/kg bw per day and 0, 14.8, 149.4 and 1456.7 mg/kg bw per day, respectively. The breeding programme was repeated with the F_1 parents (selected from the F_1 offspring) after an exposure period of at least 10 weeks. The ensuing F_2 litters were reared to weaning. The diets containing boscalid were fed continuously throughout the study. The stability and homogeneous distribution of boscalid in the diet were evaluated before the beginning of the study. Analyses to confirm correct concentrations were performed periodically during the study.

Body weights of F_0 and F_1 parents were measured once per week during the pre-mating period, after which F_0 and F_1 females were weighed on days 0, 7, 14 and 20 of gestation and on postnatal days 1, 4, 7, 14 and 21. Data on the estrous cycle were evaluated for F_0 and F_1 rats during the 3 weeks before mating until evidence of mating was obtained. Sperm parameters (motility, sperm head count and morphology) were assessed in all F_0 and F_1 rats at about the time of scheduled sacrifice. The F_1 and F_2 pups were weighed on the day of or day after birth, and on postnatal days 4, 7, 14 and 21. The state of health of the parents and the pups was checked at least once per day, and parental animals were examined for their mating and reproductive performances. Pups were sexed and evaluated as to health, and pup viability was recorded. A gross pathological examination was carried out on all parents and offspring. Histopathology was conducted on all F_0 and F_1 parents, special attention being paid to the reproductive organs.

The stability and homogeneity of the test substance in the diet and target dietary concentrations were confirmed by analysis

In the parental groups (F_0 and F_1 generations), boscalid had no adverse effects on survival and there was no evidence for treatment-related clinical changes. Food consumption among the F_0

parental rats was not markedly affected by treatment over the entire treatment period, while the food consumption of F_1 parental females was clearly reduced (by about 8%) in the group at 10 000 ppm during lactation. During the pre-mating period, male and female body-weight differences between groups were small, but differences began to increase in females during gestation. During lactation, administration of diet containing boscalid at 1000 or 10 000 ppm was associated with lower body weights and body-weight gains in F_0 females. There was a similar trend, although not so obvious and not statistically significant, in the F_1 females. In the F_1 generation, mean body weights of male rats at 10 000 ppm were significantly reduced for almost the entire treatment period. The Meeting considered that this response in male rats was related to treatment.

There was no evidence for an adverse effect of boscalid on sperm parameters, mean coital time, fertility index, conception rate, duration of gestation, or gestation index at any of the doses tested. The parental females showed regular estrous cycles over the 3-week observation period before mating and became sperm-positive within a few days after mating. There was no effect on litter size at birth that could be attributed to boscalid. Pup viability was unaffected by treatment in the F_1 generation, but there mortality of F_2 generation pups at age 0–4 days was higher at 10 000 ppm (14%; $p < 0.01$, Fisher exact test). The increased mortality in the group at 100 ppm was largely due to the deaths of all 18 pups in one litter. The Meeting considered that this event was not related to treatment.

Male and female F_1 pup body weights were significantly reduced by approximately 7% on postnatal day 21 in the group at 10 000 ppm. A similar response during lactation was observed amongst the F_2 generation pups. On postnatal day 21, the mean body weights in the group at 10 000 ppm were approximately 14% lower for males and 12% lower for female pups and, in the group at 1000 ppm, male pups were approximately 7% lighter than the controls on postnatal day 21. At autopsy of the pups, it was noted that thymus weights were reduced in female F_1 pups of the group at 10 000 ppm (10%), but not in male F_1 pups of any dosed group. In the F_2 pups, thymus weight was not significantly changed in any group of females, while there were significant reductions in all dosed groups of males, by approximately 17%, 12% and 19% in the groups at 100, 1000 and 10 000 ppm, respectively. The absence of a dose–response relationship in males and the absence of any response in females in F2 and the lack of correspondence with observations in F_1 suggested that these thymus responses are not treatment-related. Spleen weights were not affected by treatment in either male or female F_1 pups, while they were reduced in male F_2 pups of the groups at 1000 and 10 000 ppm (17% and 27%, respectively) and in female F_2 pups of the group at 10 000 ppm (18%). These reductions in spleen and thymus weights were not consistent with regard to sex and generation and therefore were not considered to be toxicologically significant.

At the autopsies of the rats in the group at 10 000 ppm, it was found that absolute and relative (to body weight) liver weights were increased in females of the F_0 and the F_1 generations. Absolute and relative liver weights increased by approximately 16% and 21%, respectively in the F_0 generation and by approximately 21% and 21% in the F_1 generation. There were no significant liver-weight changes at 1000 ppm or in male rats of any group. Histology of the liver did, however, reveal changes in both sexes at 1000 and 10 000 ppm (Table 26). Centrilobular hypertrophy occurred in all rats at 10 000 ppm, as well as in significant proportions of rats at 1000 ppm. Hepatocellular degeneration was less common and was not observed at all in any females of the F_1 generation.

Table 26. Incidences of histopathology of the liver in F_0 and F_1 parental rats fed diets containing boscalid

Histopathology	Dietary concentration (ppm)							
	Male rats				Female rats			
	0	100	1000	10 000	0	100	1000	10 000
No. of rats	25	25	25	25	25	25	25	25

F_0 generation parental								
Centrilobular hypertrophy	0	0	9	25	0	0	6	25
Hepatocellular degeneration	0	1	0	3	0	0	0	1
F_1 generation parental								
Centrilobular hypertrophy	1	0	10	25	0	0	8	25
Hepatocellular degeneration	0	0	0	8	0	0	0	0

From Schilling et al. (2001)

A number of other organ-weight changes that were apparently treatment-related were recorded in the parental rats. Absolute spleen weights were reduced in F_0 males and F_1 males and females rats of the groups at 1000 and 10 000 ppm. Absolute kidney weights were reduced in F_1 males and females at 10 000 ppm. While there were several other significant organ-weight changes (brain, ovary), the poor relationship with dose or the lack of consistency suggested that they were not treatment-related. Histological findings in organs of reproduction were incidental and assessed as having been unrelated to treatment.

The NOAEL for toxicity in adult rats was 100 ppm, equal to 10.1 mg/kg bw per day in males and 10.7 mg/kg bw per day in females, on the basis of reduced body-weight gain during lactation, liver-and spleen-weight changes and hepatic histopathology at 1000 ppm, equal to 101.2 mg boscalid/kg bw per day in males and 106.8 mg boscalid/kg bw per day in females. The NOAEL for offspring toxicity was 100 ppm, equal to 10.1 mg/kg bw per day in males and 10.7 mg/kg bw per day in females, on the basis of reduced pup-weight gain and reduced spleen weights (F_2 male pups) at 1000 ppm, equal to 101.2 mg boscalid/kg bw per day in males and 106.8 mg boscalid/kg bw per day in females. There were no effects on reproductive indices at doses up to and including 10 000 ppm, equal to 1034.5 mg boscalid/kg bw per day in males and 1062.0 mg boscalid/kg bw per day in females, the highest dose tested. The Meeting concluded that boscalid is not a reproductive toxicant in rats (Schilling et al., 2001).

(b) Developmental toxicity

Rat

In a study of developmental toxicity, groups of 25 time-mated, female Wistar rats were given boscalid (batch No. N37; purity, 94.4%) at a dose of 0, 100, 300 or 1000 mg/kg bw per day by gavage in aqueous suspension (0.5% Tylose CB 30.000 in distilled water) on days 6–19 of gestation. The day of confirmation of mating (when spermatozoa were detected) was designated day 0 of gestation. The control group of rats received vehicle only. A standard dose volume of 10 ml/kg bw was used. On day 20 of gestation, the females were killed and assessed by gross pathology. Corpora lutea were counted, and the number and distribution of implantation sites were classified. Uteri were examined for live fetuses and intrauterine deaths. The fetuses were weighed, examined for external/visceral abnormalities, sexed, eviscerated and approximately one half were stained for skeletal examination, while the others were examined for soft tissue alterations.

Analytical verification of stability and homogeneity of boscalid in 0.5% Tylose CB 30.000 in distilled water was determined before the start of the study. Verification of test concentrations was performed during the study.

Between 22 and 24 female rats per group became pregnant in the study. Treatment of pregnant Wistar rats with boscalid did not evoke clinical signs of toxicity or cause alterations in food consumption or body-weight gain. There were no treatment-related or biologically relevant differences between the

groups in conception frequencies, mean numbers of corpora lutea, implantation site number or in the values calculated for pre- and postimplantation losses. All differences were within the normal range of deviation expected of rats of this strain and age.

Examination of the fetuses after dissection from the uterus showed normal and expected values unrelated to treatment for the sex distribution, placental weight, fetal weight, external variations and, in general, external malformations. The only external malformations observed were in two fetuses from the group at 100 mg/kg bw per day.

Soft-tissue variations were found in all groups, the more common being unilateral or bilateral dilated renal pelvis and ureter, but their incidence was not treatment-related. Dilated cerebral ventricle was found in one fetus in each of the groups at 100 and 1000 mg/kg bw per day. The mean percentages (± standard deviation) of fetuses per litter with soft tissue variations in the groups at 0, 100, 300 and 1000 mg/kg bw per day were 12.6 ± 14.1%, 17.6 ± 19.4%, 13.0 ± 14.6% and 21.1 ± 18.7%, respectively. The differences were considered to be incidental and all percentages were well within the range for historical controls. These historical means and ranges in a database of 1594 fetuses were: dilated renal pelvis: mean, 13.6%, range, 7.6–21.6%; dilated ureter: mean, 1.4%, range, 0.5–3.6%; total soft-tissue variations: mean, 13.7%, range, 7.6–21.6%. Soft-tissue malformations occurred in single fetuses of each group at 0 and 100 mg/kg bw per day, but none was found in the groups at 300 or 1000 mg/kg bw per day.

Skeletal variations with or without involvement of the associated cartilaginous structures were found in all groups. These involved the skull, ribs, sternum and limbs. The mean percentages of affected fetuses per litter in the groups at 0, 100, 300 and 1000 mg/kg bw per day were 88.8%, 88.3%, 92.3% and 92.9%, respectively. All except one of the skeletal variations appeared to be without any dose–response relationship. The exception was incomplete ossification of the thoracic centra, the incidence of which showed a statistically significant increase (Wilcoxon one-sided test) at 1000 mg/kg bw per day. In spite of this finding, it appeared to be incidental to treatment because no associated evidence for incomplete ossification was found in other parts of the vertebral column. The percentages (± standard deviation) of fetuses per litter with this particular variation at 0, 100, 300 and 1000 mg/kg bw were: 3.0 ± 8.2%, 3.4 ± 8.3%, 1.6 ± 5.1% and 9.2 ± 12.0%. The mean and range for historical controls, based on two experiments carried out in 1997–1998, with updated classification of fetal findings as malformations and variations, were 2.7% and 1.8–3.5%. Skeletal tissue malformations in the form of malpositioned and bipartite ossification of sternebrae (with unchanged cartilage) occurred in individual fetuses of the groups at 0, 100 and 300 mg/kg bw per day, but none was found at 1000 mg/kg bw per day.

Thus under the conditions of this study of prenatal developmental toxicity, the oral administration of boscalid to pregnant Wistar rats from implantation until 1 day before the expected day of parturition (days 6–19 of gestation) evoked no overt maternal toxicity up to the limit dose of 1000 mg/kg bw per day. Furthermore, there were also no substance-induced, dose-related influences on the gestational parameters and no signs of boscalid-induced teratogenicity, up to and including the limit dose of 1000 mg/kg bw per day.

There were no indications for boscalid-induced teratogenicity. The NOAEL for maternal toxicity was 1000 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity was 300 mg/kg bw per day on the basis of reduced ossification of thoracic centra at 1000 mg/kg bw per day (Schilling & Hellwig, 2000b).

Rabbit

In a study of developmental toxicity, groups of 25 artificially inseminated Himalayan rabbits were given boscalid (batch No. N 37; purity, 94.4%) at a dose of 0, 100, 300 or 1000 mg/kg bw in an aqueous suspension (0.5% Tylose CB 30.000 in doubly distilled water) administered by stomach tube

on days 7 to 28 after insemination. A standard dose volume of 10 ml/kg bw was used. The control group received the vehicle only.

Food consumption and body weights were recorded regularly throughout the study period. The state of health of the animals was checked each day.

On day 29 after insemination, all surviving females were killed and assessed by gross pathology (including weight determination of the unopened uterus and the placenta). For each dam, corpora lutea were counted and number and distribution of implantation sites (differentiated as resorptions, live and dead fetuses) were determined. The fetuses were removed from the uterus, sexed, weighed and further investigated for any external, soft tissue and skeletal findings.

The stability of the test substance was proven by reanalysis. The stability and homogeneity of the test substance preparation was analytically verified. The correctness of the concentrations was analytically demonstrated.

Twenty-four to twenty-five females per group became pregnant. Treatment of pregnant Himalayan rabbits with boscalid did not cause mortality. Two deaths occurred as a result of dosing accidents, one in each of the groups at 0 and 300 mg/kg bw per day. Four females were killed after abortions, one in the group at 300 mg/kg bw per day and three at 1000 mg/kg bw per day. In addition, another rabbit in the group at 1000 mg/kg bw per day was killed after premature delivery. While the female from the group at 300 mg/kg bw per day and one of the females from the group at 1000 mg/kg bw per day that aborted showed discoloured faeces and reduced defecation a few days before abortion, the other three rabbits affected in the group at 1000 mg/kg bw per day showed no clinical signs of toxicity. Food consumption was significantly reduced in rabbits at 1000 mg/kg bw per day during several days of treatment, their total food consumption during treatment being approximately 26% less than that of the controls. Slight or occasional instances of reduced food consumption in the other groups were considered to be unrelated to treatment. Reductions in body-weight gain at 1000 mg/kg bw per day were treatment-related. Indeed, there was no body-weight gain in this group over the entire period of dosing, in spite of the pregnancies. At 100 and 300 mg/kg bw per day, body-weight gain was not different from that of the controls. There were no treatment-related differences between the groups with regard to conception frequency, mean numbers of corpora lutea, implantation site number or in the values calculated for pre- and postimplantation losses.

Examination of the fetuses after dissection from the uterus showed normal and expected values unrelated to treatment for sex distribution, placental weight, fetal weight, external variations and, in general, external malformations. The only external malformations observed were in one fetus from the group at 100 mg/kg bw per day and two fetuses from the group at 100 mg/kg bw per day. None were found at 0 or 1000 mg/kg bw per day.

Soft-tissue variations were found in all groups, the more common being malpositioned carotid branch, which is a very common finding in Himalayan rabbits. Their incidence showed no relationship to treatment. The mean numbers (± standard deviation) of affected fetuses per litter in the groups at 0, 100, 300 and 1000 mg/kg bw per day were: 5.3 ± 9.4, 7.3 ± 11.3, 6.1 ± 16.2 and 7.5 ± 10.9, respectively. Soft-tissue malformations occurred in some fetuses in each of the groups at 0, 100 and 300 mg/kg bw, but none were found at 1000 mg/kg bw per day.

Skeletal variations with or without involvement of the associated cartilaginous structures were found in all groups. These involved the skull, ribs, sternum and limbs. All except one of the skeletal variations appeared to be without any dose–response relationship. The exception was incomplete ossification of the thoracic centra, the incidence of which was statistically significantly increased (Wilcoxon one-sided test) in the group at 1000 mg/kg bw per day. In spite of this finding, it was considered to be incidental to treatment because no associated evidence for incomplete ossification was found in other parts of the vertebral column. The percentages (± standard deviation) of fetuses per litter with this particular variation in the groups at 0, 100, 300 and 1000 mg/kg bw per day were:

0.7 ± 3.5%, 1.8 ± 4.2%, 2.9 ± 9.0% and 8.3 ± 17.2%. The mean and range for historical controls, based on two experiments carried out in 1997–1998, with updated classification of fetal findings as malformations and variations, were 0.1% and 0.0–0.5%, respectively.

Skeletal tissue malformations (the most common being defect of the cardiac muscular ventricular septum) occurred in individual fetuses in the groups at 0, 100 and 300 mg/kg bw per day, but none was found at 1000 mg/kg bw per day.

There were no indications that boscalid induced teratogenicity. The NOAEL for maternal toxicity was 300 mg/kg bw per day on the basis of reduced feed consumption and body-weight gain at 1000 mg/kg bw per day. The NOAEL for developmental toxicity was 300 mg/kg bw per day on the basis of reduced ossification of thoracic centra at 1000 mg/kg bw per day.

The Meeting concluded that boscalid is unlikely to be teratogenic to man (Schilling & Hellwig, 2000a).

2.6 Special studies

(a) Neurotoxicity

(i) Neurotoxic potential

In a single-dose study of neurotoxicity, groups of 10 male and 10 female Wistar rats were given boscalid (batch No. N 46; purity, 96.3%) as a single oral dose at 0, 500, 1000 or 2000 mg/kg bw by gavage. The vehicle was a 0.5% aqueous solution of Tylose CB 30.000, and the dose administration volume was 20 ml/kg bw.

The rats were observed for up to 2 weeks after dosing. Their general state of health was examined and recorded daily. Body weight was determined 7 days before dosing, on day 0 (administration of test substance) and on days 7 and 14. Functional observational batteries (FOB) and motor activity measurements were carried out on all rats on the same days. The measurements for day 0 were made within a few hours after dosing. Five rats per sex and dose were killed, fixed by in-situ perfusion and subjected to neuropathological examinations. The remaining animals were killed with CO_2 under anaesthesia without any further examinations. The stability, homogeneity and correctness of the concentrations of the boscalid preparation were verified analytically.

There were no deaths during the study and the only clinical sign of toxicity that was treatment-related was piloerection in two female rats at 2000 mg/kg bw during the FOB on day 0. There were no other test-substance related effects at any dose. In particular, no signs of neurotoxicity were observed. The NOAEL for neurotoxicity after a single oral dose of boscalid was 2000 mg/kg bw in male and female rats (Mellert et al., 2000d).

In a multiple-dose study of neurotoxicity, groups of 10 male and 10 female Wistar rats were given diets containing boscalid (batch No. N 46; purity, 96.3%) at a concentration of 0, 150, 1500 or 15 000 ppm, equal to 0, 10.5, 103 and 1050 mg/kg bw per day in males and 0, 12.7, 124.5 and 1273 mg/kg bw per day in females, for 3 months.

Food and water consumption were determined once per week. Body weight was determined once per week and on the days when FOB were performed. A check of the general state of health was made at least daily. Furthermore, the rats were thoroughly examined and palpated once per week. FOB and measurements of motor activity were carried out in all animals 7 days before the start of the administration and on days 22, 50 and 85. Five rats per sex and dose were killed, then fixed by in-situ perfusion and subjected to neuropathological examinations. The remaining animals were killed with CO_2 under anaesthesia without any further examinations. The

stability, homogeneity and correctness of the concentrations of boscalid in the diet were verified analytically.

There were no adverse effects related to treatment at any dose. In particular, there were no signs of neurotoxicity at any dose. The NOAEL for neurotoxicity was 15 000 ppm, equal to 1050 mg/kg bw in males and 1272 mg/kg bw in females, the highest dose tested (Mellert et al., 2001c).

(ii) Delayed neurotoxicity in hens

As there were no neurotoxic effects observed in any of the experiments with boscalid, studies on delayed neurotoxicity in hens were not performed.

(iii) Developmental neurotoxicity in rats

Boscalid (batch No. N 46; purity, 96.3%) was tested for its effect on the embryonic, fetal and postnatal development of the nervous system in Wistar rats in this developmental study of neurotoxicity. Groups of 35 mated female Wistar rats were given boscalid at a concentration of 0, 100, 1000 or 10 000 ppm, equal to 0, 14, 147 and 1442 mg/kg bw per day, continuously as a homogeneous addition to the feed from day 6 postcoitum to postnatal day 21. The dams were allowed to litter and rear their offspring until day 4 (standardization of litters) or 21 after parturition. After the offspring had been weaned following lactation until day 21, the dams were killed and not examined further.

The state of health of the dams and offspring was evaluated each day. Food consumption and body weights of the dams were determined on days 0, 6, 13 and 20 of gestation and on days 1, 7, 14 and (body weight only) 21 of lactation. A detailed clinical examination outside the cage (open-field observations) was made on selected dams on days 7 and 14 of gestation and on days 7 and 14 of lactation.

The offspring were sexed and examined for macroscopically evident changes on the day of birth. They were weighed on the day after birth and on postnatal days 4, 11, 17 and 21 and after weaning, once per week at weekly intervals. Their viability was recorded. Sexual maturation (day of preputial separation/vaginal opening) of all selected offspring and their body weights were determined and recorded. A detailed clinical examination outside the cage (open field observations), measurements of motor activity and tests for auditory startle and learning and memory (water-maze test) were performed on selected offspring. All offspring not required for any of these examinations were killed and discarded without further examinations. Neuropathological examinations and determinations were carried out on selected offspring on days 11 and 60 after birth. The stability, homogeneity and concentrations of boscalid in the diet were confirmed analytically.

There were no treatment-related effects on parental females at any dose. Offspring of the group at 10 000 ppm showed slightly reduced mean body weights in males and females throughout lactation and reduced mean body-weight gain on postnatal days 1–4 and 17–21. The latter was about 5% below body-weight gain in the control group, if calculated for postnatal days 4–21. Mean body weights and body-weight gains were also reduced in the group at 1000 ppm during the early postnatal days, but there were no body-weight deficits at 100 ppm. Absolute weights of the brain and the length of the brain (male rats) were reduced only in those rats killed on postnatal day 11 at 10 000 ppm. These brain-weight and length effects were considered to be consequences of the retarded body-weight development. No similar changes were found in the groups at 100 or 1000 ppm. There were no signs of developmental neurotoxicity at any dose.

The NOAEL for maternal toxicity was 10 000 ppm, equal to 1442 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity was 100 ppm, equal to 14 mg/kg bw per day, on the basis of reduced body weights of pups during lactation at 1000 ppm, equal to 147 mg/kg bw per day. The reduction in pup body weight at the two higher doses was consistent with similar effects seen in the two-generation study in rats in which the same doses induced hepatotoxicity.

In this study of developmental neurotoxicity, boscalid had no adverse effects on the embryonic, fetal and postnatal development of the nervous system in Wistar rats at doses of up to 10 000 ppm, equal to 1442 mg/kg bw per day, the highest dose tested (Kaufmann et al., 2001). The Meeting concluded that boscalid is not neurotoxic in adult or developing rats.

The Meeting concluded that boscalid is unlikely to cause neurotoxicity in human beings.

(b) Liver and thyroid effects

The potential of boscalid (batch No. N 26; purity, 95.3%) to induce hepatic metabolizing enzymes was investigated in rats. Groups of five male and five female Wistar rats were given boscalid at a dietary concentration of 0 or 15 000 ppm for 2 weeks and a number of hepatic parameters were measured. Additional groups of three male and three female rats were also treated for 2 weeks with the same diets and subjected to perfusion fixation for subsequent electron microscopy of the liver. The stability of boscalid in the diet, the homogeneity of the dietary mixture and the correctness of the concentration were demonstrated.

Liver weights were increased by 32% in males and 23% in females at 15 000 ppm and light and electron microscopy demonstrated a centrilobular (zone 3) proliferation of smooth endoplasmic reticulum. In hepatocytes showing gross proliferation of smooth endoplasmic reticulum, glycogen storage was depleted. The only biochemical parameter showing a significant change in both sexes was the content of cytochrome P450, which was increased in male rats by 124% and in female rats by 74%. Lipid peroxidation was slightly increased in males, but not in females.

Parameters in rat liver that showed no significant differences between the groups at 0 and 15 000 ppm for either sex were: cyanide-insensitive palmitoyl-CoA oxidation, ethoxyresorufin-O-deethylase, pentoxyresorufin-O-dependentylase, and glutathione. In the absence of a change in glutathione concentration, the small change in lipid peroxidation activity in male rats only is of doubtful toxicological significance (Mellert et al., 1999). The Meeting concluded that boscalid induces proliferation of smooth endoplasmic reticulum and rat liver cytochrome P450 enzymes, although no specific isoenzymes had been identified.

Organ-specific effects of boscalid were extended to the thyroid, in view of the thyroid toxicity and possible neoplasia observed in a long-term experiment described earlier in the present monograph. It is known that possible modes of action on thyroid can involve the liver, so investigations in this study were extended to this organ, to supplement the measurements in the experiment previously described. Groups of five male and five female Wistar rats were fed diets containing boscalid (batch No. N 46; purity, 96.3%) at a concentration of 0 or 15 000 ppm, equal to 0 and 957 mg/kg bw per day for males and 0 and 1197 mg/kg bw per day for females for 4 weeks. Food consumption and body weights were determined once per week and the rats were examined for signs of toxicity or mortality at least once each day. Thyroid hormone analyses (T3, T4, TSH) were carried out on study days −3, 2, 4, 7, 14, 21 and 28. So-called phase II liver enzyme activities (*p*-nitrophenol-glucuronyltransferase, 4-methylumbeliferone-glucuronyltransferase and 4-hydroxybiphenyl-glucuronyltransferase) were measured in liver at the end of the study. The stability of the test substance over the study period was demonstrated. The stability of boscalid in the diet, the homogeneity of the dietary mixture and the correctness of the concentration were demonstrated.

There were no clinical signs of toxicity and no effects on body weight or food consumption at 15 000 ppm. Total T3 concentration was lower by approximately 15–30% of control values in male rats at 15 000 ppm throughout the study. With the exception of day 4, all these changes were seen as a trend toward reduced values. In females, reductions in the concentration of T3 of 1–25% from day 4 onwards were not statistically significant.

Total concentrations of T4 were lower by approximately 13–27% of control values in male rats at 15 000 ppm from day 4 onwards. On day 2, this finding was also seen as a trend toward reduced values. In the treated females, serum concentrations of T4 were slightly reduced by approximately 3–13% of control values from day 7 onwards, without being statistically significantly different to the control values. With the exception of day 4, all these changes were seen as a trend toward reduced values.

Concentrations of TSH were statistically significantly increased (approximately 170–280% of control values) in males at 15 000 ppm from day 14 onwards. From day 2 until day 7, this finding occurred as a trend toward increased values. With the exception of day 7, statistically significantly increased concentrations of TSH of approximately 180–280% of control values were found in the sera of the treated females throughout the study.

Liver weights were statistically significantly increased by 25% and 22% in treated males and females, respectively. *p*-Nitrophenol-glucuronyltransferase activities in the liver were statistically significantly increased in treated males and females by almost 2-fold and 1.25-fold, respectively. 4-Methylumbeliferone-glucuronyltransferase activities were statistically significantly increased in treated males and females by 2-fold and 2.4-fold, respectively. 4-Hydroxybiphenyl-glucuronyltransferase activities were statistically significantly increased in treated males and females by almost threefold in both sexes. Thus, the activities of all of the hepatic glucuronyltransferases examined were increased in male and female rats receiving boscalid at 15 000 ppm for 4 weeks. It is reasonable to assume that increased conjugation of T4 was responsible for the increase in circulating concentrations of TSH (Mellert et al., 2001e).

(c) Immunotoxicity

In a test for immunotoxicity, groups of 16 male Wistar rats were given diets containing boscalid (batch No. N37; purity, 94.4%) at a concentration of 0, 100, 1000 or 10 000 ppm, equal to 0, 7.5, 73.1 and 736.2 mg/kg bw per day for 4 weeks. These concentrations were chosen in accordance with those of the two-generation study in rats described above. The rats were about 6 weeks of age when first exposed to boscalid. As a positive control, groups of 16 male Wistar rats were given cyclophosphamide at a dose of 0 (0.5% methyl cellulose solution as vehicle control) and 3 mg/kg bw per day by gavage. Rats were observed daily for clinical signs of toxicity. Food consumption and body weight were recorded. Eight rats of each group were selected for flow cytometry analysis of lymphocytes for anti-sheep-erythrocyte immunoglobulin M (IgM) measurement at the end of the dosing period.

One rat at 10 000 ppm was killed in extremis; otherwise, there were no clinical signs of toxicity or mortalities. In the groups treated with boscalid or cyclophosphamide, food consumption and body weights were comparable to those of the negative control group throughout the treatment period. There were no significant changes in the weights of either the spleen or thymus, and cell numbers in the thymus and spleen were unaltered by treatment. Flow cytometric analysis of lymphocyte subsets from thymus and spleen did not reveal significant differences. A significant increase in the Pan T-cell subset of rats in the group at 100 ppm was noted as an incidental finding with no dose–response relationship, since no similar response was observed at 1000 or 10 000 ppm. There were no statistically significant differences in the serum anti-sheep-erythrocyte IgM antibody titres between the treatment group and control group. In contrast, treatment with cyclophosphamide caused significant decreases in the weight and cell numbers of spleen and thymus, decreases in all the splenic lymphocyte subsets and anti-sheep-erythrocyte IgM antibody titre. The results of this study showed that boscalid does not induce immunotoxicity in rats exposed at dietary concentrations of up to 10 000 ppm, equal to 736.2 mg/kg bw per day, for 4 weeks (Kosaka, 2003).

3. Observations in humans

In the short time since industrial production of boscalid has started, no occupational incidence reports suggesting substance-related adverse effects in personnel engaged in the production of active substance or formulation of preparations have been brought to the attention of the medical department of BASF Aktiengesellschaft.

Comments

Biochemical aspects

In rats given [^{14}C]boscalid as a single oral dose at 50 or 500 mg/kg bw, the radiolabel was rapidly but incompletely absorbed from the gastrointestinal tract, widely distributed, and rapidly eliminated from the body. The excretion balance of boscalid demonstrates that at doses of 50 and 500 mg/kg bw approximately 16% and 3%, respectively, of the administered is excreted via the urine. Excretion via the faeces accounted for 80% (lowest dose) to 95% (highest dose) of the dose; however, 40% of the lowest dose and 12% of the highest dose was eliminated from the body via the bile. Bioavailability decreased with increasing dose, and was estimated to be about 50% at the lowest dose, but only about 15% at the highest dose. The plasma concentration–time curve showed two maxima. The initial half-life was approximately 7–8 h and the terminal half-life was approximately 20–40 h. Tissue distribution determination showed that the largest amounts of radioactivity were in the gastrointestinal tract, liver and adipose tissue. There is no evidence for a cumulative potential of boscalid.

The dermal absorption of boscalid in rats in vivo is approximately 8% or less, depending on the duration of exposure and concentration applied. The initial rate of penetration through rat epidermal membranes was at least 7.7-fold greater than through human epidermal membranes. When the amount of radioactivity remaining associated with the skin was also taken into account, it was concluded that there is no notable difference between rats and humans in the bioavailability of boscalid applied to the skin.

After oral administration to male and female rats, the systemically available portion of boscalid was rapidly and extensively metabolized to a large number of biotransformation products. The hydroxylation of the diphenyl moiety was the quantitatively most important pathway. The second important pathway was the substitution of the chlorine of 2-chloropyridine by conjugation with glutathione. Partial cleavage of the glutathione moiety afforded the cysteine conjugate and finally the sulfhydryl compound that was subsequently methylated or oxidized. In addition, the introduction of glutathione and a second hydroxyl group into the diphenyl moiety was observed. Combinations of these reactions and the conjugation of the hydroxyl groups with glucuronic acid or sulfate, and the conjugation of the sulfhydryl group with glucuronic acid led to the large number of metabolites that have been identified. Cleavage of the amide bond appeared to be negligible because 2-chloronicotinic acid was detected only in trace amounts. No major differences were observed with regard to label, sex or dose.

Toxicological data

In male and female rats, the toxicity of boscalid was low, with values for the oral LD_{50} of > 5000 mg/kg bw, dermal LD_{50} of > 2000 mg/kg bw and inhalation LC_{50} of > 6.7 mg/l. Furthermore, boscalid is not irritating to the skin or the eyes of rabbits. In a maximization test for sensitizing potential in guinea-pigs, about one fifth of the animals developed a reaction upon challenge.

Overall, in short-term studies with boscalid, the signs of toxicity observed in mice, rats and dogs were similar, with reduced body-weight gain, changes in clinical chemistry, and liver enlargement being common features. The results of the short-term studies suggest that the increased liver weights were indicative of an adaptive response to exposure. Histopathology confirmed the liver as a target organ with observation of hypertrophy of hepatocytes, although this too is indicative of an adaptive response. Hypertrophy was accompanied by proliferation of smooth endoplasmic reticulum and the induction of cytochrome P450 protein (although no increased activities of specific CYP isoenzymes were identified) and several microsomal enzymes involved in conjugation reactions. The rat was the only species investigated for these particular effects. There were also increases in blood gamma-glutamyltransferase activity at higher doses. Furthermore, in mice and rats given diets containing high concentrations of boscalid there was increased lipid accumulation in the liver. Absolute thyroid weights were increased in male and female rats of some groups at higher doses, but not in dogs in which there were, however, some increases in relative weight of the thyroid. In male rats there was an increased incidence of thyroid follicular cell hyperplasia at doses of 2000 ppm, equal to 139 mg/kg bw, and higher, but this did not occur in female rats or in dogs of either sex. There is evidence that this effect is due to excessive perturbation of the pituitary–hypothalamus–thyroid-axis homeostatic mechanism, consisting of increases in the activity of hepatic glucuronyltransferase enzymes, some of which are involved in the metabolism of thyroid hormones, and in decreases in the concentrations of circulating thyroid hormones.

The NOAELs for the short-term dietary studies were: 90-day study in rats, 500 ppm (equal to 34 mg/kg bw per day); 90-day dietary study in mice, 150 ppm (equal to 29 mg/kg bw per day); 90-day study in dogs, 250 ppm (equal to 7.6 mg/kg bw per day); 12-month study in dogs, 800 ppm (equal to 22 mg/kg bw per day). In a 4-week study of dermal toxicity with boscalid in rats, no substance-related systemic findings were detected at 1000 mg/kg bw per day, the highest dose tested.

Long-term feeding studies with boscalid in rats and mice confirmed that the primary target organ was the liver. There was no evidence for a carcinogenic potential in mice, and a higher incidence of thyroid follicular cell tumours in rats was due to benign neoplasms, the incidence of these tumours being not statistically significantly different from that in the control group. Since no clear increase in the incidence of thyroid follicular cell tumours was observed, it was not considered necessary to investigate modes of action in a formal or detailed manner. In addition, the $BMDL_{10}$ for thyroid adenomas in male rats, according to a number of statistical models, was 76 mg/kg bw per day. This demonstration, that very high doses are required to produce proliferative responses, even in rats, reinforces the conclusion drawn from the mechanistic studies, i.e. that this is exclusively a phenomenon that occurs at high doses and is not relevant for human exposure. The NOAEL identified in long-term studies in rats was 100 ppm, equal to 4.4 mg/kg bw per day, on the basis of increased gamma-glutamyltransferase activity and hepatic centrilobular hypertrophy and eosinophilic foci in males and increased concentration of cholesterol and reduced prothrombin times in females at 500 ppm, which may be considered as an LOAEL. In long-term studies in mice, the NOAEL was 80 ppm (equal to 13 mg/kg bw per day) on the basis of decreased body-weight gain in males.

Boscalid was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence for genotoxicity was observed in any test. The Meeting concluded that boscalid is unlikely to be genotoxic.

In the absence of genotoxicity and any evidence of relevant carcinogenicity in rodents, the Meeting concluded that boscalid is unlikely to pose a carcinogenic risk to humans.

The reproductive toxicity of boscalid was investigated in a two-generation study of reproduction in rats and in studies of developmental toxicity in rats and rabbits.

Reproductive function was not affected in rats in the two-generation study with boscalid and the NOAEL for reproductive function was 10 000 ppm, equal to 1034 mg/kg bw per day, the highest dose tested. The NOAEL for systemic toxicity in the parental animals in the two-generation study

was 100 ppm, equal to 10 mg/kg bw per day on the basis of reduced body-weight gain, liver-weight increases, spleen-weight decreases and increased incidences of hepatic centrilobular hypertrophy at 1000 ppm, equal to 101 mg/kg bw per day. In pups, decreased body-weight gain and reduced spleen weights at 1000 ppm were the effects that defined the NOAEL for offspring toxicity at 100 ppm, equal to 10 mg/kg bw per day. In the two-generation study of toxicity in rats, there were reductions in spleen and thymus weight in the pups, but these were not consistent with regard to sex and generation and therefore not considered to be toxicologically significant.

In a study of developmental toxicity, rats were given boscalid at doses of up to 1000 mg/kg bw per day. No maternal toxicity was observed. There was an increased incidence of delayed ossifications (of thoracic centra), which was statistically significantly increased at 1000 mg/kg bw and exceeded the range for historical controls. The NOAEL for maternal toxicity was 1000 mg/kg bw per day, the highest dose tested, and the NOAEL for developmental toxicity was 300 mg/kg bw on the basis of reduced ossification of thoracic centra.

In a study of developmental toxicity in rabbits given boscalid at doses of up to 1000 mg/kg bw per day, significant maternal toxicity, consisting of reduced feed consumption and body-weight gain, was observed at the highest dose. There was an increased incidence of delayed ossifications (of thoracic centra) at 1000 mg/kg bw per day. Both the observed litter incidence of delayed ossifications and the number of affected fetuses per litter were statistically significantly increased above control levels and clearly exceeded the range for historical controls. The increase in fetal incidence was dose-related and exceeded the range for historical controls in all groups, including the controls. The NOAEL for maternal toxicity was 300 mg/kg bw per day and the NOAEL for developmental toxicity was 300 mg/kg bw per day on the basis of reduced ossification of thoracic centra.

No signs of neurotoxicity were noted in any studies, including specific studies for neurotoxicity.

There was no evidence of immunotoxicity in a study for immunotoxicity in adult rats exposed to boscalid at dietary concentrations of up to 10 000 ppm, equal to 736.2 mg/kg bw per day for 4 weeks.

The Meeting concluded that the existing data were adequate to characterize the potential hazard to fetuses, infants and children.

Toxicological evaluation

An ADI of 0–0.04 mg/kg bw was established for boscalid based on the NOAEL of 4.4 mg/kg bw per day, identified on the basis of increased gamma-glutamyltransferase activity and increased incidences of hepatic eosinophilic foci in male rats in a 24-month long-term dietary study of toxicity and carcinogenicity and a safety factor of 100.

The Meeting concluded that it was not necessary to establish an ARfD for boscalid in view of the well-demonstrated lack of toxicity in studies of acute toxicity, the absence of relevant developmental toxicity that could have occurred as a consequence of a single exposure, the absence of any indication of neurotoxicity and the absence of any other adverse effects that would be likely to be induced after a single or a small number of exposures in repeat-dose studies.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month study of toxicity and carcinogenicity	Toxicity	80 ppm, equal to 13 mg/kg bw per day	400 ppm, equal to 65 mg/kg bw per day
		Carcinogenicity	8000 ppm[a], equal to 1345 mg/kg bw per day	—

Rat	24-month studies of toxicity and carcinogenicity	Toxicity	100 ppm, equal to 4.4 mg/kg bw per day	500ppm, equal to 23 mg/kg bw per day
		Carcinogenicity	2500 ppm[a], equal to 116 mg/kg bw per day	—
	Two-generation study of reproductive toxicity[b]	Reproductive toxicity	10000 ppm[a] equal to 1034 mg/kg bw per day	—
		Parental toxicity	100 ppm, equal to 10 mg/kg bw per day	1000 ppm, equal to 101 mg/kg bw per day
		Offspring toxicity	100 ppm, equal to 10 mg/kg bw per day	1000 ppm, equivalent to 101 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	1000 mg/kg bw per day[a]	—
		Embryo and fetal toxicity	300 mg/kg bw[a] per day	1000 mg/kg bw per day
Rabbit	Developmental toxicity[c]	Maternal toxicity	300 mg/kg bw per day	1000 mg/kg bw per day
		Embryo and fetal toxicity	300 mg/kg bw per day	1000 mg/kg bw per day
Dog	1-year study of toxicity	Toxicity	800 ppm, equal to 22 mg/kg bw per day	2000 ppm, equal to 57 mg/kg bw per day

[a] Highest dose tested

[b] Measurements of intake of the compound are the mean of the premating phases for F_0 and F_1 females

[c] Gavage administration

Estimate of acceptable daily intake for humans

0–0.04 mg/kg bw

Estimate of acute reference dose

Unnecessary

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 boscalid

Absorption, distribution, excretion and metabolism	
Rate and extent of oral absorption	High, inverse dose-dependent bioavailability of 50–15%; two plasma T_{max} values, 1 h and 8h (rat)
Dermal absorption	Approximately 8% (rat)
Distribution	Distributed throughout the body; higher concentrations in liver and gastrointestinal tract
Potential for accumulation	No evidence
Rate and extent of excretion	High (but determined by the low rate of absorption); essentially 100% excretion in bile and urine within 168 h,
Metabolism in animals	Extensive, about 40 metabolites, little parent compound remaining
Toxicologically significant compounds (animals, plants and environment)	Parent

Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 6.7 mg/l air (4 h)
Rabbit, LD_{50}, dermal	> 2000 mg/kg bw
Rabbit, skin irritation	Not irritating
Rabbit, eye irritation	Not irritating
Guinea-pig, skin sensitization	Not sensitizing
Short-term studies of toxicity	
Target/critical effect	Liver, thyroid; body weight
Lowest relevant oral NOAEL	22 mg/kg bw per day (12-month study in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (4-week study in rats)
Lowest relevant inhalation NOAEC	No data
Genotoxicity	Not genotoxic in vivo or in vitro
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver, thyroid; body weight
Lowest relevant NOAEL	4.4 mg/kg bw per day (24-month study in rats)
Carcinogenicity	Boscalid induced a low incidence of benign thyroid tumours in rats
Reproductive toxicity	
Reproductive target/critical effect	None
Lowest relevant reproductive NOAEL	1034 mg/kg bw[a,b] per day
Developmental target/critical effect	Not teratogenic; reduced fetal body weight, delayed ossifications
Lowest relevant developmental NOAEL	300 mg/kg bw[a] per day (rat, rabbit)
Neurotoxicity/delayed neurotoxicity	
	No signs of neurotoxicity
Other toxicological studies	
	Liver xenobiotic metabolizing enzyme induction.
Medical data	
	No reports of toxicity in workers exposed during manufacture or use

Summary

	Value	**Study**	**Safety factor**
ADI	0–0.04 mg/kg bw	Rat, 2-year study of toxicity and carcinogenicity	100
ARfD	Unnecessary	—	—

[a] Highest dose tested

[b] Measurements of intake of the compound are the mean of the pre-mating phases for P and F_1 females

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