MYCLOBUTANIL

First draft prepared by
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Explanation

Myclobutanil is the International Organization for Standardization (ISO)–approved common name for (*R*,*S*)-2-(4-chlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)hexanenitrile (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service number 88671-89-0. It is a broad-spectrum fungicide of the substituted triazole chemical class of compounds. It acts by inhibiting sterol biosynthesis in fungi.

Myclobutanil was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1992, when an acceptable daily intake (ADI) of 0–0.03 mg/kg body weight (bw) was established, on the basis of a no-observed-adverse-effect level (NOAEL) of 2.5 mg/kg bw per day in a 2-year study in rats. No acute reference dose (ARfD) was established, because the establishment of ARfDs by JMPR was not common practice in 1992. Myclobutanil was reviewed by the present Meeting as part of the periodic review programme of the Codex Committee on Pesticide Residues.

New studies on acute toxicity, skin and eye irritation, skin sensitization and carcinogenicity in rats and mice as well as a reanalysis of a developmental toxicity study in rats were submitted. New studies on the acute toxicity, short-term toxicity or genotoxicity of metabolites, degradates or

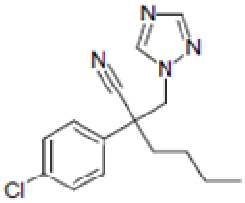
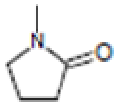
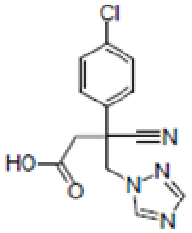
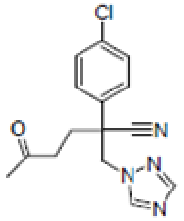
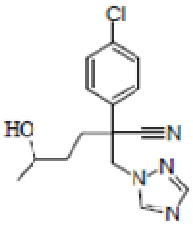
impurities were also submitted. All critical studies contained statements of compliance with good laboratory practice (GLP). Overall, the Meeting considered that the database was adequate for the risk assessment.

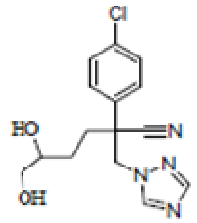
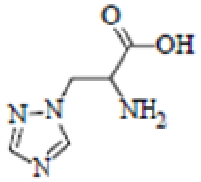
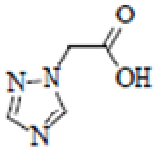
Evaluation for acceptable daily intake

1. Biochemical aspects

Structures of parent compounds, metabolites and degradation products in various crops and animals are shown in Table 1.

Table 1. Structures of parent compounds, metabolites and degradates in various crops and animals

Code/common name	Chemical name	Structural formula
Myclobutanil	(*RS*)-2-(4-Chlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)hexanenitrile	
1-Methylpyrrolidin-2-one	1-Methylpyrrolidin-2-one	
Myclobutanil butyric acid	(3*RS*)-3-(4-Chlorophenyl)-3-cyano-4-(1*H*-1,2,4-triazol-1-yl)butanoic acid	
RH-9089 (#3)[a]	(2*RS*)-2-(4-Chlorophenyl)-5-oxo-2-(1*H*-1,2,4-triazol-1-ylmethyl)hexanenitrile	
RH-9090 (#4)	(2*RS*,5*RS*)-2-(4-Chlorophenyl)-5-hydroxy-2-(1*H*-1,2,4-triazol-1-ylmethyl)hexanenitrile	

Code/common name	Chemical name	Structural formula
RH-0294 (#6)	(2*RS*,5*RS*)-2-(4-Chlorophenyl)-5,6-dihydroxy-2-(1*H*-1,2,4-triazol-1-ylmethyl)hexanenitrile	
Triazolyl alanine	(2*RS*)-2-Amino-3-(1*H*-1,2,4-triazol-1-yl)propanoic acid	
Triazolyl acetic acid	1*H*-1,2,4-Triazol-1-ylacetic acid	
1,2,4-Triazole	1*H*-1,2,4-Triazole	

[a] Numbers in parentheses refer to the metabolites in Table 2 and Fig. 1 below.

1.1 Absorption, distribution and excretion

(a) [^{14}C]Myclobutanil labelled in the chlorophenyl ring

Mice

The disposition and metabolism (see section 1.2) of [^{14}C]myclobutanil labelled in the chlorophenyl ring were investigated in a study with male and female CD-1 mice, following the administration of non-radiolabelled myclobutanil (TD83-086; lot no. LSPL0016E; purity 81.1%) for 2 weeks in the diet at 0, 10, 100 or 1000 parts per million (ppm) (equal to 0, 2.1, 22.3 and 218 mg/kg bw per day, respectively). The [^{14}C]myclobutanil (TD83-184; lot no. 424.0107; specific activity 380.4 MBq/g; radiochemical purity 99.7%) was administered as a single oral dose by gavage at 0, 2, 20 or 200 mg/kg bw, respectively, in 0.5% weight per volume (w/v) methyl cellulose. Whole blood, urine and faeces were collected and analysed. This study was conducted in compliance with GLP, but not in accordance with test guidelines.

Absorption. Radiolabel was rapidly absorbed by mice, with a half-life for absorption ranging from 0.04 hour (at 10 ppm in females) to 0.31 hour (at 1000 ppm in males), but with no clear difference between the sexes. Maximum whole blood concentrations were reached within 0.25–1 hour and were proportional to dose in both sexes. Elimination of radiolabel from blood of males at 1000 ppm was monophasic (half-life = 6.2 hours). Elimination of radiolabel from blood in both sexes of all other groups was biphasic: a rapid phase lasting 4–6 hours, with half-lives ranging from 0.63 hour (1000 ppm in females) to 0.88 hour (10 ppm in females), was followed by a more variable slow phase, with half-lives ranging from 6.0 hours (1000 ppm in females) to 30.1 hours (10 ppm in males). The area under the whole blood concentration–time curve (AUC) was proportional to dose and was similar in males and females at each dose. At 24 hours, only 0.07–0.15% of the administered radioactivity remained in the blood.

Distribution. At 1 hour after dosing, plasma concentrations of radiolabel were similar to whole blood levels at each dose, for both sexes (1.18–2.03% of the dose). Liver concentrations were also similar for both sexes at each dose (4.79–10.53% of the dose), with levels 3.9- to 11.1-fold higher than in whole blood. The liver/blood ratio of radiolabel decreased with increasing dose (6.8% or 8.4% at 2 mg/kg bw for males and females, respectively, to 4.0% or 2.9% at 200 mg/kg bw for males and females, respectively). These data suggest that the liver has a greater affinity for radiolabel, and the ability to extract or bind the radiolabel diminished with increasing dose, although this did not cause a disproportionate increase in radiolabel in the whole blood.

Excretion. Most of the administered radioactivity was recovered in the urine and faeces within 24–48 hours after dosing. By 96 hours, the proportion excreted was 61–67% of the dose for males and 67–87.2% for females. An additional significant amount of radiolabel (14.1–24.0%) was recovered in the cage washes at the end of 96 hours.

In conclusion, following its repeated oral dosing to mice, [^{14}C]myclobutanil was completely and rapidly absorbed and essentially completely excreted by 96 hours. Maximum plasma and tissue ^{14}C concentrations were achieved within 1 hour after oral administration and were dose proportional. Plasma elimination was biphasic in all but high-dose males. No significant tissue accumulation was observed 96 hours after dosing, although liver concentrations were higher than whole blood levels at 1 hour after dosing (Steigerwalt, Udinsky & Longacre, 1986b).

Rats

The disposition and metabolism (see section 1.2) of [^{14}C]myclobutanil (radiolabelled at all carbons in the chlorophenyl ring), suspended in 0.5% w/v methyl cellulose, were investigated in male and female Sprague-Dawley rats (Steigerwalt, Udinsky & Longacre, 1986a). [^{14}C]Myclobutanil was administered to rats according to three protocols: as single oral doses (10 mL/kg bw) of 1 mg/kg bw (TD83-116; lot no. 424.0102; specific activity 380.4 MBq/g; radiochemical purity 97.3%) or 100 mg/kg bw (TD83-115; lot no. 424.0103; specific activity 59.9 MBq/g; radiochemical purity 96.9%) or 100 mg/kg bw (TD83-115; lot no. 424.0103; specific activity 59.9 MBq/g; radiochemical purity 96.9%) following 2 weeks of dietary administration of 1000 ppm non-radiolabelled myclobutanil (TD83-086; lot no. LSPL0016E; purity 81.1%). An additional group of rats received a single intravenous dose (1 mL/kg bw) of [^{14}C]myclobutanil (TD83-115; lot no. 424.010 3; specific activity 59.9 MBq/g; radiochemical purity 96.9%) at 1 mg/kg bw in 20% w/v 2,5-dimethylisosorbide. Radioactivity in whole blood, plasma, various organs and tissues, urine and faeces was analysed by liquid scintillation counting after combustion.

Absorption. Practically all (89.2–114.6%) of the [^{14}C]myclobutanil was absorbed by the rats, based on the relative percentage of radiolabel excreted in urine following oral and intravenous administration. Plasma concentrations following administration of a single dose or repeated doses reached maximum levels within 1 hour after the treatment. The maximum plasma concentrations were 19.6 and 23.8 ppm in the single-dose and repeated-dose groups, respectively.

Plasma elimination of radiolabel was biphasic, with a rapid phase lasting 12–24 hours. The rapid elimination phase was faster in whole blood for the 100 mg/kg bw myclobutanil group rats. The slower phase was even slower and the AUC was slightly greater in whole blood for both single and repeated orally dosed rats. Kinetics data are shown in Table 2.

Distribution. Radiolabel rapidly appeared in all tissues of male rats, reaching maximum concentrations within 1 hour, ranging from 6.29 ppm (brain) to 56.65 ppm (liver) for single orally dosed males and from 15.01 ppm (brain) to 153.7 ppm (liver) for repeatedly dosed males. Radiolabel was rapidly eliminated from the tissues in a biphasic manner, as observed in plasma, with no evidence of significant accumulation. Less than 0.7% of the dose remained in tissues of single or repeatedly dosed rats at 96 hours.

Excretion. Following an intravenous dose of 1 mg/kg bw, 54.86–64.36% was recovered in urine (including funnel wash) and faeces within 24 hours of dosing. Total ^{14}C excretion by 96 hours post-dosing was 72.6% and 78.72% for males and females (representing 94% and 96% of the

Table 2. Kinetics data in rats

Parameter	Single dose (100 mg/kg bw)		Repeated dose	
	Serum	**Whole blood**	**Serum**	**Whole blood**
C_{max} (ppm)	19.6	26.2	23.8	19.9
$t_{½}$ (h)				
Rapid phase	5.25	1.61	1.97	2.04
Slow phase	25.7	38.5	31.5	49.5
AUC (h·µg/g)	246	276	226	289

AUC: area under the concentration–time curve; bw: body weight; C_{max}: maximum concentration; ppm: parts per million; $t_{½}$: half-life

Source: Steigerwalt, Udinsky & Longacre (1986a)

recovered radiolabel), respectively, with an equivalent distribution between both urine and faeces. In oral treatment with 1 or 100 mg/kg bw (both single and repeated dosing), 40.8–81.11% of the dose was recovered in the urine and faeces within 24–48 hours post-dosing. By 96 hours, total excretion ranged from 73.91% (1 mg/kg bw in females) to 88.12% (10 mg/kg bw in females) of the dose, representing 89–98% of recovered radiolabel, again with an equivalent distribution between both urine and faeces. Between 75% and 94% of myclobutanil was excreted within 48 hours following both intravenous and oral treatment.

In conclusion, in the single-dose oral study in rats, [^{14}C]myclobutanil was completely and rapidly absorbed (> 89%) and rapidly and essentially completely excreted. Maximum plasma and tissue ^{14}C levels were achieved within 1 hour after oral administration, and plasma elimination was biphasic. Most myclobutanil was excreted within 48 hours. No significant tissue accumulation was observed 96 hours after dosing.

This study was GLP compliant and conducted in accordance with test guidelines (Steigerwalt, Udinsky & Longacre, 1986a).

(b) [^{14}C]Myclobutanil labelled at carbons 3 and 5 of the triazole ring

Rats

A single-dose oral study in rats was conducted to define the path and rate of excretion of myclobutanil labelled with ^{14}C in the triazole ring, to identify in which organs of rats potential accumulation might occur and to characterize the excreted material for parent and metabolite compounds (see section 1.2). Four male and four female Sprague-Dawley rats were given a single oral gavage dose of 2000 ppm (equal to 150 mg/kg bw) [^{14}C]myclobutanil (lot no. 417.01; specific activity 406.3 MBq/g) radiolabelled at carbons 3 and 5 of the triazole ring. This study was not conducted in compliance with GLP (Streelman, 1984).

Excretion. Most of the radioactivity was excreted in the urine and faeces, with only a small portion appearing in the expired air as carbon dioxide. Excluding the cage washes, an average of 99.3% of the recovered radioactivity was contained in the urine (16–54%) and faeces. The distribution between urine and faeces varied considerably between rats. The excretion pattern for both males and females fit a first-order kinetic model.

Distribution. The highest concentrations of radioactivity were found in the liver, kidney, and small and large intestines. Residue levels were reduced with time and lower in the females than in the males.

In conclusion, in this study, myclobutanil was rapidly excreted from rats. There was no accumulation in any organ or tissue (Streelman, 1984).

1.2 Biotransformation

Mice

In a 2-week repeated-dose oral study in mice (see section 1.1), myclobutanil was extensively metabolized to a number of more polar metabolites, with parent compound representing only 1–7% of the administered dose. The metabolite profiles were comparable between males and females at all three doses. There were 3–4 major fractions (i.e. constituting 10% of the total) and six minor fractions detected in the excreta (Steigerwalt, Udinsky & Longacre, 1986b).

Rats

In the first single-dose study in rats (see section 1.1), myclobutanil was extensively metabolized to more polar metabolites, the parent compound representing only 1.0–3.6% of the excreted dose. In orally dosed males, five major fractions were eliminated in the excreta. The excreta of orally dosed females contained one dominant fraction that accounted for 53.0–61.1% of the excreted radiolabel. Metabolites were qualitatively similar in males and females, although quantitatively different in the amounts of certain fractions excreted (Steigerwalt, Udinsky & Longacre, 1986a).

In the second single-dose study in rats (see section 1.1), thin-layer chromatography showed a range of metabolites, all of which were more polar than the parent. The overall distribution of metabolites in urine and faeces of male and female rats is shown in Table 3. The same metabolites (metabolite fractions #2, #3, #4, #5, #6 and #7) were found in urine and faeces of both males and females, although relative distributions were different. In females, metabolite fraction #7 (sulfate of RH-9090) was a major metabolite in both urine and faeces. The major metabolic processes involved oxidation of the butyl group. Among the metabolites formed were RH-9090 and RH-9089 (metabolite fractions #4 and #3, respectively, in Table 1), the major unconjugated phenethyl triazole-containing metabolites found in plants. The distributions of RH-9090 and RH-9089 were approximately 10% or lower in the overall distribution of metabolites in rats (Streelman, 1984).

In conclusion, myclobutanil was extensively metabolized to more polar compounds (Streelman, 1984; Steigerwalt, Udinsky & Longacre, 1986a).

Table 3. Overall distribution of metabolites in the rat

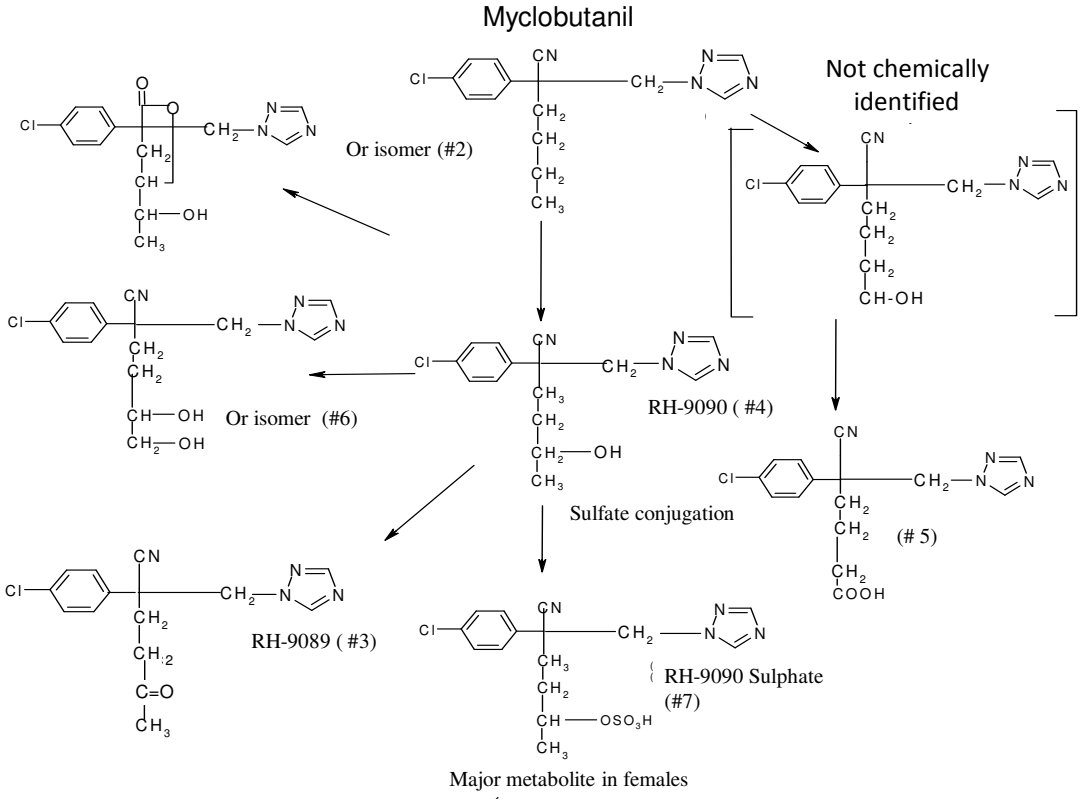
	Distribution of metabolites (%)						
	Urine		**Faeces**		**Total**		
Parent/metabolites	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Rf value**
Myclobutanil	0.6	1.7	4.5	2.2	2.8	2.0	0.66
#2[a]	8.8	2.1	14.3	0.4	11.9	1.0	0.58
#3 RH-9089	8.7	2.5	10.4	0.8	9.7	1.4	0.54
#4 RH-9090	6.4	4.7	14.3	3.5	10.8	3.9	0.50
#5	10.6	1.5	6.8	0.4	8.5	0.8	0.45
#6	26.1	18.1	12.2	5.4	18.3	9.9	0.39
#7 RH-9090 sulfate	11.9	62.3	17.8	81.5	15.2	74.7	0.20
Unknown 1	8.8	1.5	2.4	0.2	5.2	0.7	0.35
Unknown 2	3.3	1.3	3.4	0.0	3.4	0.5	0.14
Origin	9.7	2.0	9.8	0.5	9.8	1.0	–
Remainder	5.1	2.6	4.3	5.3	4.7	4.3	–

Rf: retardation factor

[a] Numbers in parentheses refer to structures given in Table 1 and Fig. 1.
Source: Streelman (1984)

A proposed metabolic scheme in rats is presented in Fig. 1.

Fig. 1. Proposed metabolic pathway in the rat



Source: Streelman (1984)

2. Toxicological studies

2.1 Acute toxicity

Studies on acute toxicity, skin or eye irritation, and skin sensitization are summarized in Table 4.

(a) Lethal doses

Mice

Three acute toxicity studies were performed. In the first study, median lethal doses (LD_{50}s) in male and female ICR mice were 1910 and 1840 mg/kg bw, respectively, and the lowest lethal dose was 1300 mg/kg bw in both sexes (Morrison et al., 1986). The LD_{50} in female ICR mice was 1360 mg/kg bw in the second study (Morrison, Murphy & Chan, 1984). In the third study, LD_{50}s in male and female ICR mice were 2270 and 2440 mg/kg bw, respectively; the lowest lethal dose in the study was 1870 mg/kg bw in both sexes (Shimizu, Tokiwa & Nakayoshi, 1987).

Rats

Two acute toxicity studies were conducted with male and female rats.

Table 4. The acute toxicities, skin or eye irritation, and skin sensitization of myclobutanil

Route (method)	Species/ strain (sex)	Purity (%)	Result	Reference
Oral, gavage	Rat/CD (M + F)	84.5	LD_{50} = 1 750 mg/kg bw (M) LD_{50} = 1 800 mg/kg bw (F)	Krzywicki (1983)
Oral, gavage	Rat/CD (M + F)	91.9	LD_{50} = 1 600 mg/kg bw (M) LD_{50} = 2 290 mg/kg bw (F)	Krzywicki & Morrison (1984)
Oral, gavage ("up and down" method)[a]	Rat/F344 (F)	95.1	LD_{50} = 3 129 mg/kg bw per day	Moore (2005b)
Oral	Mouse/ICR (M + F)	91.9	LD_{50} = 1.91 g/kg bw (M) LD_{50} = 1.84 g/kg bw (F)	Morrison et al. (1986)
Oral	Mouse/ICR (F)	91.9	LD_{50} = 1.36 g/kg bw	Morrison, Murphy & Chan (1984)
Oral	Mouse/ICR (M + F)	91.4	LD_{50} = 2.27 g/kg bw (M) LD_{50} = 2.44 g/kg bw (F)	Shimizu, Tokiwa & Nakayoshi (1987)
Dermal[a]	Rat/F344 (M + F)	95.1	LD_{50} > 5 000 mg/kg bw	Moore (2005a)
Dermal	Rabbit/NZW (M + F)	84.5	LD_{50} > 5 000 mg/kg bw	Krzywicki (1983)
Dermal	Rabbit/NZW (M + F)	91.9	LD_{50} > 5 000 mg/kg bw	Krzywicki & Bonin (1984)
Inhalation, nose only[b]	Rat/CD (M + F)	91.4	LC_{50} > 5.1 mg/L	Fisher, Emmons & Hagan (1987)
Dermal	Rabbit/NZW (M)	84.5	Not irritating	Krzywicki (1983)
Dermal	Rabbit/NZW (M)	91.9	Not irritating	Krzywicki & Bonin (1984)
Dermal[a]	Rabbit/NZW (M)	95.1	Very slightly irritating	Moore (2005c)
Eye	Rabbit/NZW (M)	84.5	Slightly irritating	Krzywicki (1983)
Eye	Rabbit/NZW (M)	91.9	Slightly to moderately irritating	Krzywicki & Bonin (1984)
Eye[a]	Rabbit/NZW (M)	95.1	Slightly irritating	Merkel (2005)
Dermal (Buehler method)[b]	Guinea-pig/ Hartley albino (M + F)	91.4	Minimally sensitizing	Bonin & Hazelton (1987)
Dermal (maximization test)	Guinea-pig/ Hartley albino (M + F)	91.4	Not sensitizing	Kreuzmann (1989)
Dermal (local lymph node assay)[a]	Mouse/BALB/c (F)	95.1	Not sensitizing	Woolhiser, Wiescinski & Anderson (2005)

bw: body weight; F: female; F344: Fischer 344; LC_{50}: median lethal concentration; LD_{50}: median lethal dose; M: male; NZW: New Zealand White

[a] Conducted in accordance with the guidelines of the Organisation for Economic Co-operation and Development, the United States Environmental Protection Agency, the European Commission and the Japanese Ministry of Agriculture, Forestry, and Fisheries.

[b] Conducted in accordance with the guidelines of the Organisation for Economic Co-operation and Development and the United States Environmental Protection Agency.

In the first oral acute toxicity study with CD rats, the LD_{50}s in males and females were 1600 and 2290 mg/kg bw, respectively (Krzywicki & Morrison, 1984). Mortality occurred at doses of 1100 mg/kg bw and above. Clinical signs of toxicity observed in the treated rats occurred from the day of dosing, at greater severity with increasing dose, and included passiveness, ataxia, prostration, emaciation, salivation, abdominal breathing, lacrimation, alopecia and scant droppings. Staining of the urogenital/anogenital area, muzzle and eyes was also observed. There were no treatment-related clinical observations in control rats, which showed some staining of anogenital areas and muzzle only (Krzywicki, 1983).

In the second study with F344 rats and using the "up and down" procedure, no treatment-related changes were detected at 175 mg/kg per bw. At 1750 mg/kg per bw, all rats survived, but clinical signs of toxicity were observed in two rats, including anogenital staining and/or hypoactivity. However, the rats recovered by day 3. At 5000 mg/kg bw, all four females died within 1 day of test substance administration. Prior to death, all rats were hypoactive and/or exhibited abnormal posture, anogenital staining, piloerection and diarrhoea (Moore, 2005b).

Based on these two studies, LD_{50} values for myclobutanil in rats were 1600–3129 mg/kg bw.

(b) Dermal irritation

In two of three skin irritation studies using male New Zealand White rabbits, no irritation was detected (Krzywicki, 1983; Krzywicki & Bonin, 1984). In one study, very slight erythema was observed at 30–60 minutes after dosing. Recovery occurred in the three rabbits by 24, 48 and 72 hours, respectively (Moore, 2005c).

Based on the three studies, myclobutanil showed no irritation to rabbit skin.

(c) Ocular irritation

Three New Zealand White rabbit eye irritation studies of myclobutanil have been made available, using unformulated material.

In the first of these, the test substance stained the skin and fur around the treated eyes. Hair loss was also observed in these areas. Signs of eye irritation in the conjunctiva (report does not state whether there was redness or chemosis) were observed only in two rabbits with unwashed eyes at 24 hours post-dosing. These effects were reversible by 72 hours, and no other effects were recorded (Krzywicki, 1983).

In the second study, slight to moderate irritation was observed in the cornea (5/6), iris (3/6) and conjunctiva (6/6) at 24 hours post-dosing for rabbits with unwashed eyes. An uneven, pitted area was observed at the centre of the cornea of one rabbit with corneal and conjunctival effects. Myclobutanil deposits were noted to be present in the eyes of 3/6 rabbits and around the eyes of the remaining three rabbits. Irritation of reduced severity was present at 48 and 72 hours in the cornea (4/6 and 3/6, respectively) and conjunctiva (6/6 at both time points). The test substance was noted to be present around the eyes of the same three rabbits as before at 48 hours and in one rabbit at 72 hours. At 7 days, irritation of the conjunctiva was still present in two rabbits. No irritation was observed at 14 and 21 days. However, a few blood vessels had extended into the cornea of one rabbit from day 7 (one of the three rabbits with test material observed in the treated eye at 24 hours) and, after dosing, a 3 mm distinct hazy yellow stained area was seen on the cornea of another rabbit (one of the three rabbits with test material observed around the treated eye at 24, 48 and 72 hours). For rabbits with washed eyes, irritation was observed in all three animals in the conjunctiva at 24 hours and only in one animal at 72 hours (Krzywicki & Bonin, 1984).

In the third eye irritation study, 1 hour after test substance instillation, all three treated eyes exhibited iritis and conjunctivitis. Corneal opacity was evident in two rabbits by 24 hours. The overall incidence and severity of irritation decreased thereafter. All animals were free of ocular irritation by 72 hours (Merkel, 2005).

On the basis of these three studies, myclobutanil was slightly to moderately irritating to the eyes of rabbits.

(d) Dermal sensitization

In a modified Buehler delayed contact hypersensitivity study in guinea-pigs (six animals of each sex per group), minimal to no erythema was observed in the naive control group at 24 and 48 hours following challenge with either myclobutanil (50% weight per weight [w/w]) or a positive control material, 1-chloro-2,4-dinitrobenzene (DNCB), at 800 ppm. In the positive control DNCB group, 7/12 and 6/12 guinea-pigs responded with erythema at 24 and 48 hours, respectively. In the myclobutanil (50% w/w) group, erythema was observed in 3/12 and 1/12 guinea-pigs at 24 and 48 hours, respectively. The decreasing erythema response to myclobutanil over time suggests either a local irritation effect or a weak sensitization effect, and hence a rechallenge phase was considered necessary. In the rechallenge study, no erythema was observed in the naive control group at 24 or 48 hours following challenge with either myclobutanil or DNCB. In the DNCB group, 6/12 and 5/12 guinea-pigs responded with erythema at 24 and 48 hours, respectively. In the myclobutanil group, 1/12 and 0/12 animals exhibited erythema at 24 and 48 hours, respectively. The same one animal responded at challenge at 24 hours, at challenge at 48 hours and at rechallenge at 24 hours, but showed no reaction at rechallenge at 48 hours. The authors concluded that the potential of myclobutanil to produce delayed contact hypersensitivity in guinea-pigs had not been determined (Bonin & Hazelton, 1987). As the Buehler method does not have the extreme sensitivity of the Magnusson-Kligman protocol, this result suggested that myclobutanil was a mild sensitizer to rabbit eyes.

In a maximization test using guinea-pigs (10 animals of each sex per group), 2/20 animals showed a minimal response to myclobutanil. Therefore, myclobutanil was considered to be not sensitizing (Kreuzmann, 1989).

In a local lymph node assay using BALB/cAnNCrl mice (six females per group), erythema was not observed in the mice treated with 5% or 20% myclobutanil, whereas 5/6 mice treated with 80% myclobutanil showed slight erythema on day 6. Topical application of 5%, 20% or 80% myclobutanil elicited proliferative responses/stimulation indices that were, respectively, 1.1-, 1.5- and 1.6-fold greater than in vehicle controls. Myclobutanil did not demonstrate dermal sensitization potential in the mouse local lymph node assay, as the lymph nodes draining the area of topical application did not demonstrate a 3-fold proliferation (stimulation index) when compared with vehicle-treated mice (Woolhiser, Wiescinski & Anderson, 2005).

Overall, in three studies, myclobutanil was equivocal for sensitization due to a lack of sensitization using the guinea-pig maximization test and the mouse local lymph node assay and mild sensitization using the Buehler method.

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Myclobutanil (TD 83-076; lot no. LSPL0016/E; purity 81.1%) was administered in the diet to nine groups of Crl:CD-1(IcCR)BR mice (10 of each sex per group) for 3 months at 0, 3, 10, 30, 100, 300, 1000, 3000 or 10 000 ppm (equal to 0, 0.40, 1.54, 4.79, 14.1, 42.7, 132, 542 and 2035 mg/kg bw per day for males and 0, 0.62, 2.11, 6.94, 22.9, 65.5, 232, 710 and 2027 mg/kg bw per day for females, respectively). This study was not conducted in accordance with test guidelines, but all the examinations required by test guidelines were conducted in this study with the exception of an ophthalmological test. Liver tissues from four randomly selected mice of each sex per group at 30 and 10 000 ppm myclobutanil were taken for liver mixed-function oxidase (MFO) assay.

At 1000 ppm, one male died at week 3, the cause being considered unrelated to treatment, because no deaths occurred at any of the higher dose levels. Nevertheless, the cause of death was not identified. No treatment-related clinical signs were observed throughout the study. There was a

statistically significant decrease in body weight gain throughout the treatment period at 10 000 ppm for both sexes; body weights for males and females were reduced by 19% and 7%, respectively, compared with controls, at 13 weeks. Male body weight gains were also statistically significantly decreased at 3000 ppm, with body weights being 7% of control values at 13 weeks. Feed consumption of males at 10 000 ppm was statistically significantly reduced during the first week of dosing (26% of controls) only and was greater than control values during weeks 9–13. For females at this dose level, feed consumption was statistically significantly decreased during week 1 (39% of controls). It was lower than the intake of controls throughout the treatment period, but not statistically significantly. The reduced feed consumption at the beginning of treatment was considered to be due to low palatability, rather than an adverse effect, as no similar change in feed consumption was observed in gavage studies (developmental toxicity studies in rats and rabbits) of myclobutanil, and no effects on the gastrointestinal tract were observed. In haematology, statistically significant decreases in the haematocrit and mean corpuscular volume (MCV) values were seen in males and females at 10 000 ppm, but not at 3000 ppm. Also at 10 000 ppm, males had significant decreases in white blood cells and the number of lymphocytes and an increase in the number of segmented neutrophils, whereas females had decreased haemoglobin and increased platelet values. There were no other treatment-related changes in haematological parameters.

At 10 000 ppm, statistically significantly increased levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT) and urea nitrogen were observed in male and female mice; ALT was also increased in males at 3000 ppm. Glucose and cholesterol levels were statistically significantly reduced in both sexes at 10 000 ppm, glucose was reduced in females at 3000 ppm and cholesterol was reduced in both sexes at 3000 ppm and in males at 1000 ppm. Blood chemistry data are summarized in Table 5. There were no other treatment-related changes in blood chemistry parameters.

Table 5. Summary of blood chemistry in the 90-day oral toxicity study in mice

	0 ppm		**1 000 ppm**		**3 000 ppm**		**10 000 ppm**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
AST (U/L)	78.3	125.0	86.0	111.2	115.2	131.1	410.3*	281.9*
ALT (U/L)	27.6	24.8	46.4	33.1	101.8*	72.7	539.4*	371.7*
ALP (U/L)	40.7	41.1	30.1	38.5	52.1	52.3	100.1*	85.6*
BUN (mg/dL)	22.88	21.06	30.14*	20.82	27.09	23.68	39.22*	28.63*
GLUC (mg/dL)	82.3	90.4	71.2	74.1	76.9	64.0*	51.7*	65.0*
GGT (U/L)	0.2	0	0.3	0	0	0	1.4*	2.3*
CHOL (mg/dL)	99.4	61.9	65.3*	52.9	37.2*	36.9*	19.1*	35.0*

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CHOL: cholesterol; GGT: gamma-glutamyltransferase; GLUC: glucose; ppm: parts per million; U: units; *: $P < 0.05$

Source: Goldman, Harris & Lampe (1986)

Statistically significant, modest treatment-related increases in hepatic MFO activity were noted at 3 months; increases in cytochrome P450 per milligram of microsomal protein at 3000 ppm and 10 000 ppm were 85% and 73%, respectively, in males and 86% and 99%, respectively, in females, compared with controls.

Liver weights (absolute and relative to body weight) were statistically significantly increased in both sexes at 3000 ppm (69% and 48% in males and females, respectively, for relative liver weights) and 10 000 ppm (152% and 141% in males and females, respectively, for relative liver weights), and relative liver weights were statistically significantly increased in both sexes at 1000

ppm (35% and 18% in males and females, respectively). Some changes in relative weights for brain, adrenal and gonads were observed as a result of decreased terminal body weights at 10 000 ppm.

At necropsy, there were enlarged livers with or without accentuated lobular architecture at 1000 ppm and above. Treatment-related microscopic changes observed are summarized in Table 6. The liver was the primary target. Microscopically, hepatocellular hypertrophy was increased in males at 1000 ppm and above and in females at 3000 ppm. The hypertrophied area increased in size with increasing dose. Swollen vacuolated centrilobular hepatocytes and single large hepatocytic vacuoles (both histopathological findings were considered fatty changes) were observed at the same doses as hepatocellular hypertrophy in both sexes. Single-cell necrosis of hepatocytes detected in the centrilobular area in males at 1000 and 3000 ppm and in females at 3000 ppm were considered to progress to necrotic hepatitis or coagulative necrosis at 10 000 ppm in both sexes. Pigmented Kupffer cells were observed in males at 3000 ppm and above and in females at 10 000 ppm, and bile duct proliferation was detected in both sexes at 10 000 ppm. Other treatment-related histopathological changes included cytoplasmic eosinophilia and/or hypertrophy of the zona fasciculata cells of the adrenal glands in males at 1000 ppm and in both sexes at 3000 ppm and above. These adrenal changes might reflect the severe stress state of the animals. Pigment was seen in the macrophages in the spleen at 3000 ppm and above. Lymphoid necrosis in the spleen was seen in one or both sexes at 3000 ppm and above. An increased myeloid:erythroid ratio, primarily involving granulocytes in the bone marrow in some females at 10 000 ppm, might be related to inflammatory or necrotic changes in the liver or lymphoid tissues. Atrophy of the uterus and absence of corpora lutea in the ovaries, suggesting no ovulation, were observed at 10 000 ppm. An increased mononuclear cell infiltration in the skin in both sexes was observed at 10 000 ppm.

The NOAEL for 90-day oral toxicity in mice was 300 ppm (equal to 42.7 mg/kg bw per day), based on hepatotoxicity, including hepatocellular hypertrophy, fatty changes and hepatocellular necrosis, in males at 1000 ppm (equal to 132 mg/kg bw per day) (Goldman, Harris & Lampe, 1986).

Rats

Myclobutanil (TD 83-076; lot no. LSPL0016/E; purity 81.1%) was administered in the diet to nine groups of CD (SD)BR rats (10 of each sex per group) for 3 months at a dietary concentration of 0, 5, 15, 50, 150, 500, 1500, 5000 or 15 000 ppm for weeks 1 and 2; 0, 7, 21, 70, 210, 700, 2100, 7000 or 21 000 ppm, respectively, for weeks 3 and 4; and 0, 10, 30, 100, 300, 1000, 3000, 10 000 or 30 000 ppm, respectively, for the remainder of the study. These dietary concentrations were equal to doses of 0, 0.52, 1.60, 5.22, 15.3, 51.5, 158, 585 and 1730 mg/kg bw per day for males and 0, 0.67, 2.03, 6.85, 19.7, 65.8, 195.2, 665 and 1811 mg/kg bw per day for females, respectively. This study was not conducted in accordance with test guidelines, but almost all examinations required by test guidelines were conducted in this study. Haematological and clinical chemistry examinations were conducted on day 32 and day 95. Samples of hepatic tissue were taken from three rats of each sex per group at 100, 300, 1000, 3000 and 10 000 ppm myclobutanil for liver MFO assay.

All rats treated with 15 000/21 000/30 000 ppm myclobutanil died during the treatment period, males during days 17–63 and females during days 18–49. Clinical signs observed prior to death included a brown-stained anogenital area, red- or brown-stained muzzle, scant faecal droppings and emaciation. One or two rats of each sex were also lethargic or ataxic prior to death. One male at 5000/7000/10 000 ppm was found dead on day 83 because of an accident. Body weights of males and females were significantly decreased at 5000/7000/10 000 ppm and above throughout the study. At 1500/2100/3000 ppm, statistically significant decreases in body weight were noted during weeks 6–12 of the study in males (8% at week 13), but only during week 9 in females. Feed consumption was significantly decreased in males and females at 15 000/21 000/30 000 ppm and in males at 5000/7000/10 000 ppm throughout the study. Feed consumption was statistically significantly decreased in females at 5000/7000/10 000 ppm for the first 7 weeks and in week 9 of the study. These constant decreases in feed consumption were considered adverse. At 1500/2100/3000 ppm, transient but statistically significant decreases in feed consumption were not considered treatment related in either sex.

Table 6. Summary of histopathological changes in the 90-day oral toxicity study in mice

	Incidence of histopathological changes									
	0 ppm		**300 ppm**		**1 000 ppm**		**3 000 ppm**		**10 000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
No. of mice examined	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Liver										
Centrilobular hepatocellular hypertrophy	6	0	5	0	10	0	10	10	0	0
Centrilobular and midzonal hepatocellular hypertrophy	0	0	0	0	0	0	0	0	10	10
Swollen vacuolated centrilobular hepatocytes	0	0	0	0	3	0	10	5	10	10
Single large hepatocytic vacuoles	0	0	0	0	3	0	6	8	5	4
Centrilobular single-cell hepatocytic necrosis	0	0	0	0	3	0	8	2	0	0
Coagulative necrosis	1	2	0	3	2	1	6	3	10	10
Centrilobular necrotic hepatitis	0	0	0	0	0	0	0	0	10	10
Pigmented Kupffer cells	0	0	0	0	0	0	3	1	10	7
Bile duct proliferation	0	0	0	0	0	0	0	0	5	7
Adrenal										
Cytoplasmic eosinophilia of the zona fasciculata cells	0	0	0	0	4	0	7	8	10	10
Hypertrophy of the zona fasciculata cells	0	0	0	0	0	0	5	2	8	6
Spleen										
Pigment in the macrophages	3	3	6	6	3	6	8	10	10	9
Lymphoid necrosis	0	0	0	0	2	0	0	2	3	3
Bone marrow										
Increased myeloid:erythroid ratio	2	0	0	0	0	0	1	0	1	5
Female reproductive tract										
Absence of corpora lutea	–	2	–	0	–	3	–	1	–	10
Atrophy of uterus	–	1	–	0	–	0	–	1	–	4

F: females; M: males; ppm: parts per million

Source: Goldman, Harris & Lampe (1986)

No treatment-related changes in ophthalmology were detected.

In haematology, haematocrit, haemoglobin or MCV was decreased at 5000/7000/10 000 ppm in both sexes at one or three time points. Increased GGT, total cholesterol and blood urea nitrogen were observed as treatment-related abnormalities in blood chemistry in both sexes at 5000/7000/10 000 ppm. ALT was slightly increased in males at 5000/7000/10 000 ppm. Many haematological and clinical chemistry abnormalities seen at 15 000/21 000/30 000 ppm in both sexes

were considered the result of the maximum tolerated dose (MTD) being exceeded, because all rats died at this dose level. No treatment-related changes were observed in the urine analysis parameters.

Statistically significant increases in hepatic MFO activity were noted at 3 months, with increases in males at 150/210/300 ppm (1.7-fold) and higher (6.5-fold at 5000/7000/10 000 ppm) and in females at 500/700/1000 ppm (2-fold) and higher (8-fold at 5000/7000/10 000 ppm). Female MFO activity was 1.4- to 1.5-fold higher than the control value at 150/210/300 ppm, but this was not a statistically significant change.

Treatment-related changes in the liver and kidneys were seen in males and females at 500/700/1000, 1500/2100/3000 and 5000/7000/10 000 ppm, as shown in Table 7. These consisted notably of statistically significant increases in absolute and relative liver weights and in relative (but not absolute) kidney weights of males and females at 1500/2100/3000 and 5000/7000/10 000 ppm.

Table 7. Organ weights in the 90-day oral toxicity study in rats

	0 ppm		**500/700/1 000 ppm**		**1 500/2 100/3 000 ppm**		**5 000/7 000/10 000 ppm**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Terminal body weight (g)	479	263	475	256	445	249	337*	224*
Absolute liver weight (g)	12.18	6.46	13.63	7.06	15.68*	8.17*	19.75*	11.30*
Relative liver weight[a]	254	246	287	276*	352*	329*	581*	504*
Absolute kidney weight (g)	3.20	1.90	3.39	1.87	3.51	1.97	3.10	1.81
Relative kidney weight[a]	67.0	72.1	71.4	73.1	79.0*	79.2*	91.9*	80.8*

ppm: parts per million; *: $P < 0.05$

[a] g × 10 000/body weight.

Source: O'Hara & DiDonato (1984)

At macroscopic examination, prominent or accentuated lobular architecture of the liver was seen at 1500/2100/3000 ppm in males and at 5000/7000/10 000 ppm in both sexes, correlating with clinical chemistry or histopathological changes. Darkened livers were seen at 1500/2100/3000 ppm in males and at 5000/7000/10 000 and 15 000/21 000/30 000 ppm in both sexes. Swollen livers were seen only at 5000/7000/10 000 ppm in both sexes. Enlarged livers were seen in males at 1500/2100/3000 and 5000/7000/10 000 ppm. Darkened kidneys were seen in males at 5000/7000/10 000 ppm and in both sexes at 15 000/21 000/30 000 ppm. Histopathological changes are summarized in Table 8. Centrilobular or panlobular hepatocellular hypertrophy, including minimal to mild single-cell necrosis of hepatocytes, was seen in both sexes at 1500/2100/3000 ppm and above. Increased Kupffer cell pigmentation was seen at 5000/7000/10 000 ppm. Zones of swollen, vacuolated hepatocytes were seen in males at 5000/7000/10 000 ppm. In the kidneys, a minimal to mild pigmentation of the convoluted tubular epithelium was seen in males at 1500/2100/3000 and above. In the adrenals, there was increased vacuolation of the adrenal cortex in males at 1500/2100/3000 ppm and above. In the thyroid, an increased number of small follicles was seen in males at 1500/2100/3000 and above. An increase in the severity of pigmentation (haemosiderosis) in the red pulp of the spleen was observed in both sexes at 1500/2100/3000 ppm and above, and an increased incidence of chronic alveolitis was seen in males at 5000/7000/10 000 ppm. No treatment-related changes were seen in the 500/700/1000 ppm dose group.

The NOAEL for 90-day toxicity in rats was 500/700/1000 ppm (equal to 51.5 mg/kg bw per day), based on increased liver and kidney weights, hepatocellular hypertrophy, single-cell necrosis in the liver and pigmentation in tubular epithelium in the kidneys at 1500/2100/3000 ppm (equal to 158 mg/kg bw per day) (O'Hara & DiDonato, 1984).

Table 8. Summary of histopathological changes in the 90-day oral toxicity study in rats

	Incidence of histopathological changes									
	0 ppm		150/210/300 ppm		500/700/1 000 ppm		1 500/2 100/3 000 ppm		5 000/7 000/10 000 ppm	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
No. of mice examined	*10*	*10*	*10*	*0*	*10*	*10*	*10*	*10*	*10*	*10*
Liver										
Centrilobular or panlobular hepatocellular hypertrophy	0	0	0	NE	0	0	10	7	10	10
Zones of swollen, vacuolated hepatocytes	0	0	0	NE	0	0	0	0	9	0
Coagulative necrosis	1	0	0	NE	0	0	0	0	2	0
Necrosis, hepatocellular, single cell	0	0	0	NE	0	0	1	3	1	1
Pigmented Kupffer cells	0	0	0	NE	0	0	0	0	1	4
Adrenal										
Vacuolation in the zona fasciculata cells	2	0	NE	NE	3	0	5	2	5	3
Kidney										
Pigmentation in tubular epithelium	0	0	0	NE	0	NE	7	0	8	0
Thyroid										
Increase in small follicles	1	1	NE	NE	2	0	3	2	4	1
Spleen										
Pigmentation in the red pulp	1	0	NE	NE	NE	NE	9	10	10	10

F: females; M: males; NE: not examined at this dose; ppm: parts per million

Source: O'Hara & DiDonato (1984)

Dogs

A dose-finding study was conducted in dogs. Myclobutanil (TD 83-087; lot no. LSPL 83-0017E; purity 84.5%) was administered in the diet to Beagle dogs (two of each sex per group) for 4 weeks at a dietary concentration of 0, 50, 250 or 1000 ppm (equal to 0, 2.0, 10.5 and 45.3 mg/kg bw per day for males and 0, 2.2, 10.6 and 39.3 mg/kg bw per day for females, respectively) and for 2 weeks at 4000 ppm (equal to 45.0–47.0 mg/kg bw per day for males and females, respectively). Acetone (100 mL) was used to facilitate premixing of appropriate amounts of myclobutanil into the diet. During the second week of treatment, granular beef bouillon was added to the 4000 ppm diets in an attempt to increase palatability. No organ weights were assessed, and histopathology was not conducted. Physical examinations included evaluation of external structure, behaviour, posture, gait, mucous membranes, body temperature, muscle tone and the presence or absence of reflexes. The thorax was examined by stethoscope for irregularities of respiration or heart rate.

There were no treatment-related effects on mortality, clinical signs, heart rate or body temperature. During both weeks of exposure to 4000 ppm, a 20% decrease in body weight was observed in both sexes. A slight decrease in body weight gain was observed in females at 1000 ppm during the first week of treatment, but their weights were comparable to those of controls during the remaining 3 weeks of treatment. Feed consumption was reduced in both sexes at 4000 ppm during the 2 weeks of treatment, by less than 4% of control values during week 2. The addition of beef bouillon to the diet did not increase feed consumption at 4000 ppm. The transitory reductions of feed consumption and corresponding decreases in body weight gains at the beginning of the treatment were considered to be due to low palatability of the test material, rather than a general toxic effect. Feed consumption for females at 1000 ppm was slightly decreased during the first week of treatment (28% less consumption than during the week prior to treatment), but was comparable to control values for the remainder of the study.

There were no treatment-related effects on haematology or clinical chemistry parameters monitored at 2 or 4 weeks. Glucose was decreased at 4000 ppm in both sexes at 12 days, but this was considered to be a reflection of the dogs' decreased feed consumption. No treatment-related gross lesions were observed at necropsy (Goldman & Emmons, 1986).

A 3-month dietary toxicity study was conducted in dogs. This study was not conducted in accordance with test guidelines, but, with the exception of urine analysis, almost all examinations required by test guidelines were conducted. Myclobutanil (lot no. LSPL0016/E; TD 83-076; purity 81.1%) was administered in the diet to five groups of 5-month-old Beagle dogs (four of each sex per group) for 3 months at a dietary concentration of 0, 10, 200, 800 or 1600 ppm (equal to 0, 0.34, 7.26, 29.1 and 56.8 mg/kg bw per day for males and 0, 0.42, 7.88, 32.4 and 58.0 mg/kg bw per day for females, respectively).

There were no deaths or signs of treatment-related toxicity. There were no treatment-related effects on heart rate or body temperature or any other unusual signs in the treated dogs when compared with controls. Myclobutanil at 1600 ppm caused a statistically significant decrease in body weight for males during the first 2 weeks and for females during the first 3 weeks. Both sexes showed an approximately 300 g reduction in body weight at day 7. After these decreases, the weight gains were comparable to those of controls. The reduced body weights at the beginning of the study in both sexes were reflective of low palatability at 1600 ppm. Feed consumption was decreased for males during the first 7 weeks (approximately 85% of controls) and for females during the entire exposure period (13–25% of controls); however, the decreases were not accompanied by body weight changes in both sexes. There were no observable ocular abnormalities attributed to treatment with myclobutanil.

In haematology, increased platelets in females at 1600 ppm (16% increases above control values at 3 months) were considered to be treatment related, as similar increases were observed in the 1-year oral toxicity study at the same dose level (see below). In blood chemistry, serum ALP in females at 1600 ppm was at least twice as high as control values (246% and 346% compared with control values at 1 and 3 months, respectively). Slight (< 50% compared with control values) and/or transitory increases for males at 1600 ppm and for males and females at 800 ppm were not considered adverse.

Absolute and relative liver weights at 800 ppm (24% increase in relative weight in males only) and 1600 ppm (42% and 31% increases in relative weight in males and females, respectively) were statistically significantly increased at 3 months. No treatment-related lesions were evident at necropsy. Microscopically, centrilobular or midzonal hepatocellular hypertrophy occurred with a dose-related severity (minimal to mild) at 200 ppm (males only, 3/4), 800 ppm (8/8) and 1600 ppm (8/8). Periportal hepatocytes were minimally enlarged in severely hypertrophied livers. Liver hypertrophy, including increased weights and/or centrilobular/midzonal hepatocellular hypertrophy, without co-expressed hepatotoxicity in males at 200 and 800 ppm was considered to be adaptive. There is a possibility that the liver hypertrophy with clearly increased ALP in females at 1600 ppm was adverse.

The NOAEL for 90-day oral toxicity in dogs was 800 ppm (equal to 29.1 mg/kg bw per day in males), based on liver hypertrophy, increased alkaline phosphatase and increased platelets at 1600 ppm (equal to 56.8 mg/kg bw per day in males) (McLaughlin & DiDonato, 1984).

A 1-year oral toxicity study was conducted in dogs. Although this study was GLP compliant, it was not conducted in accordance with test guidelines. However, it is considered that the data were adequate for the toxicological evaluation. In this study, myclobutanil (TD 84-063; lot no. 83159-7; purity 91.4%) was administered in the diet to five groups of Beagle dogs (six of each sex per group) for 1 year at a dietary concentration of 0, 10, 100, 400 or 1600 ppm (equal to 0, 0.34, 3.09, 14.3 and 54.2 mg/kg bw per day for males and 0, 0.40, 3.83, 15.7 and 58.2 mg/kg bw per day for females, respectively). Haematological and clinical biochemical examinations were conducted at predosing weeks −2 and −1 and dosing weeks 13, 25, 39 and 53.

There were no deaths or clinical signs of treatment-related toxicity. Body weight gain of male dogs at 1600 ppm was statistically significantly decreased after 1 week of treatment (80% of controls), but was comparable to that of controls throughout the remainder of the study. Group mean body weights for females at 1600 ppm were significantly below control values during the first 5 weeks of the study, with a loss in actual weight (109 g) during the first week and a reduced body weight gain during the second week (8 g compared with a gain of 194 g in controls). In males, feed consumption was reduced during the first week (approximately 200 g lower than controls), but otherwise was comparable with that of controls during the study. Feed consumption in females at 1600 ppm was consistently lower than control values throughout the study. The decreased feed consumption throughout the study in females at 1600 ppm was not accompanied by body weight changes. There were no ocular abnormalities attributed to the treatment.

In male dogs at 1600 ppm, a slight but statistically significant decrease in red blood cells (9.3–10.4%) and a constant increase in platelets were observed throughout the study. These changes were not detected in females at the same dose. In males at 400 ppm, slight but statistically significant increases in platelet counts were found at a few time points, but the individual values were consistent during the experimental period, including pretest in males, at this dose; the control values were incidentally low. No other changes were seen at 400 ppm. Therefore, the increased platelets in males at 400 ppm were not considered to be treatment related. Although statistically significant increases in mean corpuscular haemoglobin (MCH) and MCV during weeks 13–53 were seen primarily in males, the increases were very slight (within 4–6% and 2–3% of control values for MCH and MCV, respectively). The absence of any progression in these parameters with increased treatment period indicated that they were not toxicologically significant.

The activity of serum ALP in females at 1600 ppm was consistently increased to 2-fold above the control group and pretreatment values. About half the number of males in this group showed slight but consistent increases in ALP compared with pretest values, and most dogs did not indicate an age-matched decrease at 1600 ppm. Although an age-matched decrease in ALP was not observed in females at 400 ppm, the pretest values were high in two dogs in this group. ALT activity was also slightly increased in males in weeks 25 and 53 at 1600 ppm. Although the consistent increases in ALP in both sexes at 1600 ppm might indicate adverse effects on the liver, there was no progression in ALP values. The increase in ALP in females at 400 ppm was not considered adverse because there were no other parameters indicating hepatotoxicity at this dose level. Serum albumin levels were decreased in both sexes at 1600 ppm. Slight but statistically significant changes in GGT at week 53 or non-dose-dependent changes in inorganic phosphorus concentrations in serum were not considered toxicologically significant because they were slight or transitory. No treatment-related changes were observed in urine analysis.

Increased absolute and relative liver weights were observed in both sexes at 1600 ppm and in females at 100 and 400 ppm. Macroscopically, enlarged livers and/or accentuated lobular architecture were observed in one male and three females at 1600 ppm. Treatment-related histopathological changes were observed in the livers in both sexes at 400 ppm and above. Minimal to mild

hepatocellular hypertrophy was noted in males at 1600 ppm. Mild to moderate hepatocellular hypertrophy was noted in females at 400 ppm and above. No treatment-related changes were observed at 100 ppm. The distribution of the hepatocellular hypertrophy was predominantly centrilobular. At 1600 ppm, the more severely affected females showed ballooned hepatocytes in the centrilobular area of the liver. This change might indicate a degenerative process of hepatocytes resulting from severe hepatocellular hypertrophy. Hepatocellular hypertrophy and/or increase in liver weights not accompanied by indicators of hepatotoxicity in both sexes at 400 ppm were considered adaptive. Other hepatic changes and changes in other tissues were considered to be incidental and unrelated to treatment. Treatment-related liver effects are summarized in Table 9.

The NOAEL for 1-year oral toxicity in dogs was 400 ppm (equal to 14.3 mg/kg bw per day), based on increased ALP, hepatocellular hypertrophy, ballooned hepatocytes and increased platelets in males at 1600 ppm (equal to 54.2 mg/kg bw per day) (Goldman & Harris, 1986a).

(b) Dermal application

A 4-week dermal toxicity study of two formulations of myclobutanil, namely myclobutanil 2EC (TD no. 85-109; lot no. EG-0807-1) and myclobutanil 40WP (TD no. 85-110; lot no. EG-0809-1), was conducted in rats. This study complied with GLP. Three aqueous dilutions of myclobutanil 2EC at 0.07%, 0.67% and 6.67% w/v, corresponding to doses of 1, 10 and 100 mg/kg bw per day, and one aqueous dilution of myclobutanil 40WP at 6.67% w/v, corresponding to 100 mg/kg bw per day, were applied to the intact skin of six Sprague-Dawley rats of each sex per group. The doses were applied (1.5 mL/kg bw), non-occluded, once daily for 6 hours, for a total of 19–20 exposures over a 4-week period. Two additional groups of six rats of each sex were similarly treated with either distilled water or a myclobutanil 2EC formulation blank.

There were no deaths or signs of general toxicity during this study. Treatment-related effects were limited to the site of application. Skin irritation, scored as slight (days 2–25 in males and days 2–29 in females) to moderate (days 26–29), was observed in the 6.67% myclobutanil 2EC group. Females in the 2EC vehicle control group showed slight irritation on day 2 and days 20–29, and males in the 2EC vehicle control group showed slight irritation only on day 2. There were no treatment-related effects on body weight, feed consumption, haematological parameters, blood chemistry parameters or organ weights, except liver. Slight but statistically significant increases (13%) in relative liver weights were seen, but there was no dose–response relationship. Macroscopically, skin treated with both formulations of 6.67% myclobutanil and the 2EC vehicle control group showed eschar (sloughing of dead skin) and desiccation, one male in the control group had pinpoint red discolorations and another had thickening of the skin. Microscopic examination of the treated skin of the 6.67% myclobutanil 2EC group showed significant dermal irritation. Similar, but less severe, findings were seen in the 2EC vehicle control group. The skin of rats in the myclobutanil 40WP group exhibited a minimal degree of epidermal necrosis, epidermal thickening and/or subacute/chronic inflammation of the dermis. There were no treatment-related changes in any other organ. Skin irritation and/or minimal gross and microscopic changes in the treated skin were observed at the treated site with both formulations.

The NOAEL for systemic toxicity of both formulations via dermal exposure was 100 mg/kg bw per day, the highest dose tested (Bonin & Hazelton, 1986).

(c) Exposure by inhalation

Inhalation studies were not performed.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

To assess chronic toxicity and carcinogenicity, a 2-year dietary study in mice was conducted. This study complied with GLP and was conducted according to test guidelines authorized by the United States Environmental Protection Agency and the Organisation for Economic Co-operation and Development. Myclobutanil (TD no. 83-260; lot no. LAP 0298; purity 90.4%) was administered to

Table 9. Summary of blood biochemistry and liver changes in 1-year oral toxicity study in dogs

	Males					Females				
	0 ppm	**10 ppm**	**100 ppm**	**400 ppm**	**1 600 ppm**	**0 ppm**	**10 ppm**	**100 ppm**	**400 ppm**	**1 600 ppm**
No. of dogs	*6*	*6*	*6*	*6*	*6*	*6*	*6*	*6*	*6*	*6*
Platelets (10^3/mm^3)										
Week –2	292.3	298.0	296.2	313.2	320.2	285.7	321.7	361.8	386.7	316.7
Week –1	290.2	286.3	289.5	316.5	318.2	281.2	329.0	372.0	310.2	310.2
Week 13	245.7	284.5	266.5	334.3*	396.3*	304.8	309.5	369.0	368.7	368.7
Week 25	272.5	309.8	259.2	330.2	374.2*	322.3	341.2	389.8	362.0	362.0
Week 39	326.5	347.7	301.0	366.7	409.2*	371.7	390.0	385.7	425.0	418.3
Week 53	286.8	371.3*	291.8	345.7*	402.3*	337.0	367.7	363.5	397.2	384.5
ALP (U/L)										
Week –2	90.5	111.6	95.5	103.7	83.2	92.7	106.6	148.5	109.3	107.8
Week –1	88.2	107.8	91.7	98.7	81.3	85.8	103.5	139.5	106.3	103.0
Week 13	68.5	73.2	59.3	78.2	109.3*	72.2	89.0	102.2	102.2	213.7*
Week 25	55.5	60.5	41.8	66.0	104.5*	68.5	73.2	81.2	101.3	211.0*
Week 39	43.5	49.8	29.0	53.7	90.8*	57.2	57.5	58.9	90.5	218.2*
Week 53	40.0	53.2	27.7	53.2	97.3*	57.5	60.7	59.0	91.7*	187.0*
ALT (U/L)										
Week –2	22.0	19.2	19.3	19.0	19.5	22.0	15.0	20.5	23.0	19.8
Week –1	22.5	20.2	18.8	18.2	18.5	21.6	17.2	20.2	21.5	22.3
Week 13	19.8	22.7	20.2	21.5	22.0	23.2	18.3	22.8	17.7	21.8
Week 25	22.3	23.2	23.2	18.2	30.5* (36)[a]	19.8	16.3	19.8	18.2	21.0
Week 39	21.0	21.0	20.2	16.7	25.7	19.7	19.8	20.6	18.0	23.8
Week 53	23.7	23.2	23.2	20.5	29.3* (27)[a]	22.7	18.0	20.8	19.7	25.3

	Males					Females				
	0 ppm	**10 ppm**	**100 ppm**	**400 ppm**	**1 600 ppm**	**0 ppm**	**10 ppm**	**100 ppm**	**400 ppm**	**1 600 ppm**
Liver weight										
Absolute[b]	299	265	291	291	389*	226	260	281*	295*	349*
Relative[c]	295	300	294	337	424*	290	346*	330	370*	441*
Histopathology										
Hepatocellular hypertrophy, centrilobular	0	0	0	1	5	0	0	0	2	6
Ballooned hepatocyte, centrilobular	0	0	0	0	0	0	0	0	0	4

ALP: alkaline phosphatase; ALT: alanine aminotransferase; ppm: parts per million; U: units; *: $P < 0.05$

[a] Percentage of the control value.
[b] Absolute weight (g).
[c] Relative weight (absolute weight (g) / body weight (kg) × 100.

Source: Goldman & Harris (1986a)

CD-1 mice (110 of each sex per group) in the diet at a dose of 0, 20, 100 or 500 ppm (equal to 0, 2.7, 13.7 and 70.2 mg/kg bw per day for males and 0, 3.2, 16.5 and 85.2 mg/kg bw per day for females, respectively) for up to 2 years. At 3, 6 and 12 months, 10 mice of each sex per group were examined.

There were no treatment-related effects on survival or clinical signs in the treated groups. Significant decreases in body weight were seen almost throughout the study in females and after week 11 in males at 500 ppm. No treatment-related changes in feed consumption or ocular changes were seen in either sex in any dose group.

Blood samples were taken at 3, 6, 12 and 24 months for haematology and blood chemistry examinations. At all time points tested, no haematological parameters were affected in either sex of any treated group. In blood chemistry at 3 months of treatment, ALT was increased in females at 500 ppm (60% increase compared with controls). In males, total protein values were decreased at 100 ppm and above, and total bilirubin was increased at 500 ppm. However, these changes were not seen at later sampling times, suggesting that the toxicological significance of these findings is questionable. Other differences seen in blood chemistry parameters were not treatment related after 3 months. No treatment-related changes in blood chemistry parameters were seen at 6, 12 or 24 months in either sex. No treatment-related effects were found in urine analysis at any time point.

Hepatic MFO activity was significantly increased in females at 100 ppm and in both sexes at 500 ppm at 3 months. This increase was approximately 2-fold in males at 500 ppm and 1.3- and 1.5-fold in females at 100 and 500 ppm, respectively. At 6 months, hepatic MFO activity was increased 1.3- and 2.8-fold in males and 1.4- and 2.9-fold in females at 100 and 500 ppm, respectively. At 12 months, hepatic MFO activity was increased 1.5- and 3.7-fold in females at 100 and 500 ppm, respectively. MFO activity of male mice at 500 ppm was increased 1.6-fold. The increases in males at 500 ppm and in females at 100 ppm were not statistically significant.

Myclobutanil had no effect on hepatic microsomal protein content at concentrations up to and including 500 ppm at 3 months. At 6 months, hepatic microsomal protein content was increased by 28% and 21% in males and females, respectively. Hepatic microsomal protein content was not affected in males or females at 12 months.

Hepatic palmitoyl coenzyme A oxidase activity was not affected by the 12-month treatment.

Liver weight (absolute and relative) was significantly increased in both sexes at 500 ppm at 3 months. No treatment-related changes were seen in any of the organ weights after 6, 12 and 24 months of treatment.

In histopathology for non-neoplastic changes, some treatment-related changes were observed in the liver of males at 500 ppm. Hepatocellular hypertrophy was the main change seen from 3 to 12 months, but it was no longer evident at 24 months of treatment. After 6 months, pigmentation of Kupffer cells, single-cell necrosis of hepatocytes or vacuolated hepatocytes were observed in males at 500 ppm, but no progression was found in these lesions with increasing age. The total incidence of focus of hepatocellular alteration was slightly increased in males at 500 ppm at 24 months, but there was no clear difference in the type of focus among the groups. The incidence and severity of these changes were similar to those seen in control females. The treatment-related changes in the liver are summarized in Table 10. Numerous microscopic findings were encountered in mice of all groups, including the controls, but these changes were of types that are common incidental findings in laboratory mice of this age.

In histopathology for neoplastic changes, various tumours occurred at similar incidences and onset times among the control and treated groups in both sexes or at single or very low incidence. None of them was considered to be treatment related.

The NOAEL for 2-year oral toxicity in mice was 100 ppm (equal to 13.7 mg/kg bw per day), based on the histopathology related to hepatotoxicity at 500 ppm (equal to 70.2 mg/kg bw per day). The NOAEL for carcinogenicity was 500 ppm (equal to 70.2 mg/kg bw per day), the highest dose tested (Goldman & Harris, 1986b).

Table 10. Summary of histopathology in the liver in a 2-year oral study in mice

	Incidence of finding							
	Males				Females			
Time point / findings	0 ppm	20 ppm	100 ppm	500 ppm	0 ppm	20 ppm	100 ppm	500 ppm
3 months / No. of mice examined	10	10	10	10	10	10	10	10
Hypertrophy, hepatocellular, centrilobular	1	1	1	9	0	0	0	0
6 months / No. of mice examined	10	10	10	10	10	10	10	10
Hypertrophy, hepatocellular, centrilobular	2	2	1	9	0	0	0	0
Pigmentation, Kupffer cells	0	0	0	5	0	0	0	0
12 months / No. of mice examined	20	20	20	20	20	20	20	20
Hypertrophy, hepatocellular, centrilobular	5	6	5	16	1	0	1	2
Pigmentation, Kupffer cells	4	1	4	12	4	2	1	4
Individual hepatocellular necrosis, multifocal	2	1	1	6	0	0	1	2
Vacuolation, punctate, multifocal	0	0	0	4	0	1	1	3
12–24 months / No. of mice examined	66	63	65	62	64	66	66	67
Hypertrophy, hepatocellular, centrilobular[a]	8	6	5	11	1	3	2	1
Hypertrophy, hepatocytes, diffuse	5	5	1	3	2	1	1	0
Hypertrophy, hepatocytes, midzonal	0	0	0	0	0	1	0	0
Periportal punctate vacuolation	1	0	2	7	3	2	0	7
Total incidence of cellular alterations[b]	4	4	6	11	2	2	2	6

ppm: parts per million

[a] Combined minimal, slight and moderate due to no enhancement with increasing dose.

[b] Combined basophilic, clear cell, eosinophilic and vacuolated cell types.

Source: Goldman & Harris (1986b)

To confirm the carcinogenicity of myclobutanil at higher doses, a second dietary carcinogenicity study was conducted, but using only female mice. This study complied with GLP and was conducted according to test guidelines, with the deviation that only one dose level was tested. Myclobutanil (TD no. 90-016; lot no. 2-2943; purity 92.9%) was administered to 60 CD-1 female mice per group in the diet at a concentration of 0 or 2000 ppm (equal to 394 mg/kg bw per day) for up to 18 months to determine the carcinogenic potential at the MTD. Physical examinations including palpation were performed weekly.

Survival in the treated group was similar to that of controls. Numerous clinical signs were noted throughout the study, but the type and incidence were similar in control and treated mice. Treatment-related, statistically significant effects on body weight were seen in treated mice, with decreases in body weight gain (12–26%) throughout the study. Overall, the body weight gain for treated mice was 20% less than that of controls. Feed consumption was generally lower in treated mice than in controls throughout the study. Statistically significant decreases in feed consumption were seen during the first 2 weeks of the study.

A statistically significant increase (36%) in white blood cell count at 2000 ppm at 18 months was considered incidental because of a lack of increase in related lesions, including inflammation. No treatment-related changes were seen in blood chemistry. Urine analysis was not conducted. Statistically significant increases in absolute (30% and 23%, respectively) and relative (32% and 33%, respectively) liver weights were noted at 12 and 18 months. There was no notable difference in effect between the two time points. Changes observed in brain and kidney weights were considered to be a reflection of decreased terminal body weight.

No treatment-related macroscopic findings were observed at 12 or 18 months. Treatment-related microscopic changes were observed in the liver and adrenal glands at 2000 ppm at 12 and 18 months, with incidence increasing with time. Liver changes were primarily slight to moderate hepatocellular hypertrophy in most of the treated mice, minimal to slight single-cell necrosis of hypertrophied hepatocytes in several mice and slight to moderate vacuolation (fatty change) of hepatocytes. Changes in the adrenal glands included hypertrophy of the zona fasciculata of the cortex. There was no effect on the incidence or type of neoplastic changes.

Myclobutanil demonstrated no carcinogenicity up to the MTD in mice. The major target of myclobutanil in mice was the liver. Effects at 2000 ppm were qualitatively similar to those observed at lower doses in the earlier study of Goldman & Harris (1986b) described above (Anderson, O'Hara & Brown, 1993).

Rats

A 2-year oral toxicity study was conducted to investigate the chronic toxicity and carcinogenicity of myclobutanil in rats. Although this study complied with GLP, it was not conducted according to test guidelines. However, it is considered that the data were adequate for toxicological evaluation. Myclobutanil (weeks 1–15: TD no. 83-260; lot no. LAP 0298; purity 90.4%; week 16 to termination: TD no. 84-038; lot no. 83159-7; purity 91.4%) was administered to male and female Sprague-Dawley rats in the diet for up to 2 years. Hepatic MFO activity was determined in six rats of each sex per group at 3, 6 and 12 months, by in vitro enzyme assay of demethylation of aminopyrine. Hepatic palmitoyl coenzyme A activity was determined in six rats of each sex per group at 12 months. Organ weights, except liver weights, were measured at necropsy at 12, 17 and 24 months.

The experimental design is summarized in Table 11.

Table 11. Experimental design of 2-year oral toxicity study in rats[a]

Concentration of myclobutanil in diet (ppm)			Chemical intake (mg/kg bw per day)[b]		No. of rats killed at month:				
Weeks 1 & 2	**Weeks 3 & 4**	**Week 5 – termination**	**Males**	**Females**	**3**	**6**	**12**	**17**	**24**
0	0	0	0	0	10	10	20	18/10[c]	All surviving for postmortem
25	35	50	2.5	3.2	10	10	20	18/10	
100	140	200	9.8	12.8	10	10	20	18/10	
400	560	800	39.2	52.3	10	10	20	18/10	

bw: body weight; ppm: parts per million

[a] Total number of males: 114; total no. of females: 106.
[b] Mean compound intake over 24 months.
[c] Males/females.

Source: Shellenberger (1986)

Myclobutanil did not affect the survival of males or females. Although various clinical signs were found throughout the study in both sexes, all clinical signs were considered to be unrelated to the treatment. Myclobutanil treatment caused approximately 30–40 g lower body weights in males at 800 ppm between 6 and 18 months, compared with controls, but had no significant effect on body weights at 50 and 200 ppm. In females, body weights during the first and second weeks of treatment were decreased at 200 ppm and above, an effect that may be related to the small decreases (6–8 g per rat per week) in feed consumed, rather than an acutely toxic effect of exposure. Body weights in all treated groups were comparable with those of the control group throughout the first 52 weeks. During the second year, body weights in females at 800 ppm were generally lower than the control values. Feed consumption of males at 800 ppm was decreased to week 78 as a result of treatment. Feed consumption of females at 800 ppm was significantly decreased compared with that of controls at weeks 1, 14, 48 and 66. In ophthalmological examination, no treatment-related changes were observed in either sex in any treated group.

At 12 months, red blood cell counts, haemoglobin and haematocrit were slightly lower (approximately 10% in each) in males at 800 ppm than in the controls, but the differences were not statistically significant. There were no apparent differences between control and treated males at 17 months and prior to termination of the study. Haematological parameters determined in control and treated female rats were similar at all time points. The marginal decreases in haematological parameters at 12 months were considered transitory and not toxicologically significant. The blood chemistry revealed no consistent differences between control and compound-treated males or females. In urine analysis, there were no consistent differences in parameters attributed to treatment at 3, 5, 11 and 17 months and prior to termination.

A slight increase in MFO activity (34–47%) was seen in males at 800 ppm during 3–12 months. In female rats, MFO activity was statistically significantly increased by 61% and 78% at 200 and 800 ppm, respectively, at 3 months. At 6 and 12 months, slight increases (not statistically significant) were seen in females at 800 ppm at 6 and 12 months (35% and 40%). Myclobutanil had no effects on hepatic peroxisomal [^{14}C]palmitoyl coenzyme A oxidase activity in any treated group after 12 months.

There were no statistically significant differences in absolute or relative liver weights in males at any time point. In females, relative weights were slightly (approximately 10%), but statistically significantly, increased at 3 months. At other time points, statistically significant changes were not seen in relative liver weights in females. After 12 months, the absolute and relative testicular weights at 800 ppm were lower than the control values. The decreases were enhanced with increased age. At termination, absolute testes weights were statistically significantly reduced at 200 ppm and higher. The reduced testes weights at 800 ppm were correlated with testicular size reduction or soft testes (24 months only) at necropsy at 12, 17 and 24 months.

At 12 months and termination, absolute and relative organ weights for brain, heart, adrenals, kidneys and spleen were comparable with those of controls. Increases in absolute and relative ovarian weights were seen at 12 months, but were not statistically significant. There was no effect at termination. Other gross lesions were considered to be incidental findings in males and females.

In histopathology for non-neoplastic findings, a treatment-related finding was observed in the testes (Table 12). The incidence of testicular atrophy was increased at 200 ppm and above at termination after 12 months of treatment. Incidences of the atrophy in the control and 50 ppm groups were similar, but the incidence was slightly increased at 200 ppm and above, with dose dependency. Testicular atrophy was characterized as the frequent lack of spermatid formation and germ epithelium in the seminiferous tubules. In severe cases, only Sertoli cells remained in the tubules. No treatment-related effects were observed in the livers of rats at any of the time points examined. A slight increase in hepatocellular fatty change in the centrilobular area in males at 800 ppm at 17 months only was not considered to be treatment related because of its transitory nature. Various non-neoplastic and neoplastic changes observed throughout the study in either sex were considered to be incidental and not treatment related.

Table 12. Summary of testicular changes in a 2-year oral toxicity study in rats

	0 ppm	50 ppm	200 ppm	800 ppm
Testicular weight				
12 months / No. of rats examined	20	19	20	20
Absolute (g)	3.751	3.661	3.524	3.300*
Relative (testis to body weight ratio, g/100 g)	0.556	0.512	0.516	0.507
17 months / No. of rats examined	18	18	18	18
Absolute	3.341	3.393	3.655	3.017
Relative (testis to body weight ratio, g/100 g)	0.4334	0.449	0.470	0.389
24 months / No. of rats examined	17	19	20	22
Absolute	3.223	3.006	2.491*	2.430*
Relative (testis to body weight ratio, g/100 g)	0.492	0.488	0.444	0.399
Histopathology				
12 months / No. of rats examined	20	19	20	20
Atrophy – unilateral	0	1	0	0
Atrophy – bilateral	0	0	1	3
17 months / No. of rats examined	18	18	18	18
Atrophy – unilateral	2	2	2	1
Atrophy – bilateral	2	2	0	4
24 months / No. of rats examined	17	19	20	22
Atrophy – unilateral	2	3	6	2
Atrophy – bilateral	2	1	5	13

ppm: parts per million; *: $P < 0.05$

Source: Shellenberger (1986)

The NOAEL for 2-year oral toxicity in rats was 50 ppm in males (equal to 2.5 mg/kg bw per day), based on the testicular toxicity found after 12 months of treatment at 200 ppm (equal to 9.8 mg/kg bw per day). The NOAEL for carcinogenicity was 800 ppm (equal to 39.2 mg/kg bw per day), the highest dose tested (Shellenberger, 1986).

To confirm the apparent lack of carcinogenicity of myclobutanil, a second dietary carcinogenicity study was conducted using male and female rats in which myclobutanil was administered at a single dose higher than the top dose used in the earlier study. This study complied with GLP and was conducted according to test guidelines, with a deviation in that a single dose level was tested. Myclobutanil (lot no. 2-2943; purity 92.9%) was administered to 60 Sprague-Dawley rats of each sex per group at a dietary concentration of 0 or 2500 ppm (equal to 0 and 106 or 136 mg/kg bw per day for males and females, respectively) for 104 weeks. These conditions represented an anticipated MTD based on a previous 90-day toxicity study in rats. An interim kill was conducted at 52 weeks using 10 rats of each sex per group. Ophthalmological and blood chemistry examinations were not determined.

There was no apparent compound-related effect on survival throughout the study. No treatment-related clinical signs were observed in either sex throughout the study. Slight but statistically significant decreases in body weight were noted in males at weeks 26 and 52 and in

females at week 52 at 2500 ppm. No statistically significant differences in body weight were noted at week 104. There were no treatment-related changes seen in feed consumption. In haematology, statistically significant decreases in nucleated red blood cells were observed in males at 2500 ppm at week 53.

Treatment-related findings were limited to the liver and testes. A significant increase was noted in absolute and relative liver weights in males and relative liver weight in females at 2500 ppm at 12 months. At 24 months, no changes were noted in either sex. Absolute and relative weights of left testis and bilateral testes were significantly lower in males at 2500 ppm at 12 months. At 24 months, absolute weights of left testis and bilateral testes were also increased, but their relative (to body weight) weights were not different from those in control rats. Macroscopically, enlarged liver, thickened lobes in the liver and small and soft testes were increased in rats at 2500 ppm at the 12- and 24-month kills and in unscheduled deaths. The histopathological changes in the liver consisted of statistically significant increases in centrilobular to midzonal hepatocellular enlargement (equal to hypertrophy) and vacuolation (equal to fatty change) at 2500 ppm of both sexes. The changes were present at the 12-month interim kill and were not appreciably different at study termination. The liver changes were consistent with increased absolute and relative liver weights in treated rats. The incidence of liver neoplasia or foci of cellular alteration was not different in control and treated groups. Compound-related testicular changes occurred as bilateral aspermatogenesis in 22/60 treated rats, compared with 2/60 control rats; the increase was statistically significant. Normal bilateral spermatogenic activity was observed in 49/60 control and 24/60 treated rats. Decreased spermatogenic activity was associated with a significantly increased incidence of hypospermia and cellular debris in the epididymides of treated rats. Other treatment-related effects on incidences of non-neoplastic and neoplastic lesions were not seen in either sex. Liver and testicular changes are summarized in Table 13.

Myclobutanil was not carcinogenic in rats at dietary concentrations up to 2500 ppm (equal to 106 mg/kg bw per day). The primary toxicity targets of myclobutanil were liver and testis in rats, and the testicular toxicity at 2500 ppm was qualitatively in line with that detected at lower doses in the previous study (Wolfe, 1993).

2.4 Genotoxicity

The genotoxicity of myclobutanil was investigated in a comprehensive array of studies. Five in vitro and two in vivo genotoxicity tests were conducted, including a bacterial assay for reverse mutations, an in vivo chromosomal aberration assay in rat bone marrow cells, an in vitro assay for unscheduled DNA synthesis in primary cultures of rat hepatocytes, an in vitro forward mutation assay in Chinese hamster ovary (CHO) cells and in vivo mouse micronucleus tests in bone marrow (Table 14). These studies complied with GLP. Although most of studies were not conducted according to current test guidelines, they were considered to be adequate for the risk assessment. The second micronucleus test was conducted according to test guidelines.

Myclobutanil did not demonstrate any mutagenic properties in the TA98, TA100, TA1535 or TA1537 strains of *Salmonella typhimurium* tested in this study. Myclobutanil does not induce chromosomal aberrations in CHO cells in the presence or absence of metabolic activation. Treatment of male rat primary hepatocytes with myclobutanil did not result in the induction of unscheduled DNA synthesis. Myclobutanil did not induce mutations at the *Hgprt* locus in CHO cells in culture when tested in either the presence or absence of metabolic activation. In in vivo genotoxicity testing, under the conditions of this study, myclobutanil did not induce chromosomal aberrations in mouse bone marrow cells.

In conclusion, myclobutanil was negative in all genotoxicity studies in vitro and in vivo.

Table 13. Summary of organ weights and histopathological changes in the liver and testes in the second 2-year oral toxicity study in rats

	Males				Females			
	12 months		All animals		12 months		All animals	
	0 ppm	2 500 ppm	0 ppm	2 500 ppm	0 ppm	2 500 ppm	0 ppm	2 500 ppm
No. of rats examined	*10*	*10*	*60*	*60*	*10*	*10*	*60*	*60*
Liver weights								
Absolute (g)	19.57	23.48*	19.53	20.41	11.26	11.27	12.86	13.94
Relative (weight (g)/bw (g))	2.457	3.136*	2.733	2.963	2.478	3.023*	3.065	3.195
Testes weights								
Absolute (g)	3.73	2.25*	3.45	2.66*	–	–	–	–
Relative (weight (g)/bw (g))	0.470	0.308*	0.476	0.379	–	–	–	–
Histopathology, liver								
Centrilobular to midzonal hepatocellular enlargement	0	10	0	52	0	10	0	45
Centrilobular to midzonal hepatocellular vacuolation	0	8	0	32	0	2	0	13
Histopathology, testes								
Hypospermia, unilateral	0	0	4	8	–	–	–	–
Hypospermia, bilateral	0	2	5	3	–	–	–	–
Aspermatogenesis, unilateral	0	0	1	8	–	–	–	–
Aspermatogenesis, bilateral	0	6	2	22	–	–	–	–
Histopathology, left epididymis								
Immature abnormal sperm forms	0	1	12	9	–	–	–	–
Hypospermia	0	7	8	31	–	–	–	–
Lumen, debris, cellular	0	6	2	25	–	–	–	–
Histopathology, right epididymis								
Immature abnormal sperm forms	0	0	10	10	–	–	–	–
Hypospermia	0	6	6	27	–	–	–	–
Lumen, debris, cellular	0	6	2	22	–	–	–	–

ppm: parts per million; *: $P < 0.05$

Source: Wolfe (1993)

Table 14. Summary of genotoxicity studies with myclobutanil

Test	Test object	Concentration	Lot no. / purity	Result	Reference
In vitro					
Bacterial reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537	75, 250, 750, 2 500 and 7 500 µg/plate	LSPL 83/0017E / 84.5%	Negative ±S9	Byers & Chism (1983)
Bacterial reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	75, 250, 750, 2 500 and 7 500 µg/plate	LAP-0298 / 90.4%	Negative ±S9	Byers & Chism (1984)
Mammalian cytogenetics	CHO cells	0.034–1 020 µg/mL	83159 / 91.9%	Negative ±S9	Ivett (1985)
Mammalian forward mutation	UDS: Primary rat hepatocytes	0.1–1 000 µg/mL	83159-5 / 91.9%	Negative	Muller (1986)
Mammalian forward mutation	CHO cells (CHO/*Hgprt*)	25–1 000 µg/mL	LSPL-0016/E / 81.1%	Negative ±S9	O'Neill, Foxall & Byers (1984)
In vivo					
Micronucleus	Mouse bone marrow polychromatic erythrocytes	0, 65, 260 and 650 mg a.i./kg bw	LSPL-0016/E / 81.1%	Negative	McLeod & McCarthy (1984)
Micronucleus	Mouse bone marrow polychromatic erythrocytes	0, 117, 585 and 1 170 mg a.i./kg bw	83159-7 / 91.4%	Negative	Sames & Frank (1987)

a.i.: active ingredient; bw: body weight; CHO: Chinese hamster ovary; Hgprt; hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate; UDS: unscheduled deoxyribonucleic acid synthesis

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

A two-generation (two litters per generation) reproduction study was conducted in rats. This study complied with GLP, although it was not conducted according to test guidelines. However, this study generally met the requirements of current guidelines. Myclobunatil (TD no. 83-155; lot no. LSPL 83/0017E; purity 84.5%) was administered in the diet to three groups of 25 Crl:CD(SD)BR rats of each sex in the first (P_1) and second (P_2) parental generations at 0, 50, 200 or 1000 ppm. The chemical intake averaged for each generation and each group is shown in Table 15. Necropsies were performed on all P_1 and P_2 rats after pups from their second matings (F_{1b} and F_{2b} pups) were weaned. All organs, tissues and body cavities were examined, and any gross abnormalities were noted. The livers were weighed. Histopathological evaluation of the liver and reproductive organs was performed on all treated and control P_1 and P_2 rats. Necropsies were performed on all F_1 and F_2 pups that were found dead after 14 days of age. Gross observations were recorded, but no tissues were preserved.

Table 15. Average chemical intakes in two-generation reproductive toxicity study in rats

	Chemical intake (mg/kg bw per day)		
Generation	**50 ppm**	**200 ppm**	**1 000 ppm**
P_1 males	3.67	14.3	70.7
P_1 females	4.42	17.2	85.9
P_2 males	3.64	15.1	76.4
P_2 females	4.17	17.5	88.0

bw: body weight; P_1: first parental generation; P_2: second parental generation; ppm: parts per million

Source: Costlow & Harris (1985)

There were no treatment-related changes in mortality or clinical signs in either generation. Body weights in males and females at all treated doses were comparable with those of the controls in both generations. At 1000 ppm, feed consumption in males for weeks 1–4 and in females for weeks 1–2 of both generations was slightly, but not significantly, lower than in controls. The slight decreases in feed consumption by both sexes at 1000 ppm during the first few weeks were judged to be due to lower palatability at this dose, because of the lack of the same change when administration was by gavage and the lack of detected effects on the gastrointestinal tract in the present study. Feed consumption in the other treated groups was comparable with control values.

The litter data for the P_1 generation are summarized in Table 16. In reproductive performance of P_1/F_{1a}, the number of mated females that delivered was slightly, but statistical significantly, lower than in controls at 1000 ppm, whereas the number of P_1 females expressing positive signs of mating (sperm in vaginal smear), the mean number of days needed for mating to occur and the mean length of gestation were comparable with control values. This dose induced testicular toxicity; therefore, the lower number of females that delivered was possibly treatment related. Myclobutanil at 50 and 200 ppm did not affect the number of P_1 females that had positive signs of mating (sperm in vaginal smear) or the number of mated females that delivered.

In the second mating of the P_1 animals, there was no suggestion of an adverse effect on fertility in male or female rats.

In the P_1 males and females, dose-dependent increases in liver weight were observed in males (14%) and females (9%) at 1000 ppm. At 200 ppm, relative liver weights (7%) were increased in males only. In the P_1 rats, a treatment-related microscopic change was observed in the liver, consisting of centrilobular hypertrophy of hepatocytes in males (10/25) and females (8/25) and centrilobular vacuolation in males (8/25) at 1000 ppm. Increased liver weights at 200 ppm were considered to be adaptive, as there was no indication of hepatotoxicity at this dose.

In litter data for P_1/F_{1a} animals, the number of dead pups (12) was significantly higher than in controls (3) at 1000 ppm, and survival to day 4 was reduced as a consequence. Neither mean pup weight at birth (males, females or combined sexes) nor sex ratio was significantly different from control values at this dose, but the increase in the number of dead pups was judged to be treatment related. Litter data at 200 ppm and lower were comparable with those of the control group. At 1000 ppm, female pup weight was significantly less than that of controls on day 4 of lactation. By day 7 of lactation, pups of both sexes at 1000 ppm showed lower body weights. The reduced weight gain was persistent through day 21 of lactation, and the difference in weight between controls and pups at 1000 ppm was increased.

In P_1/F_{1b} animals, the number of pups born dead at 200 ppm and above was statistically significantly elevated over controls. The increase at 1000 ppm, similar to the data for the P_1/F_{1a} and P_2 animals, was judged to be treatment related. However, the number at 200 ppm was the same as for the P_1/F_{1a} animals, which was not statistically significant. The control value for F_{1b} animals was 0,

Table 16. Summary of litter data for P_1 animals in a reproductive toxicity study in rats

	F_{1a}				F_{1b}			
Litter data	**0 ppm**	**50 ppm**	**200 ppm**	**1 000 ppm**	**0 ppm**	**50 ppm**	**200 ppm**	**1 000 ppm**
No. of pups/litter at birth	13.7	12.8	13.7	12.3	13.0	13.3	13.7	14.2
Sex ratio (M/(M + F)) at birth	0.44	0.47	0.50	0.53*	0.45	0.46	0.45	0.52
No. of pups born dead	3	4	9	12*	0	6	9*	16*
Viability index[a]	98.4	97.1	96.4	92.7*	89.9	85.3	77.1	86.2
Litter size – live pups								
Birth	313	302	293	233	287	292	315	327
Day 4 pre-cull	311	297	291	227	258	249	243	282
Day 4 post-cull	226	224	209	177	208	200	202	211
Day 7	226	224	209	177	206	198	200	211
Day 14	225	224	209	177	205	198	199	210
Day 21 (weaning)	225	224	209	177	204	198	196	210
Body weight (g)/pup								
Day 0	6.0	6.1	6.2	6.3	5.9	6.0	6.1	5.9
Day 4 pre-cull	9.6	9.9	9.6	9.4	9.5	9.0	9.4	8.5
Day 7	15.3	15.1	15.0	14.3**	15.2	14.3	14.9	13.1**
Day 14	29.6	29.6	29.2	26.7**	30.4	29.1	29.8	26.9**
Day 21 (weaning)	45.7	45.9	44.4	41.9**	46.6	45.6	46.2	42.2**

F: females; F_{1a}: first mating of P_1 parents; F_{1b}: second mating of P_1 parents; M: males; ppm: parts per million; *: $P < 0.05$ for combined sex; **: $P < 0.05$ for each sex

[a] No. of pups alive at day 4/total no. born.

Source: Costlow & Harris (1985)

whereas the values for P_1/F_{1a}, P_2/F_{2a} and P_2/F_{2b} animals were 3, 6 and 5, respectively. The increase at 200 ppm was not considered to be treatment related, but was influenced by the low control value.

The second mating of the P_1 dams produced a growth response that was nearly identical to that in the first mating, but the effect was more pronounced. Pups at 200 ppm and lower were comparable with controls throughout the lactation period.

In the second generation, no treatment-related changes in mortality or clinical signs were detected in P_2 males and females. Body weights of male P_2 rats at 1000 ppm were significantly decreased throughout the 8 weeks of dosing prior to mating. Although the body weight at this dose was lower (16 g lower than in controls) prior to the treatment, the lower body weights during the first 3 weeks of postnatal growth were ascribed to treatment. At 1000 ppm, slight but consistent and statistically significant differences noted at 1000 ppm may reflect a modest degree of poor palatability of the diet of both the P_1 and P_2 animals.

A summary of reproductive performance is shown in Table 17. Although there were no statistically significant differences, the numbers of females giving birth and females weaning litters were slightly lower than control values in both the P_2/F_{2a} and F_{2b} animals. The period to mating (in days) took longer than the control value for F_{2b} animals at 1000 ppm. Gestation period was not prolonged by the treatment.

Table 17. Summary of reproductive indices for P_2 animals in a reproductive toxicity study in rats

	F_{2a}				F_{2b}			
Indices	**0 ppm**	**50 ppm**	**200 ppm**	**1 000 ppm**	**0 ppm**	**50 ppm**	**200 ppm**	**1 000 ppm**
No. of males	25	25	25	25	25	25	25	25
No. of females	25	24	25	25	25	24	25	25
No. of females mating	25	23	23	22	24	21	25	21
No. of females giving birth	23	23	24	20	23	22	25	17
No. of females weaning litters	22	23	23	18	22	21	24	15
Period to mating (days)	2.2	3.1	2.8	3.0	3.0	3.4	2.9	4.4
Gestation period (days)	21.7	21.7	21.9	22.2	21.7	22.0	21.8	21.7

F_{2a}: first mating of P_2 parents; F_{2b}: second mating of P_2 parents; ppm: parts per million

Source: Costlow & Harris (1985)

Relative liver weights were statistically significantly increased in males at 200 ppm (4% increase) and 1000 ppm (13% increase) and in females at 1000 ppm (8% increase) in P_2 rats. In the P_2 rats, centrilobular hepatocellular hypertrophy occurred in males at 200 ppm (2/25) and 1000 ppm (18/25) and in females at 1000 ppm (4/25). Centrilobular hepatocytic vacuolation was found in males at 200 ppm and above without dose dependency (3/25 and 1/25 at 200 and 1000 ppm, respectively), indicating that this change was not treatment related. Centrilobular hypertrophy associated with increased liver weights at 200 ppm was considered to be treatment related but adaptive, owing to a lack of indication of hepatotoxicity at this dose.

An increased incidence of rats with multifocal or diffuse testicular atrophy was observed in P_2 males at 1000 ppm. Associated changes, such as decreased amounts of spermatozoa, the presence of necrotic spermatocytes and spermatids in the epididymal tubules and prostatic atrophy, were noted at this dose. Testicular effects are summarized in Table 18.

In litter data for P_2/F_{2a} rats, litter size at 1000 ppm for this mating was lower than that in controls, and the number of pups born dead was higher than in controls. This response and the increase in dead pups were considered to be treatment related. Body weights for F_{2a} pups were not significantly less than those of controls at birth, but reduced body weight gain was readily apparent at day 7 and persisted through day 21 of lactation at 1000 ppm. In litter data for P_2/F_{2b} rats, litter size at 1000 ppm was less than that of controls, and the number of dead pups was higher than in controls. The increase in dead pups was not statistically significant, but was considered to be treatment related. Survival to day 4 was significantly reduced at 1000 ppm only because of the pups born dead, but there was no increased mortality between days 0 and 4 among pups born alive. Body weights for F_{2b} pups were not significantly different from those of controls at birth, but the reduced body weight gain of pups was clearly present by day 7 and persisted through day 21 of lactation. Summarized litter data are shown in Table 19.

In this two-generation reproductive toxicity study in rats, the NOAEL for parental toxicity was 200 ppm (equal to 15.1 mg/kg bw per day), based on lower body weights, histopathological changes in vacuolation and hypertrophy of hepatocytes in the liver and testicular atrophy in P_2 males at 1000 ppm (equal to 76.4 mg/kg bw per day). The NOAEL for reproductive toxicity was 200 ppm (equal to 17.5 mg/kg bw per day), based on reduced reproductive ability, including number of females mating, number of females giving birth, number of females weaning litters and prolongation of the time to mating in P_2 females at 1000 ppm (equal to 88.0 mg/kg bw per day). The NOAEL for offspring toxicity was 200 ppm (equal to 17.2 mg/kg bw per day), based on increased number of pups

born dead for both generations at 1000 ppm (equal to 85.9 mg/kg bw per day) (Costlow & Harris, 1985).

Table 18. Summary of histopathological changes in testes of the P_2 males in a reproductive toxicity study in rats

	Incidence of finding			
Findings	**0 ppm**	**50 ppm**	**200 ppm**	**1 000 ppm**
Testes (number of tissues examined)	25	25	25	25
Multifocal atrophy – unilateral	0	2	1	2
Multifocal atrophy – bilateral	3	2	3	3
Diffuse atrophy – unilateral	0	0	1	4
Diffuse atrophy – bilateral	0	1	0	4
Diffuse necrosis – unilateral	0	1	0	0
Number of rats with testicular lesions	3	5	5	11
Epididymides (number of tissues examined)	25	25	25	25
Necrotic spermatocytes/spermatids – unilateral	0	0	0	5
Necrotic spermatocytes/spermatids – bilateral	2	3	2	8
Decreased spermatozoa – unilateral	0	0	1	1
Decreased spermatozoa – bilateral	1	2	0	8
Number of rats with epididymal lesions	2	3	3	13
Prostate (number of tissues examined)	25	25	25	25
Atrophy	2	1	0	11

ppm: parts per million

Source: Costlow & Harris (1985)

Table 19. Summary of litter data for P_2 animals in a reproductive toxicity study in rats

	F_{2a}				F_{2b}			
Litter data	**0 ppm**	**50 ppm**	**200 ppm**	**1 000 ppm**	**0 ppm**	**50 ppm**	**200 ppm**	**1 000 ppm**
No. of pups/litter at birth	13.8	13.8	13.1	11.4*	15.4	14.8	13.8	13.4*
Sex ratio (M/(M + F)) at birth	0.53	0.51	0.45	0.46	0.49	0.51	0.51	0.46
No. of pups born dead	6	3	1	13*	5	6	3	12
Viability index[a]	86.8	84.9	86.7	84.6	96.9	94.2	98.5	90.8
Litter size – live pups								
Birth	314	314	314	216	349	319	341	218
Day 4 pre-cull	276	269	273	193	343	306	339	207*
Day 4 post-cull	209	215	216	169	230	207	240	155
Day 7	209	213	216	169	230	202	240	154
Day 14	208	213	216	169	230	202	240	154

Litter data	F_{2a}				F_{2b}			
	0 ppm	**50 ppm**	**200 ppm**	**1 000 ppm**	**0 ppm**	**50 ppm**	**200 ppm**	**1 000 ppm**
Day 21 (weaning)	208	213	216	169	220	202	239	144
Body weight (g)								
Day 0	5.8	6.1	6.2	6.2	6.0	5.9	6.1	5.8
Day 4 pre-cull	9.2	9.6	9.9	9.2	9.1	9.5	9.1	8.7
Day 7	14.9	15.1	15.2	13.4	14.5	15.0	14.7	13.3**
Day 14	29.1	29.2	29.2	25.3**	29.4	30.2	28.7	26.2**
Day 21 (weaning)	45.3	45.5	44.6	40.2**	46.5	48.1	46.0	41.8**

F: females; M: males; ppm: parts per million; *: $P < 0.05$ for combined sex; **: $P < 0.05$ for each sex

[a] The number of pups alive at day 4/total number born.

Source: Costlow & Harris (1985)

(b) Developmental toxicity

Rats

A developmental toxicity study was conducted with rats. This study complied with GLP, although it was not conducted according to test guidelines. However, most of the parameters tested in this study generally meet the requirements of current guidelines. Myclobutanil (TD no. 83-087; lot no. LSPL 0017/E; purity 84.5%) was administered orally in corn oil to 25 presumed-pregnant Sprague-Dawley rats from day 6 through day 15 of gestation at doses of 0, 31.3, 93.8, 313 and 469 mg/kg bw per day. Reanalysis for skeletal variations observed was conducted in compliance with GLP.

There were no deaths in this study. Treatment-related clinical signs or reactions to treatment were observed at 313 mg/kg bw per day and above, mainly at 469 mg/kg bw per day: rough hair coat, desquamation and salivation at 313 mg/kg bw per day and above and red exudate from the mouth and scant or soft faeces at 469 mg/kg bw per day. These clinical signs were found during days 8–13, mainly days 8–10. The distribution of their occurrences indicated a low possibility that they were induced by single-dose exposure. At 469 mg/kg bw per day, a statistically significantly lower maternal body weight was observed on day 10 of gestation, but not thereafter.

The litter data are summarized in Table 20. Fetal weights were not affected by the treatment in both sexes. The fertility index (the number of pregnant rats/the number of presumed-pregnant rats) was not affected by the treatment. The number of fetuses per litter was slightly, but statistically significantly, decreased at 93.8 mg/kg bw per day and above, but the litter sizes were within the range of historical control data (average, 13.46; range, 12.10–14.6). The litter size in the control group (15.3) was slightly above the historical control range. In addition, the numbers of corpora lutea and implantations in the control group were slightly above historical control values. Therefore, the decrease in litter size was not considered to be treatment related, but was influenced by the control value.

The number of resorptions (early plus late) per litter and the number of early resorptions per litter were slightly increased at 93.8 mg/kg bw per day and above. Although the decrease in the number of early resorptions per litter was very slight and showed no dose–response relationship between 93.8 and 313 mg/kg bw per day, these values were above the historical control range (mean, 5.6; range, 0.32–0.82). The numbers of late resorptions per litter and litters with late resorptions were increased at 313 and 469 mg/kg bw per day. In addition, increased resorptions were observed at 68 mg/kg bw per day and above in Sprague-Dawley rats in the dose-finding developmental toxicity study previously reviewed by JMPR in 1992, indicating the similar effect induced by myclobutanil at

similar doses in the same strain of rat. Therefore, the slight increase in early resorptions at 93.8 mg/kg bw per day and above was considered to be treatment related.

Table 20. Summary of litter data (means) in a developmental toxicity study in rats

Parameter	0 mg/kg bw per day	31.3 mg/kg bw per day	93.8 mg/kg bw per day	313 mg/kg bw per day	469 mg/kg bw per day
Number bred	25	25	25	25	25
Number non-pregnant	3	1	4	2	2
Number dead/killed moribund	0	0	0	0	0
Number of total resorptions	0	0	0	0	1
Number of viable litters	22	24	21	23	22
Number of corpora lutea/dam[a]	17.9[b]	15.2	16.6	16.4	16.8
Number of implantations/dam	16.1	14.3	15.2	15.0	15.7
Preimplantation loss (%)	10	6	8	8	6
Number of fetuses/litter	15.3[c]	13.5	13.3*	13.2*	13.1*
Number of resorptions/litter[a]	0.82	0.79	1.86	1.78	2.57
Early[a]	0.82[d]	0.71	1.76	1.35	2.04
Late[a]	0[e]	0.08	0.10	0.43	0.52
Number of litters with resorptions[a]	12	17	16[f]	18	19[g]
Early[a]	12	16	16	15	17
Late[a]	0	2	1	8	9
Fetal weights (g)	3.23	3.30	3.25	3.39	3.26
Fetal sex ratio M:F	0.87	0.98	0.92	0.99	0.88

bw: body weight; F: female; M: male; *: $P < 0.05$

[a] Not statistically analysed.
[b] Historical control data; average, 15.45; range, 14.82–17.4.
[c] Historical control data; average, 13.82; range, 12.91–14.92.
[d] Historical control data; average, 0.56; range, 0.32–0.82.
[e] Historical control data; average, 0.02; range, 0.00–0.05.
[f] One dams showed 10 early and two late resorptions.
[g] One dam showed all implantations resorbed (14 early resorptions).

Source: Costlow & Kane (1984a)

In fetal examinations, the numbers of 7th cervical ribs and 14th rudimentary ribs per litter were statistically significantly increased at 313 mg/kg bw per day and above in a dose-dependent manner. These increases in variation frequencies were considered to be treatment related. When all malformations were considered together, a marginally significant dose-related trend was noted. The control incidence was zero and required the use of Fisher's exact test for analysis of the data. Although the analysis indicated a significant increase in malformations (fetuses only) at the 469 mg/kg bw per day dose, it was not considered to be toxicologically significant. A summary of variations and total number of all malformations is shown in Table 21.

Table 21. Summary of variations and total number of all malformations in a developmental toxicity study in rats

	0 mg/kg bw per day		31.3 mg/kg bw per day		93.8 mg/kg bw per day		313 mg/kg bw per day		469 mg/kg bw per day	
Observations	**Fetus**	**Litter**	**Fetus**	**Litter**	**Fetus**	**Litter**	**Fetus**	**Litter**	**Fetus**	**Litter**
Number of fetuses (number of litters) examined										
External	337 (22)		324 (24)		280 (21)		303 (23)		301 (22)	
Visceral	114		111		95		103		100	
Skeletal	223		213		185		200		201	
Skeletal variations										
7th cervical ribs	3	2	0	0	3	3	17	10*	45	14*
14th rudimentary rib(s)	1	1	4	3	1	1	17	8*	72	18*
14th full rib(s)	0	0	0	0	0	0	0	0	1	1
Any rib variation[a]	8	5	7	6	11	7	34	16*	72	20*
Any reduced ossification[b]	150	22	103	24	93	18	123	18	125	22
Total malformed	0	0	2	2	3	2	0	0	4*	4

bw: body weight; *: $P < 0.05$

[a] Includes 7th cervical, 14th rudimentary, 14th full or 13th rudimentary rib.

[b] Includes skull bones, hyoid, vertebrae, sternebrae.

Source: Costlow & Kane (1984a)

A reanalysis of data from Costlow & Kane (1984a), focusing on the evaluation and interpretation of the aforementioned skeletal alterations, 7th cervical rib and 14th rudimentary rib, both of which are common in rats, was conducted by Carney, Tornesi & Passage (2005). The reason for the reanalysis was that the interpretation of supernumerary ribs has been controversial for many years (Kimmel & Wilson, 1973). Many investigators discount such effects based on their known association with maternal toxicity/maternal stress (Khera, 1981; Kavlock, Chernoff & Rogers, 1985; Beyer & Chernoff, 1986) and their reversibility through the process of skeletal remodelling during postnatal development (Wickramaratne, 1988; Marr et al., 1992; Foulon et al., 2000). In contrast, certain developmental toxicants (e.g. salicylate, acetozolamide, bromoxynil, actinomycin D, dinoseb) induce supernumerary ribs that persist into adulthood (reviewed in Chernoff & Rogers, 2004), which has led some to assign greater significance to such effects. Interestingly, the incidence of cervical ribs in live human fetuses (19–39%) is much higher than in the adult human (0.04–1.2%), indicating that cervical ribs are usually transient structures in humans. Whereas extra ribs in the lumbar region are relatively common in the rat, they exhibit a low incidence (0.04–2%) in both the human fetus and adult (reviewed in Chernoff & Rogers, 2004). Using a more recent criterion, supernumerary ribs were classified as such by Carney, Tornesi & Passage (2005) when their length was more than twice their width. The reanalysis on this basis confirmed that the incidences of both skeletal alterations were just slightly above expected control incidences based on published data using similar rib length criteria and therefore were considered to be a treatment-related effect (Carney, Tornesi & Passage, 2005).

The NOAEL for maternal toxicity was 93.8 mg/kg bw per day, based on clinical signs indicating ill-health or reaction to treatment at 313 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 31.3 mg/kg bw per day, based on an increased number of early resorptions per litter at 93.8 mg/kg bw per day. Myclobutanil was not teratogenic in rats (Costlow & Kane, 1984a; Carney, Tornesi & Passage, 2005).

Rabbits

A developmental toxicity study of myclobutanil in rabbits was conducted in compliance with GLP, although it was not conducted according to test guidelines. However, examinations used in this study generally meet the current guidelines. Myclobutanil (TD no. 83-260; lot no. LAP-0298; purity 90.4%) was administered orally (adsorbed onto a silica carrier [Hi-Sil 233] and suspended in 1% aqueous [w/v] methyl cellulose) to 18 artificially inseminated New Zealand White rabbits from day 7 through day 19 of gestation at a dose of 0, 20, 60 or 200 mg/kg bw per day. There were two control groups, one with distilled water and one with the methyl cellulose vehicle. Feed consumption was not assessed.

Two rabbits at 200 mg/kg bw per day died due to intubation errors. No deaths occurred in the other groups. The does of the 200 mg/kg bw per day group showed signs indicative of a toxic response, such as increased incidences of irregular-shaped faeces, bloody urine, bloody urogenital or anal area and blood and/or aborted material in the drop pan. The signs did not appear at the beginning of the treatment, and their incidences showed peaks in the middle of the treatment period. All other clinical observations occurred sporadically and were not related to treatment with myclobutanil. At 200 mg/kg bw per day, three does aborted and two does died (one pregnant). Decreased body weight gain persisted during the treatment period at 200 mg/kg bw per day. Whereas body weight changes in the other treatment groups, including the vehicle control, were slightly decreased or constant during the period, mean maternal body weight at 60 mg/kg bw per day was slightly lower than in the vehicle control and 20 mg/kg bw per day groups. The body weight on day 11 in the 60 mg/kg bw per day group was significantly lower than that in the control group. Total body weight gains during the treatment period were 0.03, −0.02, 0.04, −0.06 and −0.28 kg in the control, vehicle control, 20, 60 and 200 mg/kg bw per day groups, respectively. The decreases in maternal body weight at 60 mg/kg bw per day and above were considered treatment related.

The viability index (number of fetuses/number of implantations) summarized an increase in the number of resorptions per litter, the number of litters with more than two resorptions and the number of litters totally resorbed at 200 mg/kg bw per day. Early but not late resorptions were increased at this dose. These were treatment-related adverse effects. In fetal data, litter size (viable fetuses per litter – combined sexes) was decreased at 200 mg/kg bw per day. This resulted in a significantly lower viability index. Pairwise comparisons of the 20 and 60 mg/kg bw per day groups with combined controls revealed no significant differences in viability index or litter size. In spite of the lack of statistically significant differences, fetal weights at 200 mg/kg bw per day were below historical control values, and this depression was considered to be related to the treatment. There were no external variations in any group. The treatment with myclobutanil did not affect fetal or soft tissue variations.

The NOAEL for maternal toxicity was 20 mg/kg bw per day, based on decreased body weight gain at 60 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 60 mg/kg bw per day, based on an increased number of resorptions per litter, an increased number of litters totally resorbed and lower fetal weights at 200 mg/kg bw per day. Myclobutanil was not teratogenic in rabbits (Costlow & Kane, 1984b).

2.6 Special studies

(a) Supplementary studies for reproductive toxicity

Myclobutanil has been shown to exert some reproductive toxicity, causing testicular atrophy and also an increased number of stillborn pups. A supplementary study was conducted to determine whether or not the early embryo loss may be induced by chromosomal abnormalities of the

spermatozoa in the myclobutanil-treated rats, leading to the death of conceptuses. This study complied with GLP. Myclobutanil (TD no. 86-77; lot no. 83 159-7; purity 91.4%) was administered to groups of 25 male Crl:COBS CD(SD)BR rats at a dose of 0, 10, 100 or 735 mg/kg bw once via gavage in Kodak stripped corn oil. After the single-dose treatment, each male rat was assigned to 8 consecutive weeks of mating with intact females. On day 14 of presumed gestation, each female was caesarean-sectioned and examined for corpora lutea, implantations, resorptions and live (beating embryonic heart) or dead conceptuses. Males were examined for gross lesions and effects on the testes and epididymides.

One male was found dead at 735 mg/kg bw with severe body weight loss 2 days after the single dose of myclobutanil. Clinical signs attributed to the treatment were evident in males during week 1 post-dosing at 735 mg/kg bw. These clinical signs included chromodacryorrhoea, chromorhinorrhoea, salivation, red oral exudate or dried red perioral material and/or urine-stained abdominal fur. In other males during weeks 2 through 8 post-dosing, no treatment-related clinical signs were observed. In females, no clinical signs were detected. Body weight gains at 735 mg/kg bw showed a consistent decrease but were not remarkable at the study termination. Female body weights and body weight gains during gestation were comparable among all sets of females mated by males in all groups. At termination, no treatment-related change was observed in males or females. No differences were seen in testes weights in any group. The percentage of pregnant female rats from the first mating with males treated at 735 mg/kg bw was slightly low compared with that which occurred during any other mating of the same male rats, and the decrease was considered to be the result of decreased mating performance of these rats prior to recovery from systemic toxic effects of the test substance. There were no other differences in pregnancy incidences or in the analysis of male fertility among all groups. The caesarean-sectioning data for each week of mating did not demonstrate any dose-dependent or significant differences. Averages for corpora lutea, implantations, litter sizes (live and dead embryos), resorptions and percentage of dead conceptuses per litter were similar for females among all groups.

Whereas a single oral gavage dose of 735 mg/kg bw produced systemic toxicity in male rats, including death in one male, there was no indication of a dose-dependent effect on the incidence of embryo death, even at a dose lethal to adults. No treatment-related changes were observed at 100 mg/kg bw (Dearlove, Hoberman & Christian, 1986).

(*b*) *Toxicity studies on metabolites and impurities*

Only very limited studies on metabolites were submitted.

Acute toxicity

An oral acute toxicity study of two main metabolites of myclobutanil in plants (RH-9090 and RH-9089) and two impurities in the myclobutanil preparation (RH-8812 and RH-8813) was carried out in mice. RH-9090 and RH-9089 were also detected in rat, hen and cow. These studies complied with GLP. Male and female CD-1 mice (five of each sex per group) were given a single oral gavage dose of RH-9090 (TD 87-089; lot no. LTN 2074; purity 98%) in polyethylene glycol 400, RH-9089 (TD 87-090; lot no. LN 2616; purity 99.4%), RH-8812 (TD 87-088; lot no. WJZ 2121; purity 99.6%) or RH-8813 (TD 87-091; lot no. WJZ 2122; purity 99.8%) at 0, 300, 1000 or 3000 mg/kg bw in corn oil, following a 4-hour fast. Two vehicle control groups (polyethylene glycol 400 and corn oil) were maintained. Animals were observed for 14 days post-dosing. Body weights were measured prior to dosing, on days 1, 2, 6, 9, 12 and 14, and just prior to termination. All premature decedents were necropsied as soon as practical. All surviving animals were necropsied after the final observation period.

Their mortalities and LD_{50}s are shown in Table 22. The LD_{50} value of the parent compound, myclobutanil, was 1910 mg/kg bw in male mice and 1360 mg/kg bw in female mice (see Table 4). The LD_{50}s for RH-9090 were equal to or lower than the parent value, and those for RH-9089 showed lower values than the parent. There were no remarkable differences in mortalities between the impurities and the parent (Shimizu, Tokiwa & Nakayoshi, 1987).

Table 22. Results of acute toxicity studies of myclobutanil metabolites and impurities in mice

Test material	Lot no. / purity	Dose (mg/kg bw)	Mortality[a]		LD_{50}
			Males	Females	
RH-9090	LTN 2074 / 98%	300	0/5	0/5	300 < LD_{50} < 1 000 mg/kg bw for males, 1 000 < LD_{50} < 3 000 mg/kg bw for females
		1 000	4/5 – 2 on day 0 and 2 on day 1	3/5 – Day 0	
		3 000	5/5 – Day 0	5/5 – 4 on day 0 and 1 on day 1	
RH-9089	LN 2616 / 99.4%	300	0/5	0/5	300 < LD_{50} < 1 000 mg/kg bw for both sexes
		1 000	3/5 – Day 0	2/5 – 1 on day 0 and 1 on day 1	
		3 000	5/5 – Day 0	4/5 – Day 0	
RH-8812	WJZ 2121 / 99.6%	300	0/5	0/5	1 000 < LD_{50} < 3 000 mg/kg bw for both sexes
		1 000	1/5 – Day 2	2/5 – Day 2	
		3 000	5/5 – 3 on day 1, 1 on day 2 and 1 on day 3	5/5 - 1 on day 0 and 4 on day 1	
RH-8813	WJZ 2122 / 99.8%	300	0/5	0/5	LD_{50} > 3 000 mg/kg bw for both sexes
		1 000	0/5	0/5	
		3 000	1/5 – Day 1	1/5 – Day 1	

[a] The number of mice dead/the number of mice examined.

Source: Shimizu, Tokiwa & Nakayoshi (1987)

Treatment-related clinical signs were as follows:

- *RH-9090*: The 300 mg/kg bw males displayed piloerection of the tail and soft faeces after 3 hours. All survivors returned to normal from 5 hours post-dosing. Females showed no signs at this dose. The males given 1000 mg/kg bw displayed erecting tail after 30 minutes, which was followed by sedation, depression of spontaneous movement, prone position and piloerection. One survivor returned to normal from day 2. The females given 1000 mg/kg bw displayed depression of spontaneous movement and erecting tail just after administration, which was followed by sedation. Two survivors returned to normal from day 1. The males and females given 3000 mg/kg bw displayed depression of spontaneous movement and sedation just after administration, which were followed by salivation, convulsion, erecting tail and prone position.
- *RH-9089*: The males given 300 mg/kg bw displayed piloerection of the tail after 30 minutes. All survivors returned to normal from 4 hours. The males given 1000 mg/kg bw displayed convulsion, piloerection of the tail and prone position after 30 minutes, which were followed by sedation. The females given 1000 mg/kg bw displayed sedation after 30 minutes, which was followed by convulsion, piloerection of the tail, depression of spontaneous movement and prone position. Two male and three female survivors returned to normal from day 1. The males given 3000 mg/kg bw displayed piloerection of the tail just after administration, which was followed by depression of spontaneous movement, convulsion and prone position. The females given 3000 mg/kg bw displayed salivation and piloerection of the tail just after administration, which were followed by sedation, convulsion and depression of spontaneous movement. One female survivor returned to normal from day 1.

- *RH-8812*: The males given 1000 mg/kg bw displayed sedation after 1 hour, which was followed by depression of spontaneous movement and prone position. Females displayed depression of spontaneous movement after 30 minutes, which was followed by sedation, prone position and convulsion. Four male and three female survivors returned to normal from day 2. The males given 3000 mg/kg bw displayed depression of spontaneous movement, sedation and prone position after 1 hour. Females displayed depression of spontaneous movement after 30 minutes, which was followed by convulsion, prone position and sedation.
- *RH-8813*: The females given 300 mg/kg bw displayed piloerection of the tail after 30 minutes. All animals returned to normal from 2 hours post-dosing. The males given 1000 mg/kg bw displayed sedation after 2 hours. All animals returned to normal from day 2. The females given 1000 mg/kg bw displayed erecting tail after 30 minutes, which was followed by depression of spontaneous movement and sedation. All females returned to normal from 4 hours post-dosing. The males given 3000 mg/kg bw displayed piloerection of the tail and prone position after 2 hours, which were followed by sedation and convulsion. Females given 3000 mg/kg bw displayed piloerection of the tail after 30 minutes, which was followed by depression of spontaneous movement. Male and female survivors returned to normal from day 1.

Treatment-related body weight changes were also detected. For RH-9090 and RH-9089, body weights were decreased in both sexes given 1000 mg/kg bw and females given 3000 mg/kg bw on day 1, but increased normally thereafter. For RH-8812, body weights were decreased in males given 1000 and 3000 mg/kg bw on days 1 and 2 and in females given 1000 mg/kg bw on day 1, but increased normally thereafter. For RH-8813, body weights were decreased in both sexes given 1000 and 3000 mg/kg bw on day 1, but increased normally thereafter. No macroscopic change was observed in either sex of any group treated with each test article at necropsy at termination (Shimizu, Tokiwa & Nakayoshi, 1987).

Short-term studies of toxicity

To determine the repeated oral dose toxicity potential of myclobutanil butyric acid, a metabolite in soil, female Crl:CD(SD) rats were administered myclobutanil butyric acid by gavage once daily for 14 days at a dose of 0, 50, 150, 450, 750 or 1000 mg/kg bw per day. Clinical observations, body weight and feed consumption measurements, and urinary metabolite identification (1000 mg/kg bw per day only) were performed, followed by gross necropsy on test day 15. In addition, a separate group of four female Crl:CD(SD) rats was administered 100 mg/kg bw per day of myclobutanil via gavage for 4 days to determine if myclobutanil is metabolized to myclobutanil butyric acid in rats. Urine from all four rats, as well as two female control rats, was collected during the last 24 hours of exposure and analysed for myclobutanil butyric acid.

All animals survived. There were no treatment-related effects on clinical observations, body weights, feed consumption or gross necropsy observations at any dose level tested. Urinary metabolite identification indicated that myclobutanil butyric acid was mainly excreted unchanged, although 3–4 minor metabolites were found. In rats given myclobutanil, levels of myclobutanil butyric acid were less than 1% of the administered dose of myclobutanil, compared with the results from the myclobutanil butyric acid group.

In conclusion, myclobutanil butyric acid has no toxicity in a 2-week oral toxicity study in rats. Myclobutanil butyric acid was not a metabolite in rats (Rasoulpour & Zablotny, 2009).

Genotoxicity

The genotoxicity studies on RH-9090, RH-9089 and myclobutanil butyric acid are summarized in Table 23.

Table 23. Summary of genotoxicity studies on RH-9090, RH-9089 and myclobutanil butyric acid

Test	Test object	Concentration	Lot no. / purity	Result	Reference
In vitro					
RH-9089					
Bacterial reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 *Escherichia coli* WP2 uvrA	156–5 000 µg/plate	Not described	Negative ±S9	Food Safety Commission (2011)
DNA repair test	*Bacillus subtilis* H17, M45	200–10 000 µg/mL	Not described	Negative ±S9	Food Safety Commission (2011)
RH-9090					
Bacterial reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2 uvrA	313–5 000 µg/plate	Not described	Negative ±S9	Food Safety Commission (2011)
DNA repair test	*B. subtilis* H17, M45	100–5 000 µg/mL	Not described	Negative ±S9	Food Safety Commission (2011)
Myclobutanil butyric acid					
Bacterial reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* WP2 uvrA	100–5 000 µg/plate	V43-037424-86 / 98%	Negative ±S9	Michael & Mecchi (2009)
Mammalian forward mutation	CHO cells (CHO/*Hgprt*)	181.7–2 907 µg/mL	V43-037424-86 / 98%	Negative ±S9	Schisler & Geter (2009)
In vivo					
Myclobutanil butyric acid					
Micronucleus	Mouse peripheral blood	500, 1 000 and 2 000 (limit dose) mg/kg bw	V43-037424-86 / 98%	Negative	Schisler & LeBaron (2009)

bw: body weight; CHO: Chinese hamster ovary; DNA: deoxyribonucleic acid; Hgprt: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate

In conclusion, RH-9090 and RH-9089, which are the major metabolites of myclobutanil in rats, plant, hen and cow, were not genotoxic in vitro, and myclobutanil butyric acid, a degradate in soil, was not genotoxic in vitro or in vivo (Schisler & LeBaron, 2009; Michael & Mecchi, 2009; Schisler & Geter, 2009; Food Safety Commission, 2011).

3. Observations in humans

Myclobutanil is a triazole fungicide that was manufactured previously by contract manufacturer Rhodia Chirex in the United Kingdom and, since late 2002, by contract manufacturer Kemira Fine Chemicals in Finland. Myclobutanil is repackaged in Barranquilla, Colombia. Medical surveillance data on eight employees have not shown any abnormalities to suggest adverse health

effects; there have been no incidents or allegations of adverse effects in this operation. Myclobutanil was bottled briefly in San Lorenzo, Argentina, in 2002. No medical surveillance has been conducted on the seven workers involved. No medical surveillance data on manufacturing personnel are available.

Medical surveillance data are available from the manufacturing/formulation of myclobutanil at Mozzanica, Italy, over the time span 2000–2005 and cover 25 workers. For all 25 workers, there were no health effects related to working with myclobutanil.

In conclusion, in reports on manufacturing plant personnel, no adverse health effects were noted.

Comments

Biochemical aspects

Myclobutanil was rapidly and extensively absorbed in rats (> 89%). Peak plasma and tissue concentrations of radiolabelled myclobutanil were achieved within 1 hour after oral administration. Plasma elimination was biphasic; the half-lives were 5 hours for the rapid phase and 26 hours for the slow phase in rats exposed to a single dose. Myclobutanil was widely distributed, and no significant tissue accumulation was observed 96 hours post-dosing.

Metabolism was extensive and appeared to occur mainly through a variety of oxidation reactions of the butyl group. The major unconjugated phenethyl triazole–containing metabolites were RH-9090 ((2*RS*,5*RS*)-2-(4-chlorophenyl)-5-hydroxy-2-(1*H*-1,2,4-triazol-1-ylmethyl)hexanenitrile) and RH-9089 ((2*RS*)-2-(4-chlorophenyl)-5-oxo-2-(1*H*-1,2,4-triazol-1-ylmethyl)hexanenitrile). Myclobutanil was rapidly and mostly excreted in urine and faeces within 48 hours in rats.

Toxicological data

The oral LD_{50} for myclobutanil was greater than or equal to 1600 mg/kg bw in rats. The dermal LD_{50} was greater than 5000 mg/kg bw in rats and rabbits. The inhalation LC_{50} was greater than 5.1 mg/L in rats. Myclobutanil was not irritating to the skin but was moderately irritating to the eye of rabbits. Myclobutanil was not sensitizing in the guinea-pig maximization test or the mouse local lymph node assay and was mildly sensitizing using the Buehler method.

The liver was the interspecies target of myclobutanil in short- and long-term toxicity studies. The testis was also a target of myclobutanil in long-term toxicity studies in rats. Reductions of feed consumption and corresponding decreases in body weight gains at the beginning of treatment in short-term dietary studies in mice, rats and dogs and a reproductive toxicity study in rats are considered to be due to low palatability, rather than an adverse effect, as no similar change in feed consumption was observed in gavage studies and no effects on the gastrointestinal tract were observed.

In a 90-day toxicity study in mice administered myclobutanil in the diet at a concentration of 0, 3, 10, 30, 100, 300, 1000, 3000 or 10 000 ppm (equal to 0, 0.40, 1.54, 4.79, 14.1, 42.7, 132, 542 and 2035 mg/kg bw per day for males and 0, 0.62, 2.11, 6.94, 22.9, 65.5, 232, 710 and 2027 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 42.7 mg/kg bw per day), based on fatty changes and necrosis of hepatocytes at 1000 ppm (equal to 132 mg/kg bw per day).

In a 90-day oral toxicity study in rats, myclobutanil was administered in the diet at a concentration of 0, 5, 15, 50, 150, 500, 1500, 5000 or 15 000 ppm for weeks 1 and 2; at 0, 7, 21, 70, 210, 700, 2100, 7000 or 21 000 ppm for weeks 3 and 4; and at 0, 10, 30, 100, 300, 1000, 3000, 10 000 or 30 000 ppm for the remainder of the study. These dietary concentrations were equal to doses of 0, 0.52, 1.60, 5.22, 15.3, 51.5, 158, 585 and 1730 mg/kg bw per day for males and 0, 0.67, 2.03, 6.85, 19.7, 65.8, 195.2, 665 and 1811 mg/kg bw per day for females, respectively. The NOAEL was 500/700/1000 ppm (equal to 51.5 mg/kg bw per day), based on increased liver and kidney weights, hepatocellular hypertrophy, single-cell necrosis in the liver and pigmentation in tubular epithelium in the kidneys at 1500/2100/3000 ppm (equal to 158 mg/kg bw per day).

In a 90-day oral toxicity study in dogs administered myclobutanil in the diet at 0, 10, 200, 800 or 1600 ppm (equal to 0, 0.34, 7.26, 29.1 and 56.8 mg/kg bw per day for males and 0, 0.42, 7.88, 32.4 and 58.0 mg/kg bw per day for females, respectively), the NOAEL was 800 ppm (equal to 29.1 mg/kg bw per day), based on liver hypertrophy, increased ALP and increased platelets at 1600 ppm (equal to 56.8 mg/kg bw per day).

In a 1-year oral toxicity study in dogs administered myclobutanil in the diet at a concentration of 0, 10, 100, 400 or 1600 ppm (equal to 0, 0.34, 3.09, 14.3 and 54.2 mg/kg bw per day for males and 0, 0.40, 3.83, 15.7 and 58.2 mg/kg bw per day for females, respectively), the NOAEL was 400 ppm (equal to 14.3 mg/kg bw per day), based on hepatocellular hypertrophy, ballooned hepatocytes, increased ALP and increased platelets in males at 1600 ppm (equal to 54.2 mg/kg bw per day).

The Meeting concluded that the overall NOAEL for oral toxicity in dogs was 800 ppm (equal to 29.1 mg/kg bw per day), and the overall LOAEL was 1600 ppm (equal to 54.2 mg/kg bw per day).

In a 2-year toxicity and carcinogenicity study in mice administered myclobutanil in the diet at a concentration of 0, 20, 100 or 500 ppm (equal to 0, 2.7, 13.7 and 70.2 mg/kg bw per day for males and 0, 3.2, 16.5 and 85.2 mg/kg bw per day for females, respectively), the NOAEL for toxicity was 100 ppm (equal to 13.7 mg/kg bw per day), based on histopathological signs of hepatotoxicity at 500 ppm (equal to 70.2 mg/kg bw per day). Myclobutanil was not carcinogenic in this study.

In a second 2-year carcinogenicity study conducted to confirm the absence of carcinogenicity at high doses, female mice were administered myclobutanil in the diet at a concentration of 2000 ppm (equal to 394 mg/kg bw per day), the MTD. No carcinogenicity was observed at this dose.

In a 2-year carcinogenicity study in rats administered myclobutanil in the diet at a concentration of 0, 50, 200 or 800 ppm (equal to 0, 2.5, 9.8 and 39.2 mg/kg bw per day for males and 0, 3.2, 12.8 and 52.3 mg/kg bw per day for females, respectively), the NOAEL for non-neoplastic effects was 50 ppm (equal to 2.5 mg/kg bw per day), based on testicular toxicity found after 12 months of treatment at 200 ppm (equal to 9.8 mg/kg bw per day). Myclobutanil was not carcinogenic in this study.

A second 2-year carcinogenicity study in rats confirmed the absence of carcinogenicity of myclobutanil at a higher dietary concentration, 2500 ppm (equal to 106 mg/kg bw per day for males and 136 mg/kg bw per day for females).

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

Myclobutanil was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found.

The Meeting concluded that myclobutanil is unlikely to be genotoxic.

On the basis of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that myclobutanil is unlikely to pose a carcinogenic risk to humans.

In a two-generation reproductive toxicity study in rats administered myclobutanil in the diet at a concentration of 0, 50, 200 or 1000 ppm (equal to 0, 3.67, 14.3 and 70.7 mg/kg bw per day for P_1 males, 0, 4.42, 17.2 and 85.9 mg/kg bw per day for P_1 females, 0, 3.64, 15.1 and 76.4 mg/kg bw per day for P_2 males and 0, 4.17, 17.5 and 88.0 mg/kg bw per day for P_2 females, respectively), the NOAEL for parental toxicity was 200 ppm (equal to 15.1 mg/kg bw per day), based on lower body weights, histopathological changes of vacuolation and hypertrophy of hepatocytes and testicular atrophy in P_2 males at 1000 ppm (equal to 76.4 mg/kg bw per day). The NOAEL for reproductive toxicity was 200 ppm (equal to 17.5 mg/kg bw per day), based on reduced reproductive ability, including number of females mating, number of females giving birth, number of females weaning litters or prolonged time to mating, in P_2 females at 1000 ppm (equal to 88.0 mg/kg bw per day). The NOAEL for offspring toxicity was 200 ppm (equal to 17.2 mg/kg bw per day), based on an increased number of pups born dead for both generations at 1000 ppm (equal to 85.9 mg/kg bw per day).

In a developmental toxicity study in rats administered myclobutanil by gavage at 0, 31.3, 93.8, 313 or 469 mg/kg bw per day, the NOAEL for maternal toxicity was 93.8 mg/kg bw per day,

based on clinical signs of rough hair coat, desquamation and salivation at 313 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 31.3 mg/kg bw per day, based on an increased number of early resorptions per litter at 93.8 mg/kg bw per day.

To determine whether treatment-related early resorptions in rats were caused by chromosomal abnormalities of the spermatozoa, leading to death of conceptuses, male rats were administered a single dose of myclobutanil at 0, 10, 100 or 735 mg/kg bw and mated with untreated females. There was no evidence of treatment-related embryo death, even at a dose lethal to adults.

In a developmental toxicity study in rabbits administered myclobutanil by gavage at 0, 20, 60 or 200 mg/kg bw per day, the NOAEL for maternal toxicity was 20 mg/kg bw per day, based on decreased body weight gain at 60 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 60 mg/kg bw per day, based on an increased number of resorptions per litter, an increased number of litters totally resorbed and lower fetal weights at 200 mg/kg bw per day.

The Meeting concluded that myclobutanil is not teratogenic.

There were no studies submitted that specifically investigated neurotoxicity or immunotoxicity.

Toxicological data on metabolites and/or degradates

The oral LD_{50} ranges for RH-9090 and RH-9089, major metabolites in plants, rats, hens and cows, were between 300 and 1000 mg/kg bw in mice.

In a 2-week oral toxicity study on myclobutanil butyric acid ((3*RS*)-3-(4-chlorophenyl)-3-cyano-4-(1*H*-1,2,4-triazol-1-yl)butanoic acid), a degradate in soil, no toxicity was observed at doses up to 1000 mg/kg bw administered by gavage to rats.

Tests of the in vitro genotoxicity of RH-9089, RH-9090 and myclobutanil butyric acid and an in vivo genotoxicity assay on myclobutanil butyric acid showed no evidence of genotoxicity.

The Meeting concluded that RH-9090 and RH-9089, which are major metabolites in rats, are covered by the reference doses for myclobutanil. Myclobutanil butyric acid is of no toxicological concern, as it is of lower toxicity than the parent.

Human data

In reports on manufacturing plant personnel, no adverse health effects were noted.

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

Toxicological evaluation

The Meeting reaffirmed the ADI of 0–0.03 mg/kg bw on the basis of the NOAEL of 2.5 mg/kg bw per day in a 2-year study in rats, based on testicular atrophy at 9.8 mg/kg bw per day. A safety factor of 100 was applied. This ADI is based on the same end-point as in 1992.

The Meeting established an ARfD of 0.3 mg/kg bw for women of childbearing age only, on the basis of the NOAEL of 31.3 mg/kg bw per day in a developmental toxicity study in rats, based on an increased number of early resorptions at 93.8 mg/kg bw per day. A safety factor of 100 was applied. The Meeting concluded that it is not necessary to establish an ARfD for the remainder of the population in view of the low acute oral toxicity of myclobutanil and the absence of any other toxicological effects that would be likely to be elicited by a single dose.

Levels relevant to risk assessment of myclobutanil

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year studies of toxicity and carcinogenicity[a,b]	Toxicity	100 ppm, equal to 13.7 mg/kg bw per day	500 ppm, equal to 70.2 mg/kg bw per day
		Carcinogenicity	2 000 ppm, equal to 394 mg/kg bw per day[c]	–
Rat	Two-year studies of toxicity and carcinogenicity[a,b]	Toxicity	50 ppm, equal to 2.5 mg/kg bw per day	200 ppm, equal to 9.8 mg/kg bw per day
		Carcinogenicity	2 500 ppm, equal to 106 mg/kg bw per day[c]	–
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	200 ppm, equal to 17.5 mg/kg bw per day	1 000 ppm, equal to 88.0 mg/kg bw per day
		Parental toxicity	200 ppm, equal to 15.1 mg/kg bw per day	1 000 ppm, equal to 76.4 mg/kg bw per day
		Offspring toxicity	200 ppm, equal to 17.2 mg/kg bw per day	1 000 ppm, equal to 85.9 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	93.8 mg/kg bw per day	313 mg/kg bw per day
		Embryo and fetal toxicity	31.3 mg/kg bw per day	93.8 mg/kg bw per day
Rabbit	Developmental toxicity study[d]	Maternal toxicity	20 mg/kg bw per day	60 mg/kg bw per day
		Embryo and fetal toxicity	60 mg/kg bw per day	200 mg/kg bw per day
Dog	Thirteen-week and 1-year studies of toxicity[a,b]	Toxicity	800 ppm, equal to 29.1 mg/kg bw per day	1 600 ppm, equal to 54.2 mg/kg bw per day

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

Estimate of acceptable daily intake (ADI)

0–0.03 mg/kg bw

Estimate of acute reference dose (ARfD)

0.3 mg/kg bw (applies to women of childbearing age only)

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 myclobutanil

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapidly absorbed (> 89%)
Dermal absorption	No data
Distribution	Extensive
Potential for accumulation	No significant tissue accumulation
Rate and extent of excretion	Rapidly excreted
Metabolism in animals	Extensively metabolized, mainly through a variety of oxidation reactions
Toxicologically significant compounds in animals and plants	Myclobutanil, unconjugated phenethyl triazole–containing metabolites (RH-9089, RH-9090) (rat, hen, cow, plants)
Acute toxicity	
Rat, LD_{50}, oral	≥ 1 600 mg/kg bw
Rat, LD_{50}, dermal	> 5 000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.1 mg/L
Rabbit, dermal irritation	Not irritating to skin
Rabbit, ocular irritation	Moderately irritating to eye
Guinea-pig, dermal sensitization	Not sensitizing (maximization test and local lymph node assay); mildly sensitizing (Buehler method)
Short-term studies of toxicity	
Target/critical effect	Liver / increases in ALP and platelets (dog)
Lowest relevant oral NOAEL	29.1 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	100 mg/kg bw per day, highest dose tested (rat)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Testes/atrophy (rat)
Lowest relevant NOAEL	2.5 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic (rat and mouse); unlikely to pose a carcinogenic risk to humans
Genotoxicity	
	Unlikely to be genotoxic

Reproductive toxicity	
Target/critical effect	Testicular atrophy, increased number of pups born dead, reduced reproductive ability
Lowest relevant parental NOAEL	15.1 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	17.2 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	17.5 mg/kg bw per day (rat)
Developmental toxicity	
Target/critical effect	Fetal toxicity / increased number of early resorptions and lower fetal weights
Lowest relevant maternal NOAEL	20 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	31.3 mg/kg bw per day (rat)
Neurotoxicity	
Acute neurotoxicity NOAEL	No data
Subchronic neurotoxicity NOAEL	No data
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity	No data
Studies on toxicologically relevant metabolites	*RH-9089 and RH-9090*: Oral LD_{50}: 300–1 000 mg/kg bw (mice) Unlikely to be genotoxic *Myclobutanil butyric acid*: NOAEL: 1 000 mg/kg bw, highest dose tested (2-week study in rats) Unlikely to be genotoxic
Studies on impurities	Studies on RH-8812 and RH-8813 not relevant for dietary risk assessment
Medical data	
	No adverse effects noted in medical surveillance reports on manufacturing plant personnel

Summary

	Value	Study	Safety factor
ADI	0–0.03 mg/kg bw	Two-year study of toxicity and carcinogenicity (rat)	100
ARfD	0.3 mg/kg bw[a]	Developmental toxicity study (rat)	100

[a] Applies to women of childbearing age only.

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