

**MYCLOBUTANIL**

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**EXPLANATION**

Myclobutanil is a broad spectrum systemic fungicide of the substituted triazole chemical class of compounds. The mode of action of myclobutanil is by inhibition of sterol biosynthesis in fungi. Myclobutanil was considered for the first time by the present Meeting.

**EVALUATION FOR ACCEPTABLE DAILY INTAKE****BIOLOGICAL DATA****Biochemical aspects****Absorption, distribution, and excretion****Mice**

Male and female Crl:CD-1 mice were treated by gavage with a single oral dose of 2, 20 or 200 mg <sup>14</sup>C-myclobutanil/kg bw, radiolabelled in the chlorophenyl ring, immediately following a 2-week pretreatment period with unlabelled myclobutanil (81.1% purity) at dietary levels of 10, 100 or 1000 ppm a.i. Excretion of radioactivity after 96 h accounted for 81-107% of the administered <sup>14</sup>C-radiolabel, with the majority of the radiolabel recovered within 24-48 h post-dosing. Comparable amounts of radiolabel were found in both the urine (with cage wash: 41-57%) and faeces (31-52%) with no significant differences with respect to sex or dose level. Orally administered <sup>14</sup>C-myclobutanil was rapidly absorbed from the gastrointestinal tract, with peak blood concentrations occurring within 0.2-1 h post-dosing and with absorption half-lives of 0.04-0.3 h. Elimination was generally biphasic with half-lives of 0.6-0.9 and 6.0-30.1 h, respectively. The single exception to the elimination profile was the high-dose (200 mg/kg bw) treated male group, where only one phase of elimination (half-life: 6.2 h) was observed. Presence of <sup>14</sup>C-radiolabel in groups of mice killed one-hour after dosing revealed similar dose-related concentrations in whole blood and plasma. Concentrations of radiolabel in the liver were 4 to 11-fold higher than in the blood, although the ratio of liver to blood <sup>14</sup>C-concentration was shown to decrease with increasing dose (Steigerwalt *et al.*, 1986a).

## Rats

Four Sprague-Dawley rats/sex were administered a single oral dose of 150 mg  $^{14}\text{C}$ -myclobutanil/kg bw, radiolabelled at the 3 and 5 carbons of the triazole ring and suspended in aqueous 0.5% methyl cellulose. The major routes of elimination of the administered radiolabel, as determined 7 days post-dosing, were via the urine (48% males, 37% females) and faeces (51% males, 63% females). The level of radioactivity in expired  $\text{CO}_2$  was minimal (0.01-0.02%). Residual tissue levels were higher in males than in females, representing 0.2-0.5% of the radiolabel, with highest concentrations present in the intestine, liver and kidney (Streelman, 1984).

Myclobutanil  $^{14}\text{C}$ -labelled in the chlorophenyl ring was administered by gavage to groups of male and female Crl:CD(SD)BR rats as a single oral dose of 1 or 100 mg/kg bw or as a single high-dose of 100 mg/kg bw preceded by a 14-day repeated exposure to unlabelled myclobutanil (81.1% purity) at a dietary level of 1000 ppm a.i. An additional group of rats received a single intravenous dose of 1 mg  $^{14}\text{C}$ -myclobutanil/kg bw. Total radioactive recovery after 96 h, upon oral dosing ranged from 82-97% and following intravenous administration was 77% in males and 82% in females. The majority of the radiolabel was excreted via the urine (35-48%) and faeces (32-46% of the radiolabel dose), regardless of route of administration.  $^{14}\text{C}$ -myclobutanil was well absorbed (89-115%) following oral administration as determined by the ratio of the percentage dose excreted in the urine after oral and intravenous dosing. Peak blood and tissue levels as studied at the high-dose of 100 mg/kg bw, occurred within 1 hour post-dosing. Elimination kinetics of  $^{14}\text{C}$ -radiolabel was biphasic after a single oral dose of 100 mg/kg bw alone (plasma half-lives of 5.3 and 25.7 h) or after dietary pretreatment (plasma half-lives of 2.0 and 31.5 h). Residual tissue levels in orally treated rats after 96 hours were generally less than 1% of the dose, with highest concentrations present in the liver, kidneys, adrenals, whole blood, thyroids and bone marrow (Steigerwalt *et al.*, 1986b).

## Biotransformation

### Mice

Metabolic profiles from the urine and faeces of mice treated with  $^{14}\text{C}$ -myclobutanil (labelled in the chlorophenyl ring) at 2, 20 or 200 mg/kg bw following 2-week dietary pretreatment (Steigerwalt *et al.*, 1986a) revealed no significant sex or dose-related differences in  $^{14}\text{C}$ -metabolite patterns. Myclobutanil was extensively metabolized to more polar compounds. Unchanged parent detected in the excreta accounted for 0.7 to 7.2% of the administered dose. No further metabolic characterization was performed.

## Rats

The proposed metabolic pathway in rats is depicted in Figure 1.

<sup>14</sup>C-Myclobutanil (labelled in the 3 and 5 carbons of the triazole ring) was extensively metabolized when administered to SD rats as a single oral dose of 150 mg/kg bw (Streelman, 1984). Unchanged parent myclobutanil was estimated to represent only 2-3% of the excreted dose. The predominant pathway of metabolism has been suggested to be through a variety of oxygenation reactions of the butyl group. The major polar metabolites identified including a lactone, ketone, alcohol, carboxylate, dialcohol and sulfate conjugate, were distributed uniformly in the urine and faeces of both sexes. Quantitative differences in the metabolic profiles were apparent most notably with the major metabolite, the sulfate conjugate of RH-9090, which in females accounted for 75% and in males for only 15% of the total.

Myclobutanil, <sup>14</sup>C-radiolabelled in the chlorophenyl ring was extensively metabolized to more polar metabolites when administered by gavage to groups of male and female rats as a single oral dose of 1 or 100 mg/kg bw (Steigerwalt *et al.*, 1986b). <sup>14</sup>C-Metabolites of myclobutanil were qualitatively similar with respect to sex and dose. Unchanged parent compound represented only 1-4% of the excreted dose. In males, five fractions with more than 10% of the excreted <sup>14</sup>C were identified in the excreta compared with a single major fraction in females, which accounted for 53-61% of the radiolabel.

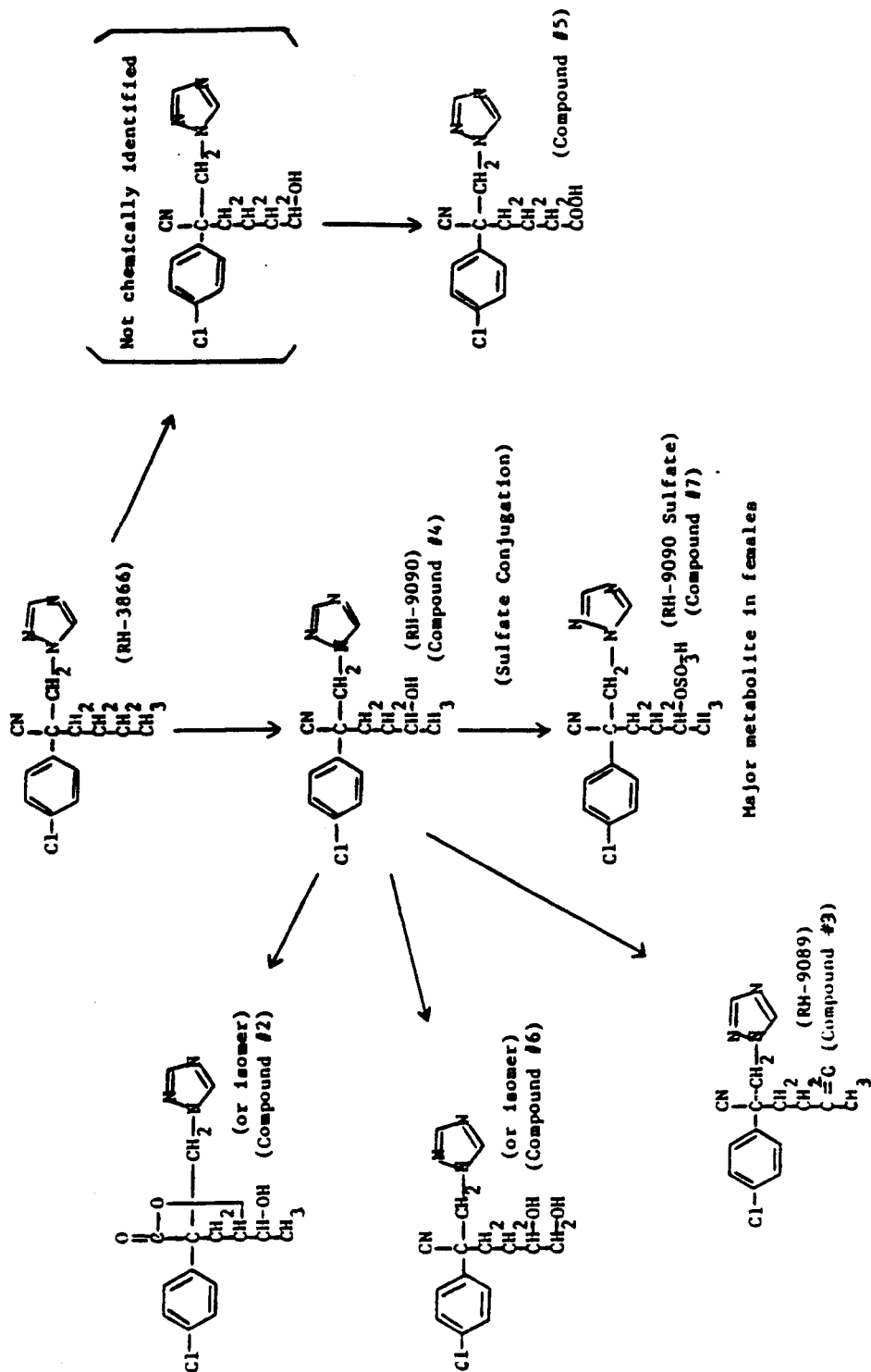
## Effects on enzymes and other biochemical parameters

### Mice

Sections of liver were taken from 4 randomly selected mice/sex from the 6 highest dietary levels: 30, 100, 300, 1000, 3000 or 10 000 ppm a.i. of the 3-month dietary toxicity study with myclobutanil in mice (Goldman *et al.*, 1986) for determination of hepatic MFO activity. Liver sections were analyzed for MFO activity using aminopyrine and benzphetamine N-demethylation assays. MFO activity per gram of liver as estimated by N-demethylation of benzphetamine was significantly increased in males (2.1 to 3.3-fold) at 1000 ppm and above, and in females (1.7 to 2.2-fold) at dietary levels of 3000 ppm and higher. Enzyme activity as measured by N-demethylation of amino-pyrene revealed increased levels in males (2.2 to 2.3-fold) at 3000 ppm and higher, and in females at 10 000 ppm (1.7-fold). Hepatic microsomal protein was increased in both sexes at dietary levels of 3000 ppm and higher.

Liver microsomal suspensions were prepared from 6 randomly selected mice/sex treated with myclobutanil at 0, 20, 100 and 500 ppm a.i. as part of the long-term carcinogenicity study (Goldman & Harris, 1986a). The microsomal suspensions were assayed for MFO activity using aminopyrene N-demethylation

Figure 1. Proposed major pathways of myclobutanil metabolism in rats



after 3, 6 or 12 months of treatment. Hepatic MFO activity per gram of liver was increased after 3 months in females (1.3-fold) at 100 ppm, and in both sexes (1.5 to 2-fold) at 500 ppm; after 6 months in both sexes at 100 ppm (1.3 to 1.4-fold) and 500 ppm (2.8 to 2.9-fold); and after 12 months in females (1.5-fold) at 100 ppm, and in both sexes (1.6 to 3.7-fold) at 500 ppm. There were no treatment-related effects on hepatic microsomal protein concentration after 3, 6 or 12 months.

Additional liver samples taken at 12 months were analyzed for hepatic peroxisomal  $\beta$ -oxidation activity by measuring the conversion of acid insoluble  $^{14}\text{C}$ -palmitoyl-CoA (substrate) to the acid soluble acetyl CoA by hepatic acyl-CoA oxidase. There were no increases in peroxisomal  $^{14}\text{C}$ -palmitoyl-CoA oxidase activity indicative of peroxisomal proliferation (Goldman & Harris, 1986a).

### Rats

Mixed function oxidase activity was measured by aminopyrine and benzphetamine N-demethylation assays on sections of liver taken from 3 randomly selected rats/sex from the control group and from dietary level groups fed myclobutanil at 100 ppm and higher as part of a 3-month study (O'Hara & DiDonato, 1984). Significant, dose-related increases in MFO activity per gram of liver were recorded at dietary levels of 300 ppm in males only (1.7 to 1.9-fold), and in both sexes at 1000 ppm (1.8 to 2.3-fold), 3000 ppm (3 to 5.1-fold) and 10 000 ppm (4.1 to 8-fold higher). Microsomal protein was increased in both sexes at 10 000 ppm and in males at 3000 ppm.

Livers from 6 randomly selected rats/sex/group were collected from interim kills scheduled after 3, 6 or 12 months of treatment with myclobutanil at dietary levels of 0, 50, 200 or 800 ppm as a component of the long-term study in rats (Shellenberger *et al.*, 1986). Hepatic MFO analyzed by aminopyrine N-demethylation revealed increased activity per gram of liver after 3 months at 200 ppm in females (1.6-fold) and in both sexes at 800 ppm (1.5 to 1.8-fold), and after 6 months in males at 800 ppm (1.5-fold). Increases in MFO activity in females at 6 months and in both sexes at 12 months of study were not significantly different from the controls. The microsomal protein concentration in the treated groups was not markedly different from the controls.

For determination of hepatic peroxisomal  $\beta$ -oxidation activity, additional sections of liver were obtained from 6 rats/sex/group at the 12-month interim kill. There was no effect of myclobutanil treatment on hepatic peroxisomal  $\beta$ -oxidation activity at dietary levels as high as 800 ppm (Shellenberger *et al.*, 1986).

## **Toxicological studies**

### **Acute toxicity studies**

Acute toxicity studies with technical myclobutanil have been performed in several animal species, the results of which are summarized in Table 1. Myclobutanil, upon oral administration was only slightly toxic to the mouse and rat with LD<sub>50</sub> values of 1.36 to > 4.42 g/kg bw and 1.6 to 2.71 g/kg bw, respectively. Myclobutanil was practically non-toxic by the inhalation and dermal routes with a LC<sub>50</sub> value greater than 5.1 mg/L in rats and LD<sub>50</sub> values greater than 5 g/kg bw in the rabbit, respectively.

### **Short-term toxicity studies**

#### **Mice**

Myclobutanil (81.1% purity) was administered daily for a period of 3 months to groups of 10 Crl:CD-1 (ICR) BR mice/sex at dietary levels of 0, 3, 10, 30, 100, 300, 1000, 3000 or 10 000 ppm a.i. (equal to 0, 0.4, 1.5, 4.8, 14.1, 42.1, 132, 542 or 2035 mg a.i./kg bw/day in males and 0, 0.6, 2.1, 6.9, 22.9, 65.5, 232, 710 and 2027 mg a.i./kg bw/day in females, respectively). A NOAEL of 300 ppm, equal to 42.1 mg/kg bw/day, was indicated based on treatment-related hepatic alterations at dietary levels of 1000 ppm and higher, manifested histomorphologically as hepatocytic inflammation, centrilobular hypertrophy, vacuolation, and necrosis. Associated hepatic changes were increases in liver weight, accentuated liver lobular architecture, increased MFO activity, increased ALAT levels and decreased cholesterol. Other microscopic treatment-related changes pertained to increased cytoplasmic eosinophilia ( $\geq 1000$  ppm males,  $\geq 3000$  females) and hypertrophy of the *zona fasciculata* cells of the adrenal gland (3000 ppm and higher in both sexes). Treatment-related effects observed at dietary levels of 3000 ppm and above were decreased body-weights, increased ASAT, decreased glucose, increased pigmentation of the liver Kupffer cells and macrophages of the spleen, and a slight increase in lymphoid necrosis of the spleen. Additional effects of treatment observed only at the highest dietary level of 10 000 ppm were related to scant faecal droppings and fluctuations in haematological (both sexes:  $\downarrow$  HCT,  $\downarrow$  MCV,  $\downarrow$  MCH,  $\uparrow$  MCHC; males:  $\downarrow$  WBC,  $\downarrow$  lymphocytes,  $\uparrow$  segmented neutrophils; females:  $\downarrow$  haemoglobin and  $\uparrow$  platelets) and blood biochemical parameters (increased ALP, GGT and BUN). Histopathological changes at 10 000 ppm were noted as bile duct proliferation, slight increase in pigmentation of renal cortical tubular cells, lymphoid necrosis of the thymus and mesenteric lymph nodes, increased myeloid/erythroid ratio of bone marrow, immaturity of the uterus and absence of corpora lutea in ovaries as well as increased mononuclear cell infiltration of the skin (Goldman *et al.*, 1986).

Table 1

Table 1. Acute toxicity of technical myclobutanil

Species (strain)	Sex	Route	Vehicle	LD <sub>50</sub> (g/kg bw)	Purity <sup>a</sup>	Reference
Mouse (CRCD-1)	M	oral	corn oil	3.23	81.1%	Krzywicki & Krajewski (1983)
Mouse (CRCD-1)	M	oral	corn oil	> 4.42	91.9%	Morrison <i>et al.</i> (1984a)
Mouse (CRCD-1)	M+F	oral	corn oil	M: 1.91 F: 1.84	91.9%	Morrison <i>et al.</i> (1986b)
Mouse (CRCD-1)	F	oral	corn oil	1.36	91.9%	Romanello <i>et al.</i> (1986a)
Mouse (CRJ: CD-1 (ICR))	M+F	oral	corn oil	M: 2.27 F: 2.44	91.4%	Shimizu (1987a)
Rat (CR(CD)SD)	M+F	oral	corn oil	M: 1.75 F: 1.80	84.5%	Krzywicki (1983)
Rat (CR(CD)SD)	M+F	oral	corn oil	M: 1.6 F: 2.29	91.9%	Krzywicki & Morrison (1984a)
Rat (CRJ: CD-1 (SD))	M+F	oral	corn oil	M: 2.62 F: 2.71	91.4%	Shimizu (1987b)
Rat (Cri:CDBR)	M+F	inhalation	-	LC <sub>50</sub> : > 5.1 mg/L air	91.4%	Fisher <i>et al.</i> (1987)
Rabbit (NZW)	M+F	dermal	-	> 5.0	84.5%	Krzywicki (1983)
Rabbit (NZW)	M+F	dermal	-	> 5.0	91.9%	Krzywicki & Bonin (1984)

<sup>a</sup> = active ingredient (a.i.)

## Rats

Groups of 10 COBS-CD(SD) BR rats/sex were fed myclobutanil (81.1% purity) for 3 months at dietary levels of 0, 10, 30, 100, 300, 1000, 3000, 10 000 or 30 000 ppm a.i. equal to 0, 0.5, 1.6, 5.2, 15.3, 51.5, 158, 585 or 1730 mg a.i./kg bw/day in males and 0, 0.7, 2.0, 6.9, 19.7, 65.8, 195, 665 or 1811 mg a.i./kg bw/day in females, respectively. A NOAEL for this study was 100 ppm, equal to 5.2 mg/kg bw/day, as revealed by increased MFO activity in males at 300 ppm and in both sexes at higher dietary levels. Increased liver weights and accentuated hepatic lobular architecture were observed at 1000 ppm and above. Treatment-related changes introduced at dietary levels of 3000 ppm were decreased body-weights, increased cholesterol, hepatic centrilobular hypertrophy and necrosis, increased kidney weights associated with renal congestion and pigmentation of the convoluted tubular epithelium, and histopathological alterations of the adrenal (increased cortical vacuolization), ovary (congestion), thyroid (increase in small follicles) and thymus (congestion). Additional effects of treatment exhibited at 10 000 ppm were decreased food consumption, slight haematological changes ( $\downarrow$  HCT,  $\downarrow$  Hb,  $\downarrow$  MCV and  $\uparrow$  RBC,  $\uparrow$  platelets), increased GGT, pigmentation of the liver Kupffer cells, hepatocytic vacuolation and coagulative necrosis, increased pigmentation in the red pulp of the spleen, and chronic pulmonary alveolitis. Treatment of rats with myclobutanil at the highest dietary level of 30 000 ppm resulted in 100% mortality. No effects of treatment were reported with respect to urinalysis and ophthalmology (O'Hara & DiDonato, 1984).

A 13-week feeding study conducted with groups of 10 Crj:CD SD rats/sex given myclobutanil (91.4% purity) in the diet at levels of 0, 100, 300 or 3000 ppm equal to 0, 6.2, 18.8 or 192 mg/kg bw/day in males and 0, 6.9, 19.6 or 225 mg/kg bw/day in females respectively, demonstrated a NOAEL of 300 ppm, equal to 18.8 mg/kg bw/day. Treatment with myclobutanil at the highest dietary level of 3000 ppm culminated in histomorphological alterations of the liver, kidney and adrenal glands. Specific organ changes were characterized in the liver as slight to moderate hepatocytic hypertrophy (10/10 males, 8/10 females) and in the kidney as slight vacuolar degeneration of the renal tubular epithelium (7/10 males). Alterations of the adrenal were described as vacuolization of the cortical cells (7/10 males), atrophy of the *zona fasciculata* (5/10 males) and fine vacuolization of the *zona glomerulosa* (1/10 males). A single male at 3000 ppm exhibited changes in the reproductive organs depicted in the testes as moderate atrophy of the seminiferous tubule(s) and giant cell-like changes with absence of sperm cells in the epididymis. Other effects of treatment with myclobutanil were decreased body-weight, blood chemistry changes (decreased bilirubin, glucose and triglycerides), increased liver and kidney weights, decreased adrenal weights and a slight increase in the number of males with round cells in the urine. Decreased food intake was observed in males during the first week of treatment. There were no treatment-related effects on haematology or ophthalmology (Shimizu, 1987c).

## Dogs

A range-finding study was undertaken with 2 beagle dogs/sex per group fed myclobutanil (84.5% purity) for a period of 4 weeks at dietary levels of 0, 50, 250, 1000 or 4000 ppm a.i. equal to 0, 2.2, 10.5, 45.3 or 45 mg a.i./kg bw/day in males and 0, 2.0, 10.6, 39.3 or 47 mg a.i./kg bw/day in females respectively. Dogs treated at the highest dietary level of 4000 ppm were sacrificed after 2 weeks of treatment due to severely depressed food intake. The NOAEL for the study was determined to be 250 ppm, equal to 10.5 mg/kg bw/day, based on slightly decreased body-weight and food consumption recorded during the first week of treatment in females at 1000 ppm. There were no treatment-related consequences on clinical signs, haematological and blood chemistry investigations (12 and 28 days), or gross pathological examination (Goldman & Emmons, 1986).

Myclobutanil (81.1% purity) was administered to groups of 4 beagle dogs/sex for a period of 3 months at dietary levels of 0, 10, 200, 800 or 1600 ppm a.i. equal to 0, 0.3, 7.3, 29.1 or 56.8 mg a.i./kg bw/day in males and 0, 0.4, 7.9, 32.4 or 58 mg a.i./kg bw/day in females respectively. The NOAEL was determined to be 10 ppm, equal to 0.3 mg/kg bw/day, based on the dose-related incidence of centrilobular or midzonal hepatocellular hypertrophy noted at 200 ppm (3/4 males) and in all animals of both sexes at 800 and 1600 ppm. Periportal hepatocytes were enlarged in a few of the more severely affected livers. Liver weights were increased in males at 800 ppm and in both sexes at 1600 ppm. Treatment-related effects on the kidney, evident as an increased incidence and severity of unilateral chronic nephritis were observed in males at 800 ppm and higher. Additional effects of treatment unveiled at the highest level of 1600 ppm were related to decreased body-weights and food consumption, due possibly to palatability of the diet. Slight changes in haematological and blood chemistry values were within range of normal variability and were not considered of toxicological consequence. There were no effects of treatment on ophthalmology. Increases in the ovarian weights at 800 and 1600 ppm were attributed to estrus (McLaughlin & DiDonato, 1984).

Groups of 6 beagle dogs/sex were administered myclobutanil (91.4% purity) daily for 12 months in the diet at levels of 0, 10, 100, 400 or 1600 ppm a.i. equal to 0, 0.3, 3.1, 14.3 or 54.2 mg a.i./kg bw/day in males and 0, 0.4, 3.8, 15.7 or 58.2 mg a.i./kg bw/day in females, respectively. The principal target organ was the liver, demonstrating a NOAEL of 100 ppm, equal to 3.1 mg/kg bw/day, based on hepatocellular hypertrophy, increased liver weights and increased serum alkaline phosphatase levels at dietary concentrations of 400 ppm and higher. At the highest level of 1600 ppm, livers displayed accentuated lobular architecture and in 4 of 6 females, the hepatocytes were expanded with large clear cytoplasmic spaces. Other effects observed at 1600 ppm were evident as decreased body-weight and food consumption, as well as changes in haematology ( $\downarrow$  RBC,  $\uparrow$  platelet count) and blood chemistry ( $\uparrow$  phosphorus,  $\uparrow$  ALAT,  $\uparrow$  GGT,  $\downarrow$  albumin). Treatment with

myclobutanil failed to elicit any adverse effects on clinical signs, ophthalmological examination or urinalysis (Goldman & Harris, 1986b).

### **Long-term toxicity/carcinogenicity studies**

#### **Mice**

A 2-year study was conducted with Crl:CD-1 (ICR)BR mice fed diets containing myclobutanil (90.4% purity) at levels of 0, 20, 100 or 500 ppm a.i. equal to 0, 2.7, 13.7 or 70.2 mg a.i./kg bw/day in males and 0, 3.2, 16.5 or 85.2 mg a.i./kg bw/day in females, respectively. A total of 110 males and 110 females per group were assigned to the chronic phase of this bioassay. Interim sacrifices were scheduled after 3 (10 mice/sex/group), 6 (10/sex/group) and 12 months (20/sex/group) of study. All surviving animals (70/sex/group, maximum) were sacrificed after 24 months of continuous treatment. Treatment with myclobutanil resulted in a NOAEL for in-life parameters of 20 ppm, equal to 2.7 mg/kg bw/day, established on the basis of increased MFO activity at 3, 6 and 12 months at dietary levels of 100 and 500 ppm. At 500 ppm, target effects of treatment, primarily on the liver were demonstrated by increased ALAT activity (females, 3 months), increased liver weights (both sexes, 3 months), and histopathological alterations comprising centrilobular hypertrophy (males, 3/6/12 months), periportal vacuolation (males, 3/6/12 months; both sexes, 24 months), Kupffer cell pigmentation (males, 6/12 months), hepatocellular necrosis (males, 12 months) and hepatocellular alteration (tinctorial and dimensional properties: both sexes, 24 months). There were no changes attributed to treatment with myclobutanil with regard to survival, clinical signs, body-weight, food consumption, ophthalmoscopy, haematology or urinalysis. Myclobutanil was not oncogenic when administered to mice for 2 years at dietary levels up to 500 ppm (Goldman & Harris, 1986a).

#### **Rats**

Groups of 110 Charles River Sprague Dawley rats/sex were treated with myclobutanil (90.4% & 91.4% purity) for a period of up to 24 months at dietary levels of 0, 50, 200 or 800 ppm a.i. equal to 0, 2.5, 9.8 or 39.2 mg a.i./kg bw/day in males and 0, 3.2, 12.9 or 52.3 mg a.i./kg bw/day in females, respectively. Interim sacrifices were performed after 3 and 6 months (10 rats/sex/group), 12 months (20/sex/group) and 17 months of study (18 males and 10 females/group). The survivors (52 male, 60 female/group, maximum) were sacrificed after 24 months of treatment. Treatment with myclobutanil indicated a NOAEL for in-life parameters of 50 ppm, equal to 2.5 mg/kg bw/day. Effects of treatment at 200 ppm were observed as decreased testes weights in association with slight testicular atrophy at 24 months. At the highest dietary level of 800 ppm, testes weights were decreased at 12 and 24 months with slight to moderate increases in the incidence of testicular atrophy. (The seminiferous tubules were reported frequently found to be devoid of spermatid formation and germinal epithelial cells; the tubules appeared smaller than normal. In severe cases only Sertoli cells remained).

Increased ovary weights in females treated at 800 ppm and sacrificed at 12 months were not correlated with any histomorphological changes. Other treatment-related effects noted at 800 ppm were decreased body-weights (both sexes) and food consumption (males), and increased liver weights (females). Increased MFO activity was recorded at 200 ppm in females (3 months) and at 800 ppm in both sexes (3 months) and in males (6 months). There were no treatment-related effects on survival, clinical signs, ophthalmoscopy, haematology, blood chemistry or urinalysis. Treatment with myclobutanil at dietary levels up to 800 ppm failed to uncover any evidence of carcinogenic potential (Shellenberger *et al.*, 1986).

## Reproduction studies

### Rats

A two-generation (two litter per generation) reproduction study was conducted with groups of 25 CRI:CD(SD)BR rats/sex fed myclobutanil (84.5% purity) at dietary levels of 0, 50, 200 or 1000 ppm a.i. equal to 0, 3.6, 14.7 or 73.6 mg a.i./kg bw/day in males and 0, 4.3, 17.4 or 87 mg a.i./kg bw/day in females respectively. In the F<sub>0</sub> generation, treatment with myclobutanil commenced 8 weeks before mating. In the F<sub>1</sub> generation, the F<sub>1a</sub> litter-derived parental animals were exposed to the test material throughout weaning and for a minimum period of 8 weeks post-weaning. In both generations, treatment continued throughout the reproductive phases. Treatment-related effects on reproduction were denoted at the highest dietary level of 1000 ppm by a decreased number of females delivering litters (F<sub>0</sub>→F<sub>1a</sub> and both matings from second generation), decreased mean number of pups per litter (first mating of second generation) and an increased number of stillborn pups (all matings of both generations). An increase, albeit minimal in the proportion of dead pups in both matings of the first generation was similarly recorded at 200 ppm when compared to the controls. Effects on the reproductive organs were evident in the second generation F<sub>1</sub> males treated at 1000 ppm as multifocal or diffuse atrophy of the testes, decreased spermatozoa and/or necrotic spermatocytes of the epididymides, as well as atrophy of the prostate. Systemic toxicity was observed at 200 ppm as increased liver weights in males of both parental generations in association with centrilobular hepatocytic hypertrophy in the F<sub>1</sub> generation males. At 1000 ppm, increased liver weights and hepatocytic hypertrophy were observed in males and females of both generations. Decreased body-weight in males and depressed food intake in both sexes were recorded at 1000 ppm in both parental generations. Body-weights were similarly decreased at 1000 ppm in both sexes of all filial generations. The NOAEL for this study was determined to be 50 ppm, equal to 3.6 mg/kg bw/day, for systemic and reproductive effects (Costlow & Harris, 1985).

## Special studies on teratogenicity

### Rats

A range-finding study was performed with groups of 8 mated female Crl:CD(SD)BR rats treated orally by gavage with myclobutanil (81.1% purity) at 0 (vehicle, corn oil), 32, 68, 100, 215, 464 or 700 mg a.i./kg bw/day on days 6 to 15 of gestation. Day 0 of gestation was considered the day sperm were evident in the vaginal smear. All surviving dams were killed on day 20 of gestation. The NOAEL for maternal toxicity was 215 mg/kg bw/day. At dose levels of 464 and 700 mg/kg bw/day, treatment with myclobutanil resulted in mortality (25% and 100%, respectively), decreased body-weights and clinical signs of toxicity manifest as scant faeces, chromodacryorrhea, red exudate around mouth, rough and urine-stained hair coat, and salivation. Embryofetal toxicity was expressed at levels of 68 mg/kg bw/day and higher as increased resorptions and decreased viability indices (viable fetuses/implantation sites). Decreased fetal weights were recorded at 464 mg/kg bw/day. In the absence of detailed visceral and skeletal examinations of the fetuses, the NOAEL for developmental toxicity was 32 mg/kg bw/day (Costlow & Kane, 1984a).

Myclobutanil (84.5% purity) was administered orally by gavage at 0 (vehicle, corn oil), 31, 94, 310 or 470 mg a.i./kg bw/day to groups of 25 presumed pregnant Crl:CD(SD)BR rats from days 6 to 15 of gestation. The day on which sperm were found in the vaginal smear was considered day 0 of gestation. All surviving dams were killed on day 20 of gestation. A NOAEL for maternal toxicity was indicated at 94 mg/kg bw/day based on clinical signs of toxicity (rough hair coat, desquamation and salivation) at doses of 310 mg/kg bw/day and higher. At 470 mg/kg bw/day, red exudate around the mouth, scant faeces and decreased body-weights were also observed. Decreased viability indices (viable fetuses/implantation sites) and a slight trend toward increasing resorption rate were recorded at 94 mg/kg bw/day and higher resulting in a NOAEL for embryofetal toxicity of 31 mg/kg bw/day. An increased incidence of skeletal variations of the ribs (7th cervical and 14th rudimentary ribs) was observed at 310 mg/kg bw/day and higher. Treatment with myclobutanil failed to reveal any evidence of teratogenic potential (Costlow & Kane, 1984b).

### Rabbits

A range-finding study was conducted with myclobutanil (84.5% purity) administered orally by gavage at 0 (vehicle, 1% methyl cellulose), 10, 31.6, 100, 215, 464 or 700 mg a.i./kg bw/day to groups of 6 artificially inseminated New Zealand white rabbits on days 7 through 19 of gestation. The day of artificial insemination was designated as day 0 of gestation. All surviving animals were killed on day 29. Myclobutanil was lethal at doses of 464 mg/kg bw/day and higher resulting in 100% mortality. Maternal toxicity represented by clinical signs

(irregular faeces and red-stained urine) and decreased body-weights was noted at 215 mg/kg bw/day. An increased incidence of resorptions and decreased litter size (viable fetuses/litter) was observed at 215 mg/kg bw/day, resulting in an overall NOAEL of 100 mg/kg bw/day. Viable fetuses from surviving rabbits appeared normal upon gross examination (Costlow & Kane, 1984c).

Groups of 18 artificially inseminated female New Zealand white rabbits were administered myclobutanil (90.4% purity) at 0 (distilled water control), 0 (vehicle, 1% methyl cellulose), 20, 60 or 200 mg a.i./kg bw/day orally by gavage from day 7 through 19 of gestation. The day of insemination was designated as day 0 of gestation. All surviving rabbits were killed on day 29. The NOAEL for maternal toxicity was 20 mg/kg bw/day, based on minimal, transient body-weight loss at 60 mg/kg bw/day. Maternal animals at the high-dose of 200 mg/kg bw/day experienced body-weight loss and clinical signs reported as irregular faeces and blood-stained urine. Embryofetal toxicity was manifest at 200 mg/kg bw/day as an increased frequency of abortion and total litter resorption, an increased incidence of litters with resorptions as well as reduced litter size and fetal weight. Myclobutanil was not teratogenic when administered to pregnant rabbits at dose levels of up to 200 mg/kg bw/day (Costlow & Kane, 1984d).

#### **Special studies on genotoxicity**

Myclobutanil did not reveal any evidence of genotoxic potential when investigated in a battery of assays specific for gene mutation in microbial and mammalian cells or for detection of chromosomal aberrations in cytogenetics studies. Myclobutanil did not induce unscheduled DNA synthesis in isolated rat hepatocytes and was negative in a DNA repair test with *Bacillus subtilis*. A dominant lethal study in rats was also negative. The results of the genotoxicity studies are presented in Table 2.

#### **Special studies on irritation and sensitization**

Eye irritation potential was studied in 9 male New Zealand white rabbits given technical myclobutanil with a purity of 78.4% (Krzywicki, 1983) or 91.9% (Krzywicki & Bonin, 1984). Treatment of the eyes with higher purity technical material produced both corneal and conjunctival effects suggestive of moderate to severe irritating potential. Myclobutanil with a purity of 78.4% was only slightly irritating to the eyes of rabbits resulting in reversible conjunctival effects.

A 0.5 ml aliquot of technical myclobutanil was applied dermally to the shaved backs of 6 male New Zealand rabbits under occluded conditions for a 4-hour exposure period. Myclobutanil with a purity of 78.4% (Krzywicki, 1983) or 91.9% (Krzywicki & Bonin, 1984) was practically non-irritating to the skin of male rabbits.

Table 2. Genotoxicity of technical myclobutanil

Test	Test system	Concentration (vehicle)	Purity*	Results	Reference
Reverse Mutation ( <i>in vitro</i> )	<i>S. typhimurium</i> TA 98, 100, TA 1535, TA 1537	75, 250, 750, 2500, 7500 µg/plate 75, 250, 500, 1000, 5000 µg/plate 250, 750, 1500, 2500, 7500 µg/plate (DMSO)	99%	negative 1., 2.	Byers & Lohse (1983)
	<i>S. typhimurium</i> TA 98, 100, 1535, 1537	75 - 7500 µg/plate (DMSO)	84.5%	negative 1., 2.	Byers & Chism (1983a)
	<i>S. typhimurium</i> TA 98, 100, 1535, 1537	75, 250, 750, 2500, 7500 µg/plate (DMSO)	90.4%	negative 1., 2.	Byers & Chism (1983b)
Point mutation ( <i>in vitro</i> )	<i>S. typhimurium</i> TA 98, 100, 1535, 1537	125, 250, 500, 1000, 2000 µg/plate (DMSO)	91.4%	negative 1., 2.	Sutou (1987)
	<i>E. coli</i> WP2 uvrA	(DMSO)			
Chromosome aberration ( <i>in vitro</i> )	Chinese hamster ovary, K1BH <sub>4</sub> cell line - HGPRT locus	1. 120-175 µg/ml 2. 25-90 µg/ml (DMSO)	81.1%	negative 1., 2.	O'Neill <i>et al.</i> (1984)
	Chinese hamster ovary WBI cells	1. 20, 30, 40, 50 µg/ml 2. 25, 50, 75 µg/ml (DMSO)	91.9%	negative 1., 2.	Ivett (1985)
Chromosome aberration ( <i>in vivo</i> )	Mouse (male CR CD-1) bone marrow	0, 65, 260, 650 mg/kg bw (corn oil)	81.1%	negative	McLeod & McCarthy (1984)
	Mouse (male and female CrI:CD-1(ICR)), bone marrow	0, 117, 585, 1170 mg/kg bw (corn oil)	91.4%	negative	Sames & Frank (1987)

Table 2 (cont'd)

Test	Test system	Concentration (vehicle)	Purity <sup>a</sup>	Results	Reference
DNA repair ( <i>in vitro</i> )	<i>Bacillus subtilis</i> H17, M45	312.5, 625, 1250, 2500, 5000 ug/plate	91.4%	negative 1., 2.	Sutou (1987)
Unscheduled DNA synthesis ( <i>in vitro</i> )	Rat (CRCD, Cri:CDBR male) hepatocytes	0.1 - 1000 ug/ml (DMSO)	91.9%	negative	Muller (1986)
Dominant lethal ( <i>in vivo</i> )	Rat, male Cri:COBS(SD)BR	0, 10, 100, 735 mg/kg bw (corn oil)	91.4%	negative	Dearlove <i>et al.</i> (1986)

DMSO = dimethyl sulfoxide

1. = in the presence of metabolic activation

2. = in the absence of metabolic activation

<sup>a</sup> = active ingredient (a.i.)

The potential of technical myclobutanil to induce delayed contact hypersensitivity in the Hartley guinea-pig using a modified Buehler procedure, could not unequivocally be ascertained due to a low incidence of animals with erythema response following challenge doses with the test material (Bonin & Hazelton, 1987a). Technical myclobutanil, when administered to guinea-pigs according to the method of Magnusson and Kligman did not produce delayed contact hypersensitivity (Kreuzmann, 1989).

### **Observations in humans**

No information was available.

### **COMMENTS**

Myclobutanil was rapidly absorbed when administered orally to rats and mice. The principal routes of excretion were via the urine and faeces, with no significant residual tissue accumulation. The toxicokinetic model in the rodent did not differ markedly with respect to species, sex, single versus repeated exposures or doses.

In both the rat and mouse, myclobutanil was extensively metabolized to more polar compounds. The proposed metabolic pathway of myclobutanil in the rat was through oxidation of the butyl group. The major excretory metabolites were qualitatively similar with respect to sex and dose.

Myclobutanil was only slightly toxic upon acute oral administration to rats and mice. WHO has classified myclobutanil as slightly hazardous (WHO, 1992).

The primary target organ upon repeated dietary exposure to myclobutanil was the liver. Histomorphological changes, characterized predominantly by centrilobular hepatocytic hypertrophy in association with increased liver weights, were observed in all species investigated. Microscopically, there was accentuated lobular architecture, hepatocytic vacuolation, inflammation, necrosis, and pigmentation of the Kupffer cells. Increased hepatic enzyme activity in serum (ALAT, ASAT, GGT, ALP) were also observed. Hepatic microsomal MFO activities in rats and mice were increased correspondingly. There were no similar increases in hepatic peroxisomal  $\beta$ -oxidation activity that would have suggested peroxisomal proliferation.

A three-month dietary study with myclobutanil in the mouse at levels of 0, 3, 10, 30, 100, 300, 1000, 3000 or 10 000 ppm revealed hepatic alterations at dietary levels of 1000 ppm and higher, resulting in a NOAEL of 300 ppm, equal to 42.1 mg/kg bw/day.

Two 3-month studies in rats fed myclobutanil at levels of 0, 10, 30, 100, 300, 1000, 3000, 10 000, or 30 000 ppm and 0, 100, 300, or 3000 ppm indicated a

NOAEL of 100 ppm, equal to 5.2 mg/kg bw/day, based on treatment-related hepatic effects.

The NOAEL for myclobutanil-related liver effects in dogs treated for 3 months at 0, 10, 200, 800 or 1600 ppm was 10 ppm, equal to 0.3 mg/kg bw/day. Treatment of dogs with myclobutanil for 12 months at dietary levels of 0, 10, 100, 400, or 1600 ppm resulted in a NOAEL for hepatic effects of 100 ppm, equal to 3.1 mg/kg bw/day. Myclobutanil administered to two dogs per sex at dietary levels of up to 1000 ppm, equal to 39 mg/kg bw/day, did not produce any hepatic changes after a period of 4 weeks.

Long-term dietary treatment of mice with myclobutanil for two years at 0, 20, 100 or 500 ppm revealed a NOAEL of 20 ppm, equal to 2.7 mg/kg bw/day, based on increased MFO activity at 100 ppm as well as more pronounced liver toxicity at 500 ppm and above. Myclobutanil was not carcinogenic in mice.

A 24-month long-term toxicity/carcinogenicity study in rats at dietary concentrations of 0, 50, 200 or 800 ppm revealed a NOAEL of 50 ppm, equal to 2.5 mg/kg bw/day, based on findings of testicular atrophy and increased MFO activity at 200 ppm and above. Myclobutanil was not carcinogenic in rats.

A two-generation reproduction study in rats at dietary concentrations of 0, 50, 200 or 1000 ppm revealed a NOAEL of 50 ppm, equal to 3.6 mg/kg bw/day, based on increased liver weights and an increase in numbers of stillborn pups at 200 ppm and above. At 1000 ppm atrophy of the testes and prostate were observed.

An oral teratogenicity study in rats at gavage doses of 0, 31, 94, 310, or 470 mg/kg bw/day demonstrated clinical signs of toxicity at 310 mg/kg bw/day and above, indicating a NOAEL of 94 mg/kg bw/day. The NOAEL for embryofetal toxicity was 31 mg/kg bw/day. There was no evidence of teratogenicity at doses up to 470 mg/kg bw/day.

Myclobutanil was not teratogenic when administered to the rabbit at gavage doses of 20, 60 or 200 mg/kg bw/day. A NOAEL for maternal toxicity was 20 mg/kg bw/day, based on decreased body-weight at 60 mg/kg bw/day and above. Embryofetal toxicity was evident at 200 mg/kg bw/day.

After reviewing the available genotoxicity data, the Meeting concluded that myclobutanil was not genotoxic.

An ADI was allocated on the basis of NOAELs in two-year feeding studies in mice and rats, a reproduction study in rats and a one-year study in dogs, using a 100-fold safety factor.

## TOXICOLOGICAL EVALUATION

### Level causing no toxicological effect

- Mouse: 20 ppm, equal to 2.7 mg/kg bw/day (two-year study)
- Rat: 50 ppm, equal to 2.5 mg/kg bw/day (two-year study)  
50 ppm, equal to 3.6 mg/kg bw/day (two-generation reproduction study)
- Dog: 100 ppm, equal to 3.1 mg/kg bw/day (one-year study)

### Estimate of acceptable daily intake for humans

0 - 0.03 mg/kg bw

### Studies which will provide information valuable in the continued evaluation of the compound

1. Results of ongoing long-term studies in mice and rats known to be in progress.
2. If the results of (1) show a carcinogenic response, studies (a) to determine whether myclobutanil acts as a tumour promoter in the two-stage rat liver bioassay and (b) whether it causes inhibition of intercellular communications.
3. Observations in humans.

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