

BITERTANOL

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Explanation	39
Evaluation for acceptable daily intake	39
Biochemical aspects	39
Absorption, distribution, and excretion	39
Biotransformation	41
Toxicological studies	42
Acute toxicity	42
Short-term studies of toxicity	44
Long-term studies of toxicity and carcinogenicity	47
Genotoxicity	49
Reproductive toxicity	49
Multigeneration reproductive toxicity	49
Developmental toxicity	50
Special studies	51
Effects on the central nervous system	51
Toxicity in combinations	52
Effect on the liver	52
Studies on metabolites	53
1-(Triazol-1-yl)-1-(4'-phenylphenoxy)-3,3-dimethylbutan- 2-one (plants, soil)	53
Bitertanol benzoic acid (soil)	53
1,2,4-triazole (photodegradation, soil)	53
Observations in humans	54
Comments	54
Toxicological evaluation	56
References	58

Explanation

Bitertanol was previously evaluated toxicologically by the Joint Meeting in 1983, 1987, and 1988 (Annex 1, references 40, 50, and 53). The 1983 JMPR allocated a temporary ADI of 0–0.005 mg/kg bw and requested studies on metabolism in order to clarify the metabolic pathway of bitertanol, a study of toxicity in dogs treated orally for a minimum of one year, and a long-term study of toxicity and carcinogenicity in rats at appropriate doses. Relevant data were submitted for evaluation by the 1987 JMPR, when an ADI of 0–0.003 mg/kg bw was established on the basis of a NOAEL of 10 ppm in a one-year study in dogs. In 1988, the Meeting concluded that, upon further consideration of the data from two-year and one-year studies of toxicity in dogs, the NOAEL was 25 ppm (equal to 1 mg/kg bw per day). Therefore, an ADI of 0–0.01 mg/kg bw was allocated using a safety factor of 100. The compound was reviewed at the present Meeting within the CCPR periodic review programme. This monograph summarizes new data on bitertanol and data that were not previously reviewed and includes relevant data from the previous monographs (Annex 1, references 41 and 52).

Evaluation for acceptable daily intake

1. Biochemical aspects

(a) *Absorption, distribution, and excretion*

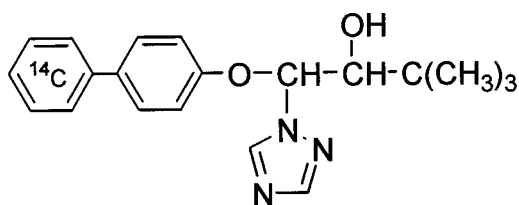
Groups of five male and five female Wistar rats received [¹⁴C-phenyl]-bitertanol as a solution in ethylene glycol as a single oral dose of 100 or 1000 mg/kg bw or a single intravenous dose

of 100 mg/kg bw as well as 14 daily oral doses of unlabelled chemical at 100 mg/kg bw, followed by a single oral dose of ^{14}C -bitertanol at 100 mg/kg bw. Urine and faeces were collected 6 and 24 h after dosing and then at subsequent 24-h intervals until sacrifice seven days later. Serial blood samples were collected during this period.

Total absorption of radiolabel was found to depend on the dose over the range studied. Faecal excretion of radiolabel after intravenous administration showed that biliary excretion was predominant. Urinary excretion represented 4–11% of the administered dose, while no radiolabel was detected in expired air. The total recovery of radiolabel was >92%. Pharmacokinetic analysis indicated that absorption of bitertanol after oral administration follows a first-order pattern for single and repeated doses of 100 mg/kg bw but not at the higher dose (1000 mg/kg), suggesting saturation of absorption, distribution, or elimination. The highest concentrations detected in tissues were in the liver (17.9 ppm, females) and kidney (5.9 ppm, males) of animals at the high dose. Seven days after dosing, 0.2–0.4% of the administered radiolabel remained in the body (Puhl & Hurley, 1983).

The biokinetic behaviour of bitertanol in rats was investigated in studies requested by the Japanese registration authority, which were conducted in compliance with standards of good laboratory practice. The compound was uniformly labelled with ^{14}C in the phenyl moiety, as shown in Figure 1 and was administered orally to male and female rats at a dose of 10 mg/kg bw. Radiolabel was determined over time in the excreta and plasma, and the body and individual organs and tissues were assayed for total radiolabel at sacrifice. Male rats were also given the same dose intraduodenally after bile fistulation, and total radiolabel was assayed in excreta, including the bile, and in the body at sacrifice. Total radiolabel was also determined over time in various organs of male rats after oral administration of a single dose of 10 mg/kg bw.

Figure 1. Labelling of bitertanol for studies of its biokinetic behaviour



About 84% of the dose was absorbed after oral administration. Absorption commenced immediately, and the plasma concentration increased from 25 to 75% of the peak value within 1–2 h. The radiolabel was eliminated rapidly and almost completely from the body within the 72-h test period. More than 90% of the recovered radiolabel was excreted with the faeces, and about 7% with the urine. On average, the residual radiolabel in the body (excluding the gastrointestinal tract) after 72 h represented 0.5% of the administered dose. The results obtained after bile fistulation indicate that the largest fraction of faecally eliminated radioactivity was first absorbed and then eliminated into the gut lumen with the bile (about 77%). Most of the radiolabel eliminated in the bile underwent enterohepatic circulation. The radiolabel was rapidly distributed from the intravascular space to the peripheral tissues. The maximum concentration in plasma was reached after 3–8 h. The terminal phase of elimination of radiolabel from the plasma was described by linear regression analysis, which yielded a half-life of about 26 h. The distribution of total radiolabel in individual organs of male rats at various times after administration of a single oral dose of 10 mg/kg was generally similar to that observed 72 h after administration. The highest concentrations were found in the liver and kidney (Klein, 1988a,b, 1989).

After a 50% formulation of ^{14}C -labelled bitertanol was applied to the skin of adult male and female albino rabbits, the mean level of dermal penetration was around 10%. There were

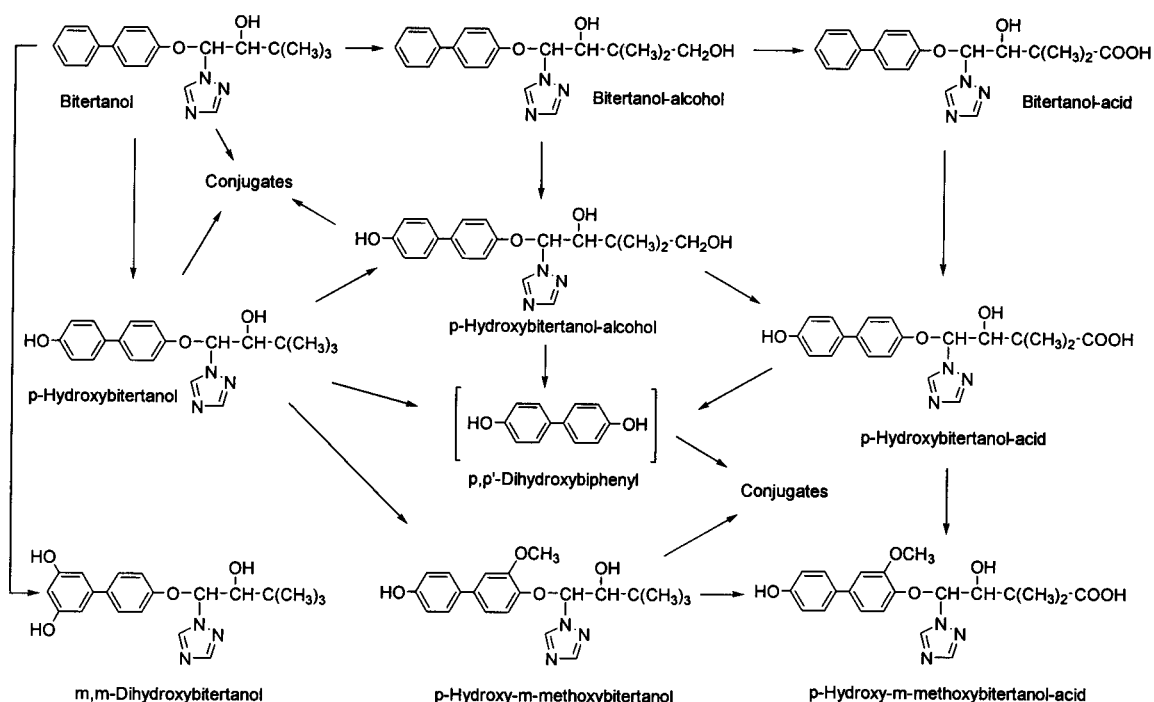
no marked sex-specific differences. Since this experiment was designed to simulate 'worst-case' conditions, lower absorption rates might be expected in practice (Hixson, 1984).

(b) *Biotransformation*

In the first study described above, bitertanol was extensively metabolized, and the metabolite profile was similar in the groups receiving 100 or 1000 mg/kg bw. The relative amounts of metabolites were also similar, except that the animals receiving the highest oral dose eliminated much more unchanged parent compound than the others. Fourteen metabolites (plus bitertanol), representing 38–76% of the recovered radiolabel, were identified or characterized. Hydroxylation of the *para* position of the biphenyl and methyl groups of the *tert*-butyl moieties gave rise to phenolic and diol metabolites. Although one diastereomer of the latter was detected, it underwent oxidation to the corresponding butanoic acid and subsequent ring hydroxylation. The parahydroxylated diol was also detected, as was 4,4-dihydroxy-biophenyl, and other hydroxylated metabolites were also tentatively identified. The metabolic reactions thus included ring monohydroxylation, ring dihydroxylation, aryl *O*-methylation, aliphatic hydroxylation, aliphatic oxidation to carboxylic acids, and ether cleavage. In addition to the metabolites shown in Figure 2, glucuronide and sulfate conjugates of some metabolites, including *para*-hydroxybitertanol, were also detected in faeces and urine (Puhl & Hurley, 1983).

In the second set of studies described above, the structures and amounts of metabolites were determined in faeces, urine, and bile and in the liver, kidneys, and perirenal fat at various times after treatment. The metabolism of bitertanol began immediately after absorption from the gastrointestinal tract lumen. The parent compound was not detected in urine or bile, and the only metabolite identified in the bile was *para*-hydroxybitertanol. Excretion of the

Figure 2. Reaction pathways involved in metabolism of bitertanol in rats after administration of 100 or 1000 mg/kg bw



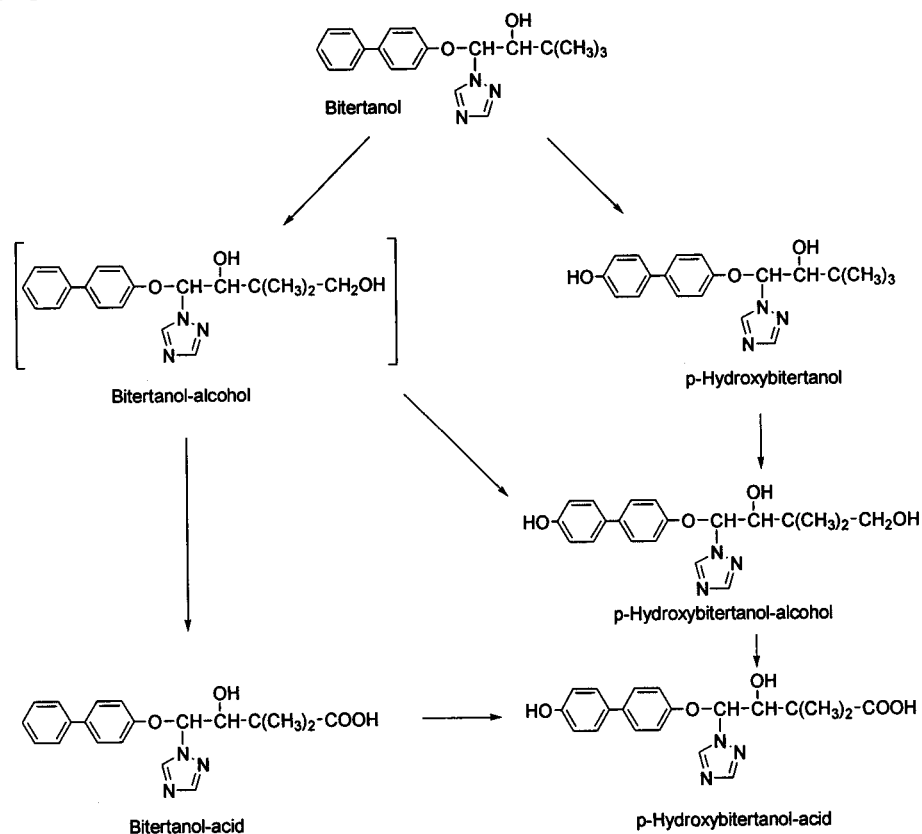
unchanged parent compound in the urine was unlikely because of its lipophilic character. Metabolic degradation of bitertanol in the organs was rapid: within 8 h, the concentration in the liver fell from around 15% to about 2% of the total radiolabel in the organs. The main metabolite in the liver was also *para*-hydroxybitertanol; smaller amounts of *para*-hydroxybitertanol acid, *para*-hydroxybitertanol alcohol, and bitertanol acid were also identified. The distribution of metabolites in the kidneys was similar: total organ radiolabel fell from around 14% to about 2.5% within 8 h; the main metabolite was again *para*-hydroxybitertanol, which represented 30–50% of the organ radiolabel. Furthermore, the amounts of metabolites in the liver and kidneys were similar. The total amount of radiolabel in fat samples was too low to permit reliable quantification or identification of possible metabolites, due mainly to the small amount of fat available in the young animals. A proposed metabolic pathway for bitertanol in rats is given in Figure 3. The parent compound found in the faeces of orally treated rats was probably due to the unabsorbed fraction of administered radiolabel, representing about 15% of the original dose. The main metabolite in the liver and kidneys, *para*-hydroxybitertanol, was also identified in the faeces. The amounts of the other biotransformation products in the organs were probably too low for detection in the excreta (Klein, 1988b, 1989).

2. Toxicological studies

(a) Acute toxicity

The methods used in the studies summarized below complied to a certain extent with OECD guidelines. At the time that most of the studies were performed, compliance with good

Figure 3. Proposed metabolic pathway for bitertanol in rats after administration of a single oral dose of 10 mg/kg bw



laboratory practice (GLP) was not compulsory. The results of studies on the acute toxicity of bitertanol are summarized in Table 1. Bitertanol had very low acute toxicity in rats, mice, and dogs treated orally. No significant sex difference was observed. The LD₅₀ values are 4000–5000 mg/kg bw or higher. The toxicological properties of the A and B isomers are similar. The symptoms included nonspecific signs, such as deteriorated general condition, isolation from the group, and piloerection; however, central nervous system effects were also seen, including sedation, spasms, and respiratory disturbances. Lobulation of the liver and irritation of the glandular stomach mucosa were observed at necropsy of orally treated rats. Further signs in dogs were vomiting and diarrhoea. Sheep were more susceptible, with an oral LD₅₀ of about 1000 mg/kg bw. Bitertanol was moderately toxic to rats and mice after intraperitoneal injection.

Dermal exposure to a dose of 5000 mg/kg bw was tolerated by rats with no observed signs. It was not toxic to rats after inhalation in an aerosol, even at the highest test concentration. An initial test showed no primary irritation of the intact or abraded skin of rabbits inspected 24 and 72 h after the beginning of exposure (Thyssen & Kimmerle, 1977a). In a study in rabbits, the active ingredient was found to be slightly irritating to intact and abraded skin (Iyatomy, 1981).

Table 1. Acute toxicity of bitertanol

Species	Strain	Sex	Route	LD ₅₀ or LC ₅₀ (mg/kg bw or mg/m ³ air)	Purity (%)	Reference
Rat	NR	M	Oral	> 5000	99.1 A	Mihail (1978a)
Rat	NR	M	Oral	> 5000	97.1 B	Mihail (1978b)
Rat	Wistar	M/F	Oral (fasted)	> 5000	96.5	Thyssen & Kimmerle (1977a)
Rat	NR	M	Oral	> 5000	97.3	Flucke (1978)
Rat	NR	M	Oral	4000	95.0	Flucke (1979)
Rat	NR	M	Oral	3700	95.0	Iyatomy (1980)
		F	Oral	3900		
Rat	Wistar	F	Oral (fasted)	> 5000	95.1	Flucke (1980)
Rat	NR	M	Oral	> 5000	96.7	Heimann (1981)
		M	Oral (fasted)	> 5000		
Rat	NR	M	Oral (fasted)	4800	99.1	Mihail (1982a)
Rat	NR	M	Oral (fasted)	4800	97.1	Mihail (1982b)
Rat	NR	M	Oral	> 5000	NR	Heimann (1983a)
Rat	NR	M	Oral (fasted)	> 5000	97.6	Heimann (1984a)
Mouse	NMRI	M	Oral (fasted)	4500	96.5	Thyssen & Kimmerle (1977a)
		F	Oral (fasted)	4200		
Mouse	NR	M	Oral	3500	95.0	Iyatomy (1980)
		F	Oral	3200		
Dog	Beagle	M/F	Oral (fasted)	> 5000	95.0	Hoffmann (1981a)
Sheep	Blackface	M/F	Oral	~ 1000	95.0	Hoffmann (1981b)
Rat	Wistar	M/F	Dermal	> 5000	96.5	Thyssen & Kimmerle (1977a)
Rat	NR	M/F	Dermal	> 5000 ^a	95.0	Iyatomy (1980)
Mouse	NR	M/F	Dermal	> 5000	95.0	Iyatomy (1980)
Rabbit	New Zealand	M/F	Dermal	> 2000	94.9	Hixson (1979)
Rat	Wistar	M/F	Inhalation 1 h	> 720	96.5	Thyssen & Kimmerle (1977a)
Rat	Wistar	M/F	Inhalation 4 h	> 550	96.5	Thyssen & Kimmerle (1977a)
Rat	Wistar	M/F	Inhalation 5 x 4 h	> 380	96.5	Thyssen & Kimmerle (1977a)
Rat ^b	Sprague-Dawley	M/F	Inhalation 4 h	> 1200	95.7	Shiotsuka (1987)
Rat	Wistar	M	Intraperitoneal	1200	96.5	Thyssen & Kimmerle (1977a)
		F	Intraperitoneal	720		
Rat	NR	M	Intraperitoneal	700	95.0	Iyatomy (1980)
		F	Intraperitoneal	560		
Mouse	NR	M	Intraperitoneal	570	95.0	Iyatomy (1980)
		F	Intraperitoneal	610		
Rat	NR	M/F	Subcutaneous	> 1000	95.0	Iyatomy (1980)
Mouse	NMRI	M/F	Subcutaneous	> 5000	96.5	Thyssen & Kimmerle (1977a)
Mouse	NR	M/F	Subcutaneous	> 1000	95.0	Iyatomy (1980)

NR, not reported; M, male; F, female; A, diastereomer A; B, diastereomer B

^a Slight local irritation present

^b The study was conducted in compliance with good laboratory practice

A five-minute treatment of the eye of rabbits did not cause primary irritation of the mucous membranes. Slight to moderate reddening of the conjunctivae, which persisted for about 48 h, was observed after a 24-h exposure (Thyssen & Kimmerle, 1977a). A reaction that was reversible within four days was seen in rabbit conjunctivae; the cornea and iris were unaffected (Iyatomy, 1981).

After an initial administration of 0.05 mg bitertanol per animal to female Pirbright guinea-pigs, nine repeated intracutaneous injections of emulsified bitertanol (0.1 mg) over three consecutive weeks had no sensitizing effect. Intracutaneous injection of an additional 0.05 mg after a further two weeks also failed to produce signs of dermal sensitization (Thyssen, 1977).

Magnusson and Kligman tests in 20 male and 20 female Pirbright guinea-pigs with Freund's adjuvant provided no evidence of sensitizing effects of bitertanol at a concentration of 1% for intradermal induction, 25% for topical induction, and 25% for challenge (Flucke, 1981).

(b) *Short-term studies of toxicity*

Rats

Groups of 20 male and 20 female Wistar rats were given bitertanol (purity, 96.5%) by gavage at doses of 0, 30, 100, or 300 mg/kg bw per day for 28 days. Half of the animals were then sacrificed, and the other half were observed for a further 28 days. The method used in this study complied to a certain extent with OECD guideline 408; at the time the study was performed, compliance with GLP was not compulsory.

Doses of 100 mg/kg per day and higher had a dose-related adverse effect on body-weight development. At 300 mg/kg per day, behavioural disturbances (isolation from the other animals, dirty coat) and hair loss were observed in the female rats; however, all females had normal behaviour near the end of the treatment period. Animals at this dose had moderate leukocytosis, and females had reduced haemoglobin and thrombocyte counts. Increased alkaline phosphatase activity was observed only in the females. The relative liver weights of animals of each sex at 100 mg/kg bw per day and above were increased. At 300 mg/kg bw per day, the absolute liver weights were elevated and the absolute weights of the heart, kidneys, adrenals, and ovaries depressed in female rats at the termination of treatment. The relative weights of the thyroid and testes in males and of the spleen in females were also elevated, whereas the relative weights of the heart, adrenals, and ovaries were depressed in females. Hyperkeratoses and parakeratoses, distended epithelial cells, slight inward growth of the papillary body, and cellular infiltration in the epithelial and subepithelial layers were observed histopathologically in the forestomachs of four female animals at 300 mg/kg bw per day that were sacrificed at the end of treatment. The appearance of the digestive tract was unexceptional at the end of the recovery period, and all of the changes in the rats at the high and intermediate doses had likewise reverted. The NOAEL was 30 mg/kg bw per day (Thyssen & Kaliner, 1977).

Groups of 20 male and 20 female Sprague-Dawley rats were given bitertanol (purity, 95%) at doses of 0, 100, 400, or 1600 ppm, equal to 7, 28, and 110 mg/kg bw per day in males and 7.4, 30, and 110 mg/kg bw per day in females, for 28 days. The test procedures complied to a certain extent with OECD guideline 407; at the time the study was performed, compliance with GLP was not compulsory. Half of the animals were sacrificed and examined after four weeks, whereas the other half were observed without treatment for a further four weeks.

The dose of 400 ppm had an adverse effect on body-weight development and food intake, and an adverse effect on the red blood cell population was seen from decreases in the haematocrit and haemoglobin readings. The relative liver weight was slightly elevated in animals of each sex. Those at 1600 ppm had ataxia, reduced food intake and body weight, and depressed red blood cell parameters (haemoglobin, haematocrit, erythrocyte count), with a simultaneous increase in the reticulocyte count. A slight increase in the serum cholesterol concentration and a significant increase in the relative liver weight were found in animals of each sex.

Histopathological examination revealed weak irritation of the gastric mucosa and slightly altered ovaries, adrenals, and pituitaries. All of the observed effects were reversible within the four-week recovery period. The NOAEL was 100 ppm, equal to 7 mg/kg bw per day (Hatanaka et al., 1981).

Bitertanol (purity, 90.2%) was administered to groups of 20 male and 20 female Wistar rats at concentrations of 0, 150, 600, or 2400 ppm, equal to 12, 48 and 300 mg/kg bw per day in males and 13, 58, and 310 mg/kg bw per day in females, for three months. This study was conducted before enactment of prevailing regulatory guidelines; however, the procedures complied to certain extent with OECD guideline 408.

Although appearance and behaviour were unaffected at doses up to 600 ppm, 2400 ppm caused decreased motility and reduced food intakes. Dose-related growth retardation was observed in females at 600 and 2400 ppm and in male animals at all concentrations; however, the 150 ppm dose decreased body weights only temporarily. The dose of 2400 ppm had adverse effects on various haematological parameters, including reduced erythrocyte and leukocyte counts, decreased haemoglobin content and haematocrit reading, increased reticulocyte counts, a relative increase in the polymorphonuclear leukocyte count, and a relative decrease in the lymphocyte count. The changes in clinical chemical parameters of the blood also found at this concentration were elevated alkaline phosphatase, aspartate aminotransferase, and glutamate dehydrogenase activities in the female animals. The blood protein level was slightly depressed and the cholesterol level slightly elevated. The finding of increased liver weight in females at 2400 ppm also indicated an effect on the liver. The NOAEL was 150 ppm, equal to 12 mg/kg bw per day (Bomhard & Löser, 1978).

In a supplementary study, bitertanol (purity, 90.8%) was administered to groups of 15 male and 15 female Wistar rats at concentrations of 0, 30, 100, or 300 ppm, equal to 2.5, 8.1, and 25 mg/kg bw per day in males and 3.3, 10, and 32 mg/kg bw per day in females, for three months. The method used in this study complied to a certain extent with OECD guideline 408; at the time the study was performed, compliance with GLP was not compulsory.

The appearance, behaviour, and mortality rate were unaffected at all concentrations. At 300 ppm, the rats gained less weight than the controls. Haematological, clinical chemical, gross and histopathological examination, and urinary analyses provided no evidence of adverse effects. The NOAEL was 100 ppm, equal to 8 mg/kg bw per day (Krötlinger et al., 1978).

In a further study, bitertanol (purity, 95%) was administered to groups of 15 male and 15 female Sprague-Dawley rats at concentrations of 0, 40, 200, or 1000 ppm, equal to 3.1, 16, and 82 mg/kg bw per day in males and 3.6, 19, and 88 mg/kg bw per day in females, for three months. Five males and five females were subjected to urinary, haematological, and clinical chemical determinations. The method used in this study complied to a certain extent with OECD guideline 408; at the time the study was performed, compliance with GLP was not compulsory.

The dose of 200 ppm retarded weight gain in male and female animals and slightly increased the activities of alkaline phosphatase and aspartate aminotransferase in the serum of females. The dose of 1000 ppm reduced body-weight gain and decreased food and water intakes in animals of each sex. The erythrocyte count was also depressed, and the reticulocyte count elevated in male and female animals. In addition, the leukocyte and thrombocyte counts were elevated, and the haemoglobin content and haematocrit reading were decreased in females. Elevated lactate dehydrogenase, aspartate aminotransferase, and alkaline phosphatase activities and a slightly elevated cholesterol concentration were found in the serum of females. The absolute weights of the liver and spleen were increased in females and the relative weights in males and females. The following lesions were found histopathologically in animals of each sex: increased swelling and fatty degeneration of hepatocytes, hyperkeratoses of the oesophageal and gastric mucosal epithelium and/or erosions of the glandular stomach, swelling, and fatty degeneration of adrenal cortical cells. Isolated bile-duct proliferation was also seen in females. The NOAEL was 40 ppm, equal to 3 mg/kg bw per day (Yonemura et al., 1981).

Groups of 10 male and 10 female Wistar rats were exposed to bitertanol at mean analytically determined concentrations of 18, 63, or 200 mg/m³ air for 6 h per day, five days per week for three weeks. Only the head and nose of the animals were exposed, under dynamic conditions, and the inhaled bitertanol was dissolved in a 1:1 blend of ethanol:polyethylene glycol 400. The method used in this study complied to a certain extent with OECD guideline 412; at the time the study was performed, compliance with GLP was not compulsory.

The concentrations of 18 and 63 mg/m³ air were tolerated by male and female rats with no observed effect. Exposure to 200 mg/m³ air caused deterioration of the general condition of female rats, and the growth of male rats in this group was significantly decreased. Although the relative weights of the lung, liver, kidney, and adrenal in males and females at the high concentration were sometimes higher than control values, no evidence of damage due to exposure to bitertanol was seen histopathologically. The NOAEL was 63 mg/m³ air (Mihail & Kimmerle, 1977).

Dogs

Groups of four male and four female beagles received bitertanol (purity, 90.2%) at a dose of 0, 1, 5, or 25 mg/kg bw per day in gelatin capsules. The method used in this study complied to a certain extent with OECD guideline 409; at the time the study was performed, compliance with GLP was not compulsory.

Doses of 5 mg/kg bw per day and above led to signs of dose-related adverse effects on the skin and mucous membranes. The effects on the skin were manifested by increased reddening, with local inflammation, desquamation, and hair loss; and those on the mucous membranes by increased reddening and slight inflammatory phenomena in regions of the oral cavity (gingiva) and eyes (conjunctiva). They were evident histologically as slight broadening of the epithelial layer of the skin and slightly enhanced cornification in some cases. Evidence for superficial involvement of the cornea (keratitis), apparently resulting from conjunctival irritation, was observed in a few animals at 25 mg/kg bw per day. The behaviour of the controls and treated animals was similar; in contrast, the food intake and body-weight development of animals at the high dose showed an adverse effect. The results of laboratory tests indicated treatment-related interference with liver function (elevated alkaline phosphatase and alanine aminotransferase activities) in dogs at 5 and 25 mg/kg bw per day. Elevated *N*-demethylase activity and an increase in the cytochrome P450 concentration in liver homogenates were found in the high-dose group at study termination. The weights of the thymus in females at 25 mg/kg per day and of the prostate (relative at 5 mg/kg per day and both relative and absolute at 25 mg/kg per day) were statistically significantly lower than those of the controls. The changes in prostate weights correlated with dose-related histopathological findings of retarded development. The NOAEL was 1 mg/kg bw per day (Hoffmann & Schilde, 1979).

In view of the dermal phenomena observed in the previous study, tests were performed to determine whether they represented a sensitization effect. Beagle dogs were first treated orally 10–42 times with 35 or 70 mg/kg bw bitertanol, which led to marked irritation and dermal lesions, particularly in the area of the head. After a treatment-free interval of four to six weeks, when the lesions had healed, the animals were treated with a dose of 1.75 mg/kg bw, previously found to induce no reaction, for 14 days. Hair loss and a slightly increased incidence of reddened gingiva were determined in isolated animals only at termination of treatment. The study gave no evidence for sensitization, and the delay in development of the effect specifically argues against such an effect (Hoffmann, 1977).

No evidence for sensitization was found in a study in which beagles were exposed to a concentration of 28.8 mg/m³ air for 4 h per day, five days per week for three weeks, left untreated for 10 days, and subsequently re-exposed to a concentration of 47.1 mg/m³ air for one week. All dogs tolerated the exposures with no signs, and no evidence was found for either an irritating effect on visible mucous membranes or for a Type I (immediate anaphylactic type) allergy (Thyssen & Kimmerle, 1977b).

Cats

Groups of three male and three female cats weighing 2–3 kg received whole-body exposure to aerosols of bitertanol (active ingredient, 95.8%) in a 3-m³ inhalation chamber for 6 h per day for four weeks (20 x). The mean analytically determined bitertanol concentration was 27 mg/m³ air. Analysis of the generated aerosols showed that more than 90% of the aerosol particles had a mass-related and more than 99% a particle-related aerodynamic diameter smaller than 5 µm. The exposed animals were compared with negative controls (air) and positive controls (dichlozoline, 28 mg/m³ air). The animals were observed over a recovery period of 12 weeks after termination of the exposure. Their eyes were examined with an ophthalmoscope before exposure and each week throughout the four-week exposure and the recovery period and for gross and histopathological alterations at the end of the study. The method used complied with OECD guideline 412; at the time the study was performed, compliance with GLP was not compulsory.

The exposed cats tolerated the treatment with no signs of toxicity, damage to the eyes, or cataractogenesis. Under comparable experimental conditions, the animals exposed to dichlozoline showed signs of lenticular opacity (cataracts), starting from the end of the second week of exposure. Lenticular opacity that had not reverted up to the end of the recovery period was observed in all animals of this group at the end of the second week of the recovery period. Cats exposed to bitertanol had no cataracts (Pauluhn et al., 1983).

Rabbits

Groups of six male and six female New Zealand rabbits received applications of bitertanol (purity, 95.8%) in an aqueous suspension (0.5 ml) at concentrations of 0, 50, or 250 mg/kg bw on the intact or abraded dorsal and lateral skin for 6 h per day, five times per week for three weeks. The method used in this study complied to a certain extent with OECD guideline 411; at the time the study was performed, compliance with GLP was not compulsory.

Bitertanol had no apparent effect on the appearance, behaviour, body weight, or survival of the rabbits. Transient erythema developed on the exposed areas, initially on abraded skin but later on intact skin. There was no effect on skin-fold thickness or on haematological, clinical chemical, or urinary parameters. At necropsy, there were no gross pathological findings. Histopathological examination showed slight epidermal thickening only in exposed areas of all treated animals. Preparations of liver showed no evidence of induction of microsomal *N*- or *O*-demethylation or cytochrome P450 content. The dermal application had no effect on any of the parameters investigated. The NOAEL was 250 mg/kg bw (Heimann & Vogel, 1984).

(c) Long-term studies of toxicity and carcinogenicity

Mice

Bitertanol (purity, 94–95%) was administered to groups of 50 male and 50 female CF1/W74 mice at doses of 0, 20, 100, or 500 ppm, equal to 2, 25, and 130 mg/kg bw per day in males and 7, 37, and 180 mg/kg bw per day in females, for two years. The study was conducted before enactment of prevailing regulatory guidelines; however, the test procedures complied to a certain extent with OECD guideline 453 but with no attempt to monitor ocular changes.

Behaviour, feed consumption, mortality, and haematological parameters were not affected by treatment. In animals at 500 ppm, body weights were reduced and serum alkaline phosphatase activity was significantly elevated; females at this dose had an increased incidence of enlarged and greenish-coloured livers at necropsy. The liver weights were also increased, particularly in the female mice, and the livers had increased numbers of eosinophilic foci. No evidence was found for a carcinogenic effect at any concentration, which extended into the toxic range. The NOAEL was 100 ppm, equal to 25 mg/kg bw per day (Bomhard & Löser, 1981a).

Rats

Bitertanol (purity, 94% in weeks 1–18; 95% from week 19) was administered to groups of 50 male and 50 female SPF Wistar rats at doses of 0, 20, 100, or 500 ppm, equal to 1, 4.9, and 26 mg/kg bw per day in males and 1.3, 6.6, and 34 mg/kg bw per day in females, for two years. The study was conducted before the enactment of prevailing regulatory guidelines; however, the test procedures complied to a certain extent with OECD guideline 453.

The 500 ppm concentration led to retarded growth in animals of each sex. Ocular changes were not recorded. Haematological, blood chemical, and urinary parameters were not significantly affected, and the mortality rate was not adversely influenced. No treatment-related effects were seen on organ weights or the gross or microscopic appearance of the tissues. In particular, the type, location, incidence, and time to occurrence of the tumours observed in the treated groups were comparable to the control findings and to the spontaneous findings for this strain of rat. The NOAEL was 100 ppm, equal to 4.9 mg/kg bw per day (Bomhard & Löser, 1981b).

Dogs

Groups of four male and four female beagle dogs received bitertanol (purity, 95–97.3%) in the diet at concentrations of 0, 10, 40, or 160 ppm, equal to 0.3, 1.2, and 4.9 mg/kg bw per day, for two years. The method used in this study complied to a certain extent with OECD guideline 452; at the time the study was performed, compliance with GLP was not compulsory.

Although the control dogs gained more weight than did treated dogs, there was no association with treatment. A good nutritional status was maintained by dogs in all groups, and there was no significant difference between groups in food or water consumption. No abnormalities were seen in selected reflexes, body temperature, or pulse rate; however, three of eight dogs at the high dose developed bilateral cataracts. Urinary and haematological examinations conducted at approximately three-month intervals revealed no abnormalities, although serum alanine aminotransferase and alkaline phosphatase activities were increased at 40 and 160 ppm. At necropsy, the mean liver weight of dogs at the high dose was markedly increased. Histological examination showed mild to moderate vacuolation of the adrenal zona reticularis epithelia at doses at and above 40 ppm. The NOAEL was 10 ppm, equal to 0.3 mg/kg bw per day (Hoffmann & Gröning, 1983).

Groups of six male and six female beagles were fed diets containing 0, 3, or 25 ppm bitertanol (purity, 96.3–96.7%) for 12 months, equal to 0.1, 1, and 7.6 mg/kg bw per day. A further group was maintained on a diet containing 200 ppm bitertanol for 20 months to permit continued ophthalmoscopic examination. The method used in this study complied to a certain extent with OECD guideline 452; at the time the study was performed, compliance with GLP was not compulsory.

Treatment had no apparent effect on behaviour, appearance, or nutritional status, and food consumption and body-weight gain were also unaffected. Pulse rates, body temperature, and selected reflexes were unchanged. Ophthalmoscopy, conducted on animals at 0, 3, or 25 ppm at three-month intervals, showed no changes; however, one dog at the high dose developed severe bilateral lenticular cataracts, which were observable from week 58. Four other animals had slight lenticular opacification by week 85. Dogs in this group also had signs of conjunctivitis, with nasociliary discharge and incrustation. Intermittent increases in serum alanine aminotransferase, glutamate dehydrogenase, and alkaline phosphatase activities were also seen in the dogs at the high dose. Other clinical chemical, haematological, and urinary parameters were unaffected by treatment. At necropsy, there were no gross abnormalities. The weights of the adrenals of the dogs at the high dose were apparently greater than those of controls, and lipid vacuolation was observed in the zona reticularis epithelia. The NOAEL was 25 ppm, equal to 1 mg/kg bw per day (Hoffmann & Vogel, 1983).

(d) *Genotoxicity*

No genotoxic or mutagenic potential of bitertanol was found in lower organisms or in mammalian cells or systems *in vivo* or *in vitro*. The results of assays for the genotoxicity of bitertanol are summarized in Table 2.

(e) *Reproductive toxicity*(i) *Multigeneration reproductive toxicity*

The effect of bitertanol (purity, 95.0%) on fertility, lactation performance, and pup development was examined in a three-generation study in Long-Evans FB 30 rats with two litters per generation. The test substance was administered throughout the study period at concentrations of 0, 20, 100, or 500 ppm, equivalent to 1, 5, and 25 mg/kg bw per day. Each of the mating groups consisted of 10 male and 20 female rats. The animals were five to six weeks old at the beginning of the study and were treated for 70 days before the first mating. The F_{3b} generation pups and their parents (F_{2b} generation) were sacrificed and examined histopathologically after a four-week lactation period. The method used in this study complied to a certain extent with OECD guideline 416; at the time the study was performed, compliance with GLP was not compulsory.

No adverse effects were seen on appearance, behaviour, or mortality in any group or generation. Bitertanol at a concentration of 20 ppm in the diet did not affect reproductive performance. Administration of 100 or 500 ppm led to reductions in pup survival rates during the lactation period in several matings and to pups with lower birth weights. At 100 ppm, retarded pup growth was also seen in the F_{2a} and F_{2b} generations. At 500 ppm, general retardation of growth was seen. The NOAEL was 20 ppm, equivalent to 1 mg/kg bw per day (Löser & Eiben, 1981).

Table 2. Results of assays for the genotoxicity of bitertanol

Test system	Test object	Concentration	Purity (%)	Results	Reference
<i>In vitro</i> Reverse mutation ^a	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	4–2500 µg/plate	93.7	Negative	Herbold (1979)
Reverse mutation ^a	<i>E. coli</i> WP2 hcr <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	1–5000 µg/plate	95.0	Negative	Shirasu et al. (1981)
Forward mutation ^a	Mouse lymphoma L5178Y tk ^{+/–}	1–25 µg/ml ^b 1–20 mg/ml ^c	95.0	Negative	Bootman & Rees (1983)
DNA repair ^d	<i>B. subtilis</i> H17 rec ⁺ , M45 rec [–]	20–5000 µg/disc	95.0	Negative	Shirasu et al. (1981)
DNA repair ^{a,d}	<i>E. coli</i> W3110 pol A ⁺ ; P3470 pol A [–]	0.1–33.3 mg/plate	95.0	Negative	Riach (1981)
Aneuploidy	<i>Sordaria brevicollis</i>	0.1–5.0 mg/L	95.0	Negative	Bond & McGregor (1981)
Cytogenetic alterations ^{a,d}	Chinese hamster lung cells	3.3 x 10 ^{–6} –3.3 x 10 ^{–4} mol/L ^b 1.0 x 10 ^{–5} –1.0 x 10 ^{–3} mol/L ^c	97.1	Negative	Sasaki (1987)
<i>In vivo</i> Micronucleus formation	NMRI mice	2 x 1000 mg/kg bw 2 x 2000 mg/kg bw	93.7	Negative	Herbold (1978a)
Dominant lethal mutation	Male NMRI mice	1000 mg/kg bw	93.7	Negative	Herbold (1978b)

^a With and without exogenous metabolic activation

^b Without exogenous metabolic activation

^c With exogenous metabolic activation

^d Conducted in compliance with good laboratory practice and to a certain extent with OECD guidelines

(ii) *Developmental toxicity*

Rats

Groups of 20–23 Long-Evans rats were given bitertanol (purity, 96.5%) orally at doses of 0, 10, 30, or 100 mg/kg bw per day on days 6–15 *post coitum*. The method used in this study complied to a certain extent with OECD guideline 414; at the time the study was performed, compliance with GLP was not compulsory. The body-weight gain of dams at 30 or 100 mg/kg bw per day was significantly reduced during treatment and throughout the gestation period for those at 100 mg/kg bw per day. Resorption rates, fetal deaths, placental weights, and sex ratio were unaffected by treatment; however, fetal weights were significantly reduced at 100 mg/kg bw per day and significant fetal stunting occurred at doses of 30 mg/kg bw per day and higher. At 100 mg/kg bw per day, skeletal ossification was retarded, and a significantly increased incidence of malformations was observed, which included cleft palate, hydrocephalus, malformed tails, dysplasia, and synostosis of the ribs. A simple case of hydrocephalus occurred at 30 mg/kg bw per day. The NOAEL was 10 mg/kg bw per day (Machemer, 1977).

Groups of 22 or 23 Sprague-Dawley rats were given bitertanol (purity, 96%) orally at doses of 0, 10, 25, or 65 mg/kg bw per day on days 6–15 *post coitum*. The study was conducted in compliance with GLP standards and with OECD guideline 414. Reduced weight gain was seen in dams at daily doses of 25 mg/kg bw and higher, which persisted throughout the gestation period in those at 65 mg/kg bw per day. The number of corpora lutea, implantation rates, resorption rates, live fetuses, sex ratio, and fetal and placental weights were unaffected by treatment. An increased incidence of a fourteenth (lumbar) rib relative to controls was observed at daily doses of 25 mg/kg bw (32% incidence) and 65 mg/kg bw (70% incidence). No teratogenic effects were observed. The NOAEL was 10 mg/kg bw (Nagumo et al., 1987).

Pregnant Wistar rats were given single oral doses of 100, 500, or 1000 mg/kg bw bitertanol on day 9, 10, 11, or 13 of gestation. The doses were calculated to represent 1/5, 1/10, and 1/50 of the reported LD₅₀ value of 5000 mg/kg bw. The method used complied to a limited extent to OECD guideline 414. The largest deviation was the short application period; no information was given on compliance with GLP. The study was available only in abstract form and is therefore of only limited value for risk evaluation. Bitertanol induced congenital anomalies when given on day 9, 10, or 11 at 500 or 1000 mg/kg bw. The malformations consisted of microcaudia and acaudia and, in rare cases, exophthalmus, hypognathia, and cleft palate. The NOAEL was 100 mg/kg bw (Vergieva, 1990).

In two studies, groups of 25 Long-Evans rats were exposed to bitertanol (purity, 93.7%) by inhalation for 4 h per day on days 6–15 *post coitum* at a mean analytical concentration of 0, 2.9, 6.4, or 22 mg/m³ air in one study and 0, 27, 60, or 120 mg/m³ air in the other. The method used complied to a certain extent with OECD guideline 414; at the time the study was performed, compliance with GLP was not compulsory. No adverse effects were observed on dams exposed at any concentration, but the average fetal weights were reduced significantly by exposure to 120 mg/m³. Fetuses with below-average weights were found increasingly at aerosol concentrations greater than 22 mg/m³ air, without a clear dose–response relationship. Implantation rates, resorption rates, placental weights, and sex ratio were unaffected by treatment. No embryolethal or teratogenic effects were observed. The NOAEL was 22 mg/m³ air (Machemer & Thyssen, 1979).

Rabbits

Twelve Himalayan rabbits, were given bitertanol (purity, 96.7%) at daily oral doses of 0, 10, 30, or 100 mg/kg bw on days 6–18 *post coitum*. The method used complied to a certain extent with OECD guideline 414; at the time the study was performed, compliance with GLP was not compulsory. The dose of 100 mg/kg bw led to reduced weight gain and isolated

clinical signs, such as reduced food intake, diarrhoea, and blood in the urine; one dam died on day 29 *post coitum*. This dose also resulted in increased embryonal and fetal mortality and reduced fetal and placental weights, and three individual malformations occurred, which deviated from those seen in controls in their nature (epignathus, pulmonary hypoplasia, and aplasia) but not in their number. The NOAEL was 30 mg/kg bw per day (Roetz, 1982).

Groups of 15 Himalayan rabbits were given bitertanol (purity, 93.9%) at daily oral doses of 0, 10, 30, or 100 mg/kg bw on days 6–18 *post coitum*. The method complied to a certain extent with OECD guideline 414; at the time the study was performed, compliance with GLP was not compulsory. Treatment had no effect on the behaviour, appearance, or body-weight gain of the dams. At 100 mg/kg bw per day, a significantly higher resorption rate and reduced numbers and weights of fetuses were seen, and the number of malformed fetuses was increased, with rare types of malformation such as cleft palate and pigeon chest. The NOAEL was 30 mg/kg bw per day (Schlüter, 1983).

Groups of 16 fertilized chinchilla rabbits were given bitertanol (purity, 96.9%) at a daily oral dose of 0, 10, 50, or 250 mg/kg bw on days 6–18 of gestation. Because of the very high rate of post-implantation loss at 250 mg/kg bw per day, doses of 0 and 150 mg/kg bw per day were used in a supplementary study. The study was conducted in compliance with OECD guideline 414 and with GLP standards. A dose-related reduction in food consumption was seen in dams at ≥ 50 mg/kg bw per day. The dose of 250 mg/kg bw per day significantly reduced body weights from day 7 to the end of the experiment and significantly reduced corrected body-weight gain. One dam at 150 mg/kg bw per day and two at 250 mg/kg bw per day died during the study. Isolated hair loss and enlarged and heavier livers at necropsy were observed in dams at the high dose. A dose-related increase in the rate of post-implantation losses was observed at ≥ 150 mg/kg bw per day. Two dams at 150 mg/kg bw per day and 13 at 250 mg/kg bw per day completely resorbed their embryos, and the fetuses at these doses showed dose-related reductions in body weight and a dose-related increase in the incidence of incompletely or unossified phalangeal nuclei and calcanei. The NOAEL was 50 mg/kg bw per day (Becker et al., 1987).

(f) *Special studies*

As the studies in this section were published in the open literature, the level of detail needed for full evaluation was not available.

(i) *Effects on the central nervous system*

The effect of bitertanol on the central nervous system was examined in pharmacological screening tests in mice and rats, which included tests for potentiation of anaesthesia in mice (hexobarbital sleep period), stimulation of spontaneous motility in mice, an Irwin behaviour test in mice, the novel box response in rats, the open field test in mice, and a test for reserpine ptosis in mice. In these investigations, bitertanol was administered as a single oral dose of 0.075, 0.6, or 4.8 mg/kg bw. The results showed a slight stimulating effect of bitertanol on the central nervous system in mice, but no specific pharmacological effects, such as potentiation of amphetamine effects or antagonism to reserpine ptosis, were determined (Polacek, 1983).

In a pharmacological test programme, groups of 10 male mice were given a single oral dose of 0.02, 0.2, 2, 20, or 200 mg/kg bw bitertanol. The highest dose significantly increased spontaneous motor activity, with marked effects when treatment was given during the dark period. In mice treated with 20 mg/kg bw per day, motor activity tended to increase but not significantly, and lower doses had no effect. No further effects on behaviour were observed at any dose (Kaneto, 1986a). In a further test, male mice received bitertanol at 1 or 100 ppm for one week or one month. No increase in spontaneous motor activity and no potentiating effect in amphetamine-pretreated animals were seen after repeated dosing (Kaneto, 1986b).

In a study designed to determine whether bitertanol has similar behavioural effects on the fixed-interval response rate and motor activity of rats, doses of 10–300 mg/kg bw were given intraperitoneally to rats maintained under a multiple fixed-interval 1-min schedule of reinforcement. Intermediate doses increased the response rates and disrupted response patterning in both fixed-interval components. The same doses of bitertanol did not increase motor activity (Allen & MacPhail, 1983).

In rats placed in an actographic device designed for continuous measurement of the locomotor component of spontaneous motor activity, bitertanol increased motor activity at 200 mg/kg bw after administration either orally or intraperitoneally. The activity peaks at the low dose of 100 mg/kg bw coincided with normal night and morning activity maxima (Frantik et al., 1996).

On the basis of previous results showing that acute exposure to the triazole fungicide triadimefon affects central nervous system catecholamines and induces a transient syndrome in rats that consists of hyperactivity and stereotyped behaviour, a study was designed to determine whether this type of toxicity is characteristic of other triazoles. Dose–effect functions were determined for 14 triazoles or structurally related pesticides, including bitertanol, in adult male Long-Evans rats. All of the chemicals were administered orally in corn oil. Hyperactivity was measured for 2 h in figure-eight mazes. Only triadimefon and triadimenol induced hyperactivity, suggesting a very rigid structure–activity relationship for the hyperactivity syndrome. The absence of an effect of bitertanol may be due to steric hindrance of benzene-ring substitution for the halogen on the benzene-ring structure of triadimefon and/or triadimenol. Alternatively, bitertanol may lack halogen substituents on the benzene rings and thus be less polarized. The lack of activity of bitertanol is probably not due to differences in absorption kinetics (Crofton, 1996).

(ii) *Toxicity in combinations*

Acute tests were performed to determine whether bitertanol has superadditive (potentiating) effects when administered in combination with triadimenol, captan, fuberidazole, or dodine. At the time the studies were performed, OECD methods were not available and compliance with GLP was not compulsory. The study involved administration of single equitoxic oral (with triadimenol, captan, or fuberidazole) or intraperitoneal (with dodine) doses of the active ingredients to male rats. A factor greater than 1 between observed and expected LD₅₀ values indicates a superadditive effect. Bitertanol plus triadimenol, bitertanol plus fuberidazole, and bitertanol plus dodine had no superadditive effects but only additive toxic effects (Mihail, 1982b; Flucke, 1980; Heimann, 1984b). In contrast, the active ingredient combination of bitertanol plus captan had slightly superadditive action, with a potentiation factor of 1.9 (Mihail, 1982a).

(iii) *Effect on the liver*

Groups of 10 male and 10 female Wistar rats received bitertanol (purity, 95.8%) suspended in distilled water with Cremorph EL by gavage at doses of 0, 30, 100, or 300 mg/kg bw per day for 14 days. On sacrifice, blood was collected for detailed haematological and clinical chemical testing, and liver samples were taken for detailed histopathological and enzyme studies. During treatment, 1/10 female rats at the intermediate dose and 9/10 at the high dose showed hair loss, and some lost weight, while females at the low dose had reduced body-weight gain in comparison with control animals. Female rats also had a dose-related tendency to mild thrombocytosis, which was significant at the intermediate and high doses. Serum γ -glutamyl transpeptidase activity and bilirubin concentration were slightly increased in females at the high dose. There were no gross pathological findings at necropsy. The liver weights tended to be increased at the intermediate and high doses, especially in females. Slight-to-moderate bile-duct proliferation with peribiliary infiltration of monocytes or polynucleocytes

was seen histologically in animals at the intermediate and high doses. These changes were sometimes accompanied by parenchymal Councilman bodies or, more occasionally, mitoses. The hepatocytes of animals at the high dose were occasionally swollen, with finely granular cytoplasm. Little fatty infiltration was seen. The results of studies *in vitro* were consistent with induction of hepatic microsomal enzymes, as the cytochrome P450 content increased in a dose-related manner, especially in males. Aminopyrene *N*-demethylase activity was increased in males and females at the high dose and in males at the intermediate dose, while *O*-demethylase activity was increased in males at the intermediate and high doses and in females at the high dose. The hepatic triglyceride content was not affected by treatment. Bitertanol thus caused mild hepatotoxicity, with modest induction of hepatic microsomal activity in rats at 100 and 300 mg/kg bw per day. The NOAEL was 30 mg/kg bw per day (Mihail & Luckhaus, 1985).

3. Studies on metabolites

(a) *1-(Triazol-1-yl)-1-(4'-phenylphenoxy)-3,3-dimethylbutan-2-one (plants, soil)*

1-(Triazol-1-yl)-1-(4'-phenylphenoxy)-3,3-dimethylbutan-2-one, a keto analogue of bitertanol, has very little acute toxicity in rats when given orally or dermally. It induced signs of effects on the central nervous system, with initial sedation and respiratory disturbances and later stimulation. The highest technically administrable oral dose, 1750 mg/kg bw, caused no deaths. A dermal dose of 5000 mg/kg bw and the highest dose administered by inhalation (dynamic dust nebulization) were tolerated with no observed signs. The LC₅₀ value in male and female rats was > 500 mg/m³ air after a 1-h exposure or a 4-h exposure, respectively. In rabbits, the compound was slightly irritating to the skin only after contact for 24 h and was mildly irritating to the mucous membranes of the eyes (Thyssen & Kimmerle, 1978).

(b) *Bitertanol benzoic acid (soil)*

Bitertanol benzoic acid had little acute toxicity when given orally. The highest administered dose, 5000 mg/kg bw, was tolerated by fasted male rats with no observed signs or deaths (Heimann, 1983b). The compound was not mutagenic to *Salmonella typhimurium* in the presence of an exogenous metabolic activation system (Herbold, 1983).

(c) *1,2,4-Triazole (photodegradation, soil)*

1,2,4-Triazole has moderate or low acute toxicity when given orally (LD₅₀ value, 1600 mg/kg bw in males and females) or dermally (LD₅₀ values, 4200 mg/kg bw in males and 3100 mg/kg bw in females). At high oral and dermal doses, the compound had effects on the central nervous system. In tests of inhalation of air enriched with vapours of the test substance, male rats and mice tolerated exposure for 4 and 6 h, respectively, with no observed signs. No dermal irritation was observed on rabbits exposed for 24 h or on the skin of five male volunteers exposed for 8 h. 1,2,4-Triazole was strongly irritating to the mucous membranes of the rabbit eye (Thyssen & Kimmerle, 1976).

Groups of 15 male and 15 female rats were given 1,2,4-triazole (purity, 99.6%) at a dose of 0, 100, 500, or 2500 ppm for three months. The study was conducted before enactment of prevailing regulatory guidelines, but the test procedures complied to a certain extent with OECD guideline 408. Treatment at 2500 ppm resulted in a temporary decrease in food intake, a reduction in body weight, and transient, slight palpospasm in isolated animals. Significant reductions in the haemoglobin, haematocrit, mean corpuscular volume, and mean corpuscular haemoglobin values in male rats at 2500 ppm indicated an effect on the blood. In this group, slight to moderate fat accumulation was found in the cells of the liver parenchyma in three of 15 males, which was attributed to the treatment. The NOAEL was 500 ppm, equal to 38 mg/kg bw per day (Bomhard et al., 1979).

1,2,4-Triazole at concentrations of 10–5000 µg/plate did not induce point mutations in *Salmonella typhimurium* TA1535, TA1537, TA98, or TA100, with or without metabolic activation (Poth, 1989).

In two studies, groups of 25 fertilized Wistar rats were given daily oral doses of 1,2,4-triazole (purity, 95.3% and 94.0%, respectively) on days 6–15 of gestation at doses of 0, 10, 30, or 100 mg/kg bw in one study and 0, 100, or 200 mg/kg bw in the other. The studies were conducted in compliance with GLP standards and OECD guideline 414. Maternal toxicity was indicated by decreased weight gain of dams at 100 and 200 ppm relative to those in the control group. Reduced fetal weights, retarded osteogenesis, and increased numbers of runts were found at ≥ 100 mg/kg bw per day. An increased resorption rate was found in dams at 200 mg/kg per day, but the rate of fetuses with retarded ossification was not increased. The types and incidences of the malformations observed in this group (including cleft palate and malformed extremities) indicate that 1,2,4-triazole has teratogenic potential. The NOAEL was 30 mg/kg bw per day (Renhof, 1988a,b).

4. Observations in humans

No health impairment was observed in male or female employees engaged in formulating bitertanol and using the customary safety precautions, who had regular medical examinations (Miksche, 1981).

Slight, transient prurient reddening of the forearms, which regressed spontaneously after a few days, developed in rare, isolated cases after direct dermal contact during packaging of a powder of the pure active ingredient. Unequivocal differentiation between an allergic dermal reaction and mechanical–toxic skin irritation was not possible. There was no tendency to relapse (Faul, 1986).

Comments

After oral administration bitertanol is rapidly and extensively absorbed (about 84%) and distributed. Excretion is also rapid (rats, almost complete within 72 h) and occurs mainly in the faeces (about 90%) by biliary excretion, owing to the lipophilic nature of the parent substance. The liver and kidneys are the main sites of tissue accumulation in both male and female rats. Although some statistically significant sex-related differences were seen, they were of minor physiological importance. The substance has a relatively low rate of dermal penetration. The metabolic profile was similar at the various doses tested. The main metabolic pathways are hydroxylation of the phenyl ring in the *para* position and oxidation of the *tert*-butyl moiety, leading to bitertanol alcohol and the corresponding carboxylic acid; metabolites derived from the two pathways combined were also observed. The metabolites occur in both free and conjugated forms. The parent compound was not detected in urine or bile. There was no toxicological concern with regard to the metabolic profile in plants.

Bitertanol had very low acute toxicity in rats, mice, and dogs when given orally and after dermal application or by inhalation. It was of moderate to low toxicity in rats and mice after intraperitoneal injection. Females appeared to be slightly more sensitive than males, but only in some studies. This was perhaps due to slightly different absorption characteristics in animals of the two sexes, as seen after oral administration. In view of the toxic signs (including respiratory disturbances, sedation, spasms, and tremor) and the findings of the pharmacological screening tests, it may be inferred that bitertanol has central nervous system activity; however, no specific pharmacological effects, such as potentiation of amphetamine action, antagonism of reserpine ptosis, or an effect on hexobarbital anaesthesia, were observed.

Bitertanol induces no, or only very slight, dermal irritation. It induces slight to moderate reactions of the ocular mucosa but has no effect on the cornea or iris. No evidence for a sensitizing effect was observed in any study

Bitertanol has been classified by WHO as unlikely to present an acute hazard in normal use (WHO, 1996).

In medium- and long-term tests for toxicity, the liver is regarded as the main toxicological target organ in dogs and rats at doses of 1.2 and 28 mg/kg bw per day and above, respectively. Liver weight was found to be the most sensitive indicator. Corresponding patterns of disruption of liver function were observed in rats and dogs. The activities of the transaminases, alkaline phosphatase, and glutamate dehydrogenase in the serum were increased. In addition, a rise in cholesterol level was observed in several studies in rats at doses of 61 mg/kg bw per day and above. The ability of bitertanol to induce mixed-function oxidases was verified in both species. It is therefore likely that the effect on weight is essentially due to hypertrophy of the endoplasmic reticulum in hepatocytes at doses of 100 mg/kg bw per day and above. Morphological changes in the liver were seen only at relatively high doses and consisted of hepatocytic swelling, bile-duct proliferation, perilobular fatty degeneration, eosinophilic foci, and fibrous structures. The induced effects corresponded to toxic liver damage with bile-duct involvement.

Evidence for haemotoxicity was found at doses of 28 mg/kg bw per day and above in rats; this may be classified as an effect on the peripheral red blood cell population. The decreases in erythrocyte count, haemoglobin concentration, and packed cell volume and the compensatory rise in the reticulocyte count argue for this interpretation. No evidence was found for damage to the haematogenic organs.

Slight increases in the leukocyte count were also seen in a few studies in rats at doses of 61 mg/kg bw per day and above. The increased leukocyte counts are probably attributable to inflammatory processes, since the increase occurred in studies and at doses at which inflammatory processes were also observed. Hyperkeratosis of the oesophageal epithelium and glandular stomach and/or erosions of the glandular stomach as well as parakeratosis of the stomach wall were observed in rats. The histopathological picture included distended epithelial cells, slight inward growth of the papillary body, and cellular infiltration of the epithelial and subepithelial layers in the affected animals. These changes are probably attributable to irritation of the mucous membranes by the active ingredient.

Effects on the skin were observed in dogs, sheep and rats. The effects in dogs at doses of 5 mg/kg bw per day and above consisted of reddening, with localized inflammation, desquamation, and hair loss, and increased reddening and slight inflammatory phenomena in the mucous membranes of the oral cavity and eyes. Histological examination showed broadening of the epithelial layer, enhanced cornification, and minor erosions. The dermal effects were apparently accompanied by pruritus. The keratitis observed in dogs was considered to be secondary to conjunctivitis. Hair loss was also observed in rats and sheep, which occurred after administration of bitertanol by capsule or gavage at doses of 300 mg/kg per day and above; this was considered to be a systemic effect.

Pathological changes were seen in the adrenals of dogs and rats at 1.2 and 81 mg/kg bw per day and above, respectively, consisting of swelling and fatty degeneration of the adrenal cortical cells, particularly in the zona reticularis and zona fasciculata. These alterations were considered to be due to inhibition of sterol biosynthesis by triazole derivatives. It is highly probable that this effect, which also represents the biological, antimycotic action of the substance, leads to an effect on corticoid metabolism with corresponding morphological effects in the cells of the adrenal cortex.

In feeding studies in dogs, lenticular opacity seen at doses of 4.9 mg/kg bw per day and above were considered to be related to treatment. No lenticular alterations attributable to administration of bitertanol were seen in any other study or species. The exact mechanism of the cataractogenesis resulting from long-term administration of triazole fungicides is presently unknown. The ocular lens undertakes its own de-novo synthesis of cholesterol, which is isolated from lipoproteins circulating in the blood; other substances that inhibit cholesterol synthesis can induce cataracts.

No evidence for any carcinogenic potential of bitertanol was found in long-term studies of toxicity and carcinogenicity in rats and mice treated in the diet. The highest doses tested were 130 mg/kg bw per day in mice and 26 mg/kg bw per day in rats.

In a series of studies, bitertanol had no genotoxic potential in lower organisms or in mammalian cells or systems *in vitro* or *in vivo*.

In a three-generation study of reproductive toxicity in rats, adverse effects on the pups (reductions in survival rates during the four-week lactation period, reduced weight at birth, and retarded growth) were observed at parentally toxic doses of 100 or 500 ppm, equivalent to 5 or 25 mg/kg bw per day. The NOAEL was 1 mg/kg bw per day.

In studies of developmental toxicity, treatment with bitertanol led to several embryotoxic and teratogenic effects, depending on the animal species, route of administration, and dose. In rats, an oral dose of 10 mg/kg bw per day was tolerated with no observed effect. Doses of 25 mg/kg bw per day and above led to retardations and variations (e.g. increased incidence of the 14th rib). Malformations were observed at an oral dose of 100 mg/kg bw per day, which was clearly maternally toxic. Exposure of pregnant rats to concentrations of 27 mg/m³ air and above by inhalation resulted in retardation effects; no malformations were observed. In rabbits, doses of 50 mg/kg bw per day and above were maternally toxic; fetotoxic effects were seen at doses of 100 mg/kg bw per day and above. Teratogenic effects were observed in Himalayan rabbits at 100 mg/kg bw per day, while in the second strain tested (Chinchilla), even a dose of 250 mg/kg bw per day did not lead to malformations.

The ADI established at the 1988 Meeting of 0–0.01 mg/kg bw, based on a combined NOAEL of 1 mg/kg bw per day from the two-year and the one-year study in dogs, was maintained. The ADI is supported by the NOAEL of 20 ppm (equivalent to 1 mg/kg bw per day) in a three-generation study in rats.

An acute RfD was not allocated because bitertanol has been classified by WHO as unlikely to present an acute hazard in normal use and it has not shown any specific adverse effects (teratogenicity, neurotoxicity) after single doses 100 times the lowest relevant NOAEL in long- and short-term studies that were used to establish the ADI. Therefore, the Meeting concluded that the acute intake of residues is unlikely to present a risk to consumers.

Toxicological evaluation

Levels that cause no toxic effects

- | | |
|---------|--|
| Mouse: | 100 ppm, equal to 25 mg/kg bw per day (toxicity in a two-year study of toxicity and carcinogenicity) |
| Rat: | 100 ppm, equal to 4.9 mg/kg bw per day (toxicity in a two-year study of toxicity and carcinogenicity)
20 ppm, equivalent to 1 mg/kg bw per day (reproductive and parental toxicity in a three-generation study)
10 mg/kg bw per day (maternal and developmental toxicity in a study of developmental toxicity) |
| Dog: | 1 mg/kg bw per day (overall NOAEL in one-year and two-year studies of toxicity) |
| Rabbit: | 50 mg/kg bw per day (maternal and developmental toxicity in a study of developmental toxicity) |

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of acute reference dose

Not allocated (unnecessary)

Studies that would provide information useful for continued evaluation of the compound

Further observations in humans

List of end-points relevant for setting guidance values for dietary and non-dietary exposure

<i>Absorption, distribution, excretion, and metabolism in mammals</i>			
Rate and extent of oral absorption		Commenced immediately, about 84% absorbed	
Dermal absorption		About 10%	
Distribution		Highest concentrations in liver and kidneys	
Potential for accumulation		None	
Rate and extent of excretion		About 90% excreted with bile, 10% with urine	
Metabolism in animals		No parent compound in bile or faeces; extensively metabolized to 14 metabolites (ring monohydroxylation, ring dihydroxylation, aryl <i>O</i> -methylation, aliphatic hydroxylation, aliphatic oxidation to carboxylic acids, and ether cleavage)	
Toxicologically significant compounds (animals, plants and environment)		Parent compound	
<i>Acute toxicity</i>			
Rat: LD ₅₀ , oral		> 5000 mg/kg bw	
Rat: LD ₅₀ , dermal		> 5000 mg/kg bw	
Rat: LC ₅₀ , inhalation		> 550 mg/m ³ (4 h)	
Skin irritation		Not irritating	
Eye irritation		Not irritating	
Skin sensitization		Not a sensitizer (Magnussen & Kligman test)	
<i>Short-term toxicity</i>			
Target/critical effect		Liver, red blood cells, adrenals, digestive tract	
Lowest relevant oral NOAEL		Dog: 90 days: 1 mg/kg bw per day	
Lowest relevant dermal NOAEL		Rabbit: 3 weeks; 250 mg/kg per day	
Lowest relevant inhalation NOAEL		Rat: 3 weeks, 63 mg/m ³	
<i>Genotoxicity</i>		No genotoxic or mutagenic potential	
<i>Long-term toxicity and carcinogenicity</i>			
Target/critical effect		Liver	
Lowest relevant NOAEL		Dog: 1 year and 2 years: 1 mg/kg bw per day	
Carcinogenicity		No evidence of carcinogenic potential	
<i>Reproductive toxicity</i>			
Reproductive target/critical effect		Reproductive effects (reduced litter size, pup growth rate, and pup survival) at parentally toxic doses	
Lowest relevant reproductive NOAEL		Rat: 1 mg/kg bw per day	
Developmental target/critical effect		Fetotoxic and teratogenic effects at maternally toxic doses	
Lowest relevant developmental NOAEL		Rat: 10 mg/kg bw per day	
<i>Neurotoxicity/Delayed neurotoxicity</i>		No relevant effects	
<i>Other toxicological studies</i>		Induction of hepatic microsomal activity	
<i>Medical data</i>		No health impairments observed in employees subjected to regular medical examinations	
Summary	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	1 and 2 years in dogs	100
Acute reference dose	None allocated (unnecessary)		

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