

**FENBUTATIN OXIDE**

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**EXPLANATION**

Fenbutatin oxide was previously evaluated by the JMPR in 1977 (Annex I, reference 28) when an ADI of 0-0.03 mg/kg bw was allocated. The data reviewed in 1977 consisted of studies on pharmacokinetics, short-term toxicity in mice, rats, and dogs, long-term toxicity in mice and rats, reproductive toxicity in rats, neurotoxicity in rats, developmental toxicity in rabbits, and genotoxicity. The present review evaluates studies conducted since the 1977 review. Relevant portions of the previous monograph have been incorporated into this toxicological monograph.

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

Groups of 2 male and 2 female rats were fed an average of 19.5 ppm <sup>119</sup>Sn-labelled fenbutatin oxide (with or without a 3 day withdrawal period before killing following 3 or 6 days exposure) for 1, 3 or 6 days to study the absorption, distribution and excretion of fenbutatin. Most of the administered <sup>119</sup>Sn was rapidly excreted in the faeces following multiple dosing (80-90% of the administered <sup>119</sup>Sn). Essentially no <sup>119</sup>Sn was excreted via the urine. The GI tract contained approximately 30, 15 and 5% of the administered <sup>119</sup>Sn in animals sacrificed after 1, 3 or 6 days of dosing, respectively. The highest tissue concentrations of <sup>119</sup>Sn were found in liver (0.015-0.09 mg/kg), kidneys (0.015-0.055 mg/kg) and fat (< 0.01-0.065 mg/kg). No <sup>119</sup>Sn was detected in brain or bone tissue (Loeffler, 1972).

The pharmacokinetics of fenbutatin oxide following oral administration was studied using groups (5/sex) of Crl:CD BR rats. One group received a single low dose of 10 mg <sup>119m</sup>Sn-labelled fenbutatin oxide/kg bw. A second group received

a single high dose of 500 mg/kg bw of radiolabelled compound. A third group received multiple doses (14 daily doses) of unlabelled compound followed by a single dose of radiolabelled compound. Urine and faeces were collected over 7 days at which time rats were sacrificed and tissues collected.

Over a 5-7 day period, 83%-100% of the administered dose was excreted in the faeces with less than 1% of the radiolabel detected in the urine. Excretion was somewhat slower following the single high dose compared to the low-dose exposure. For all dosage regimens, excretion was almost complete within 72 h. The excretion pattern was similar for all three dosage regimens and for males and females. Tissue residues were low 5-7 days after treatment. Highest radioactivity was found in liver, kidney, and heart. Concentrations ranged from 0.2 to 3 ppm following the low dose (single or repeated) and high dose, respectively. The elimination half-life was estimated to be 24 h following the low dose (single or repeated) and 40 h following the high dose (Reynolds, 1989).

### **Biotransformation**

#### **Rats**

Analysis of the faeces from rats dosed with  $^{119}\text{Sn}$ -labelled fenbutatin oxide indicated that  $\beta,\beta$ -dimethylphenethylstannic acid and 1,3-dihydroxy-1,1,3,3-tetrakis(2-methyl-2-phenylpropyl)-distannoxane are the major metabolites (Loeffler, 1972)

The metabolism of fenbutatin oxide was studied in rats by analyzing faeces obtained in the pharmacokinetics study described above (Reynolds, 1989). Metabolites were identified by co-chromatography with reference compounds using TLC. The majority (86-96%) of the extracted radiolabel co-chromatographed with unchanged fenbutatin oxide. Two minor metabolites were found in faecal extracts, one of which was unidentified. The other metabolite was tentatively identified as 1,3-dihydroxy-1,1,3,3-tetrakis(2-methyl-2-phenylpropyl)-distannoxane (IN-CG200). Traces of IN-CG200 were detected as an impurity of the test material prior to dosing (Reynolds, 1989).

### **Effects on enzymes and other biochemical parameters**

In starch gel electrophoretic studies on sera from rats fed either 0 or 600 ppm fenbutatin oxide in the diet for 6 months (2 buffer systems) control and test animal sera were identical. The effect of 1-phenylalanine or heat was similar on both sera. Comparisons with intestinal, bone, liver and kidney alkaline phosphatase indicated that the serum alkaline phosphatase was similar to intestinal alkaline phosphatase. Further studies on rat sera derived from animals fed 0 or 600 ppm fenbutatin oxide in the diet for one year, utilizing heat inactivation, selective substrates and inhibitors, confirmed the absence of any difference

between control and test animal sera, as well as confirming the similarity of the serum alkaline phosphatase to intestinal alkaline phosphatase (Pickering, 1973a).

In a further series of studies, the elevation of serum alkaline phosphatase observed in rats after administration of 300 ppm fenbutatin oxide in the diet for 90 to 120 days was shown to be completely reversible following one month's withdrawal from exposure. Limited data indicate that the reversal of elevated serum alkaline phosphatase in the test rats may be completed within 7 days of withdrawal of the fenbutatin oxide (Pickering, 1973b).

Fenbutatin oxide and other tri-substituted tin compounds were shown to inhibit uptake and stimulate release of 5-hydroxytryptamine (5-HT) in rat platelets *in vitro* (at 10  $\mu$ M) and to a lesser extent in platelets isolated after intraperitoneal injection (2.5 mg/kg bw). Interference with membrane ATP was suggested as the site of inhibition of 5-HT uptake by organotins (Johnson & Knowles, 1983).

Fenbutatin oxide (10  $\mu$ M) did not affect aggregation of rat platelets *in vitro* (Knowles & Johnson, 1986).

### **Toxicological studies**

#### **Acute toxicity studies**

Acute toxicity studies are summarized in Table 1. Fenbutatin oxide has low toxicity by the oral route but is highly toxic by the inhalation route. Inhalation exposure produced necrosis of bronchiolar epithelium, lung congestion and edema, and lesions in renal tubule epithelium (Parker, 1981).

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

##### **Mice**

Groups of 5-10 young or mature male Swiss-Webster mice were administered fenbutatin oxide (purity unspecified) at 137, 411, or 1230 ppm (260, 780, or 2340  $\mu$ eq/kg diet), equal to 21, 62 or 190 mg/kg bw/day, for 7 days. Mice were sacrificed 4 or 7 days after treatment. In young mice (13-14 g bw), a dose level of 137 ppm had no effect on body, brain, heart, liver, or spleen weight 7 days after treatment. In mature mice (27-29 g bw), a dose level of 411 ppm had no effect on body-weight, haemoglobin, haematocrit, or erythrocyte count 4 days after treatment. Spleen weight was reduced 20% and WBC count was reduced 11% compared to control values. At 1230 ppm, body-weight, spleen weight, and WBC count were decreased and haemoglobin, haematocrit, and erythrocyte count were increased. A number of other triorganotin compounds were more potent than fenbutatin oxide producing effects on body-weight, spleen weight, and blood composition at lower dose levels. The NOAEL was 137 ppm (equal to 21 mg/kg

bw/day) based on slight effects on the spleen and WBC count at 411 ppm (Ishaaya et al., 1976).

**Table 1 Acute toxicity of fenbutatin oxide**

Species	Strain	Sex	Route	LD <sub>50</sub> mg/kg bw	LC <sub>50</sub> mg/l	Reference
Rat	Crl:CD BR	M	oral	4400 <sup>1</sup>		Sarver (1988)
	Crl:CD BR	F	inhalation (4 hr exp)		0.072 <sup>1,2</sup>	Valentine (1987)
	Sprague-Dawley	M&F	inhalation (4 hr exp)		0.23 <sup>1</sup> 0.14 <sup>3</sup>	Parker (1981)
	CFE	M&F	i.p.	33 <sup>4</sup>		Cassidy (1978)

<sup>1</sup> Purity of technical fenbutatin oxide > 98%.

<sup>2</sup> LD<sub>50</sub> for males could not be calculated. Lowest achievable concentration of 0.046 mg/l produced 1/5 deaths.

<sup>3</sup> Concentration corrected for particles in respirable range (<4.5 µm).

<sup>4</sup> Purity of test material unspecified.

## Rats

Five groups of 5 male and 5 female rats were fed 0, 30, 100, 300 or 1000 ppm fenbutatin oxide in the diet for 5 weeks. Body-weight was significantly reduced, as was food intake at 300 and 1000 ppm. Reductions in absolute brain, heart, liver, spleen and kidney weights were noted in both sexes at 1000 ppm. Spleen weight was reduced in males at 300 ppm and brain, liver, spleen and kidney weights were reduced at 300 ppm in females. Serum alkaline phosphatase was increased in both sexes and SGPT levels were elevated in females at 1000 ppm (Simpson, 1972a).

Three groups of five male rats were used in a paired feeding study, one group being a normal control, the second being fed 600 ppm and the third paired-fed at the food intake levels of the 600 ppm group. The study ran for 5 weeks. Results (daily body-weights) indicated that the reduction in body-weight at 600 ppm can be totally accounted for by reduced food intake (Simpson, 1972b).

Groups of 6 male and 6 female rats were fed 50, 100, 300 or 600 ppm fenbutatin oxide (97%) in the diet for 3 months. Concurrent controls comprised 12 male and 12 female rats. Health and behaviour were comparable in all groups. Body-weight gain was reduced in males at 600 ppm and in females at 300 and 600 ppm. Food intake was reduced in males at 600 ppm and in females during the first 3 or 4 weeks of the study at 300 and 600 ppm. Absolute organ weights of liver and kidney were reduced in both sexes, heart in females, and brain and

spleen in males at 600 ppm. At 300 ppm, liver, spleen and kidney absolute weights were reduced in males. Organ/body-weight ratios were elevated for brain in males at 600 ppm and in females at 300 and 600 ppm. Heart and liver to body-weight ratios were elevated in females at 600 ppm and the heart to body-weight ratio was increased at 300 ppm. The testes to body-weight ratio was elevated in males at 300 and 600 ppm. Haematology parameters were comparable in all groups. Clinical chemistry determinations were comparable for total protein, SGOT and electrolytes ( $K^+$ ,  $Na^+$  and  $Cl^-$ ). BUN and SAP in males were elevated at 300 and 600 ppm in males, as was SGPT at 300 ppm. In females, BUN was elevated at 100 ppm and above. Serum protein fractions were comparable in all groups. No compound-related gross or histopathological effects were noted (Simpson & Thorpe, 1973).

Groups of rats, 20/sex/control group or 10/sex/test group, were fed 0, 3, 10, 30, 100 or 300 ppm of 99% 1,3-dihydroxy-1,1,3,3-tetrakis(2-methyl-2-phenylpropyl)-distannoxane (a metabolite of fenbutatin oxide) for 90 days. Health and behaviour were comparable in all groups. Body-weights of 300 ppm males were significantly decreased throughout the study. This was also true for the females in the 30, 100 and 300 ppm groups, but the suppression was not significant. Slight reductions in haemoglobin, erythrocyte count and packed cell volume occurred in the 300 ppm males. No changes were noted in any of the other groups of either sex. The other clinical values of animals killed after 13 weeks showed no dose-related differences when compared to the control values. Gross and microscopic examination of a wide range of tissues from all control, 100 and 300 ppm animals revealed no consistent changes associated with exposure to the metabolite. Organ weights of males at 13 weeks did not differ from control weights, while the organ body-weight ratios were increased for brain, heart, liver, kidneys and testes in this group and for the liver in the 100 ppm male group. Brain, spleen and kidney weights were reduced in the 300 ppm females (Simpson & Dix, 1972).

### Rabbits

Groups of 5-10 male and 5-10 female New Zealand white rabbits received dermal applications (6 h/day) of technical fenbutatin oxide (98% purity) at doses of 0.05, 0.5, or 5 mg/kg bw for 21 consecutive days. Control groups received dermal applications of the vehicle, dimethyl phthalate. Doses of 0.5 and 5 mg/kg bw produced mild to severe skin irritation, which was not completely reversed after a 14-day recovery period. Slight irritation observed sporadically in control and 0.05 mg/kg bw groups was attributed to the wrapping technique and the vehicle. No systemic toxicity was observed. The NOAEL for systemic toxicity was greater than 5 mg/kg bw (Brock, 1988).

## Dogs

Five groups of 2 male and 2 female beagle dogs were orally dosed (gelatin capsules) with 0, 10, 30, 100 or 300 mg fenbutatin oxide/kg bw/day for 5 weeks. Slight vomiting and diarrhoea occurred at 100 and 300 mg/kg bw/day which was associated with decreased body-weight gain. At 30 mg/kg bw/day and above, serum alkaline phosphatase was elevated. No pathological changes were observed (Simpson, 1972a).

Groups of dogs (8/sex/control group and 4/sex/test group) were administered, by capsule, 0, 2.5, 5, 15, 30 or 60 mg/kg bw/day 97% fenbutatin oxide for 2 years. Blood samples were taken every six weeks to measure BUN, glucose, plasma protein, sodium, potassium, chloride, SGPT, SGOT and alkaline phosphatase. Every 12 weeks haemoglobin, packed cell volume, RBC, WBC, differential WBC counts, prothrombin and kaolin-cephalin coagulation times were determined. Urine analysis was carried out every 3 months. Emesis and diarrhoea occurred in most of the dosed dogs. These effects continued in some dogs in the 30 and 60 mg/kg bw/day groups throughout the study, although at a reduced frequency towards the end of the study. Lower dosage groups showed none of these effects after the first year. Convulsions occurred in 5 dogs, predominantly female, during the second year (control, one female; 2.5 mg/kg bw/day, one female; 15 mg/kg bw/day, one female and one male; and 30 mg/kg bw/day, one female). None of the 60 mg/kg bw/day animals exhibited this effect. EEGs with simultaneous single lead monitoring of ECGs in one female each in the 0, 2.5 and 30 mg/kg bw/day groups revealed a normal EEG for the control dog, while the other 2 dogs showed normal waking rhythms but abnormal activity during light sleep. The investigators contended that the lack of a dose-response relationship suggests a diagnosis of spontaneous epilepsy not related to exposure to fenbutatin oxide. General health and behaviour of all other dogs were similar to control dogs. Significant reductions in rate of weight gains occurred in both sexes in the 60 mg/kg bw/day group. Males receiving 30 mg/kg bw/day exhibited no significant rate of gain. This effect was not evident in females of this group. Haematological and clinical chemistry and urine data showed no differences between control and test groups. Necropsies revealed no gross changes. Microscopic examination, including frozen sections of liver and kidney stained for lipid, revealed no compound-related pathological changes in a wide range of tissues. Organ weights were not affected by exposure to the test compound. The NOAEL was 15 mg/kg bw/day (Granville & Dix, 1973).

## Long-term toxicity/carcinogenicity studies

### Mice

Groups of 48 male and 48 female Carworth Farm No. 1 strain mice were fed 50, 100, 300 or 600 ppm fenbutatin oxide in the diet for 18 months. A control

group comprised 96 males and 96 females. All animals were examined grossly at death. Microscopic examination was undertaken on all animals dying during the study and on all 600 ppm mice, on 10 mice/sex at 50, 100 and 300 ppm, and on 45 controls/sex at 18 months. Survival rate was comparable in all groups and exceeded 75%. There was a high incidence of tumours in all groups. However there was no indication of compound or dose-related tumour induction (Granville *et al.*, 1973).

## Rats

Groups of rats (114/sex/control group and 72/sex/test group) were fed 0, 50, 100, 300 or 600 ppm 97% fenbutatin oxide for two years. Rats were killed at 3 and 12 months (12/sex/control group and 6/sex/test group) and at 6 months (24/sex/control group and 12/sex/test group). Organ weights of brain, heart, liver, spleen, kidney and testes were recorded and a wide variety of tissues examined from all rats dying or killed at interim periods and from all rats from the control, 300, and 600 ppm groups. Tissues were examined from 10 animals/sex in the 50 and 100 ppm groups. Blood determinations were made at each kill period for haemo-globin, PCV, RBC, WBC and WBC differential counts; prothrombin and kaolin-cephalin coagulation times; BUN; total protein; potassium, sodium and chloride, SGPT, SGOT and alkaline phosphatase. Serum proteins were fractionated electrophoretically and estimates made of albumin and  $\alpha$ ,  $\beta$  and  $\tau$ -globulin concentrations.

Fenbutatin oxide rendered the diet unpalatable to both sexes causing, in the early months of the study, significant reductions in food consumption and body-weight gains. Males were more affected than females. For the remainder of the study all groups exhibited similar rates of gain to the control group except both sexes receiving 300 and 600 ppm, which exhibited reduced body weights throughout the study. BUN levels were increased at the higher dose levels for the first 6 months but remained within normal limits for the remainder of the study. Serum alkaline phosphatase activity was increased in both sexes at 300 and 600 ppm throughout the study, in the 100 ppm males at 6 months, and in the 100 ppm females at 3 months and 2 years. All other haematological and clinical chemistry values remained within normal limits at all times. Survival and behaviour were not affected by administration of the compound. No compound-related tissue lesions nor any increase in tumour incidences were found. Organ weights revealed no compound-related differences except kidney-weight reduction in all treated males and the 600 ppm females at 2 years. No specific compound-related lesions were seen in the kidney of any treated group. Absolute and relative testes weights, unaccompanied by hypertrophy, were noted in the 300 and 600 ppm groups at 2 years. The NOAEL was 50 ppm, equivalent to 2.5 mg/kg bw/day (Simpson *et al.*, 1973).

## Reproduction studies

### Rats

In a standard 3 generation, 2 litter/generation reproduction study, groups of 10 male and 20 female rats were fed 0, 50, 100 or 300 ppm fenbutatin oxide (98% purity). Rats of the F<sub>0</sub> generation commenced on diet and were mated at 100 days of age, and litters were culled to 10 pups/litter on post-partum day 5. Necropsy was performed on 10 male and 10 female F<sub>3b</sub> weanlings from the 0 and 300 ppm group, and on 5 weanlings/sex from the 50 and 100 ppm groups. In the 300 ppm group, parents and pups were smaller, hyperactive and irritable; mean litter size was slightly reduced in the F<sub>1b</sub> litters, and in the two F<sub>3</sub> generations survival to weaning was reduced at 300 ppm and the testicular organ/body-weight ratio was reduced in the F<sub>3b</sub> weanlings. Necropsies of F<sub>2b</sub> adults did not show any compound-related changes. No consistent effects were observed at 50 or 100 ppm with respect to fertility, gestation, viability, or lactation indices, litter size, litter weight, or necropsy of F<sub>3b</sub> weanlings (Hine *et al.*, 1973).

Technical fenbutatin oxide (99.4%) was administered continuously to Crl:CD BR rats over two generations at 0, 40, 75, 250, or 500 ppm in the diet, (equal to 2.8, 5.2, 17 or 38 mg/kg bw/day for males and 3.2, 6.0, 20 or 44 mg/kg bw/day for females). The first parental (F<sub>0</sub>) animals (30/sex) were treated beginning 72 days prior to mating through weaning of the F<sub>1</sub> offspring. Selected F<sub>1</sub> offspring (30/sex) were treated beginning 105 days before mating through weaning of the F<sub>2</sub> offspring. During the pre-mating period, body-weight, body-weight gain, and food consumption were reduced in F<sub>0</sub> and F<sub>1</sub> males and females receiving 500 ppm and females receiving 250 ppm. Body-weights of F<sub>0</sub> and F<sub>1</sub> females were also reduced during lactation at 500 ppm. Reproductive performance of F<sub>0</sub> and F<sub>1</sub> rats was unaffected. During lactation, mean body-weights of F<sub>1</sub> and F<sub>2</sub> offspring were reduced at 250 and 500 ppm. The NOAEL was 75 ppm, equal to 6.0 mg/kg bw/day, based on reduced body-weight of parental animals and offspring at 250 and 500 ppm (Bentley, 1990).

### Special studies on embryo/fetotoxicity

#### Rats

The developmental toxicity of fenbutatin oxide was studied in Wistar rats. Fenbutatin oxide (98.7% purity) was administered by oral gavage to groups of 27 mated rats at doses of 0, 15, 30, or 60 mg/kg bw/day. Controls received the vehicle, aqueous carboxymethylcellulose. Rats were treated on days 6-15 of gestation and sacrificed on day 20. The incidence of diarrhoea was increased at the high dose of 60 mg/kg bw/day. Body-weight was reduced (4-8%) at 30 and 60 mg/kg bw/day during the treatment period. Body-weight of the high-dose group remained lower than controls post-exposure (day 20). The only other finding was

a slight increase in pre-implantation loss at 60 mg/kg bw/day (1.1 per litter) compared to controls (0.6 per litter). Because treatment began around the time of implantation, the increase in pre-implantation loss may or may not have been related to treatment. The NOAEL for maternal toxicity was 15 mg/kg bw/day, based on reduced weight gain at 30 mg/kg bw/day. The NOAEL for embryo-fetal toxicity was 30 mg/kg bw/day based on increased pre-implantation loss at 60 mg/kg bw/day (Dix, 1981a).

## Rabbits

Three groups of 15 pregnant rabbits were dosed by gelatin capsule, on days 6-18 inclusive of gestation (mating day = day 0), with 3 or 10 mg fenbutatin oxide/kg bw/day, or with 37.5 mg thalidomide/kg bw/day. Twenty-six rabbits were used as capsule-only treated controls. Rabbits were killed on day 28 of pregnancy. A repeat experiment differed only in that 20 pregnant females/dose level and 30 controls were used, autopsy being on day 29. Fetuses were incubated for 24 h following removal from the uterus, after which approximately 1/3 were examined for visceral abnormalities, and 2/3 for skeletal defects. Prior to clearing for skeletal examination, viscera from these animals were also examined for defects. Although some maternal deaths occurred, they were random between groups. Mean live litter size, resorption rate, fetal loss, survival of incubated fetuses and incidence of anomalies were not affected by fenbutatin oxide at either dose level. Crown-rump length and fetal weight were comparable to controls except at 3 mg/kg bw/day, in the repeat experiment, where a significant increase was noted. The positive control group behaved as expected (Dix & Wilson, 1973).

The developmental toxicity of fenbutatin oxide was studied in New Zealand white rabbits. Fenbutatin oxide (98.7% purity) was administered orally in capsules to groups of 18-23 artificially inseminated rabbits at doses of 0, 1, 5, or 10 mg/kg bw/day. Rabbits were treated on days 6-18 of gestation and sacrificed on day 29. Maternal toxicity was observed at 5 and 10 mg/kg bw/day. The high dose was extremely toxic producing anorexia and substantial weight loss during and after the treatment period. The group receiving 5 mg/kg bw/day also experienced anorexia. Anorexia may have been the result of gastric irritation. One-third of the does treated with 5 or 10 mg/kg bw groups showed lesions in the gastric mucosa at necropsy. Twelve of 20 does (60%) receiving 10 mg/kg bw/day aborted compared to 3/11 (27%) controls. Another 5/20 (25%) at the high dose had litters with no live fetuses compared to 2/11 (18%) controls. At 5 mg/kg bw/day, 2/17 (12%) aborted and 4/17 (24%) had litters with no live offspring. Mean post-implantation loss per litter was 1.0, 1.2, 2.8, and 3.2 for the 0, 1, 5, and 10 mg/kg bw/day groups, respectively. Litter weights were reduced at the high dose. The NOAEL for maternal toxicity and embryo-fetal toxicity was 1 mg/kg bw/day based on clinical signs and gastric lesions in does and increased post-implantation loss at 5 mg/kg bw/day (Dix, 1981b).

### Special studies on genotoxicity

The results of genotoxicity studies are summarized in Table 2. Fenbutatin oxide tested negative in assays for point mutation in bacteria and mammalian cells, chromosome aberrations *in vitro* and *in vivo*, and unscheduled DNA synthesis.

### Special study on male reproduction

#### Rabbits

The effect of a single high dose of fenbutatin oxide on the reproductive system of male rabbits was investigated. The study was conducted as a follow-up to a report of a reduced spermatogenesis and increased multinucleate spermatid formation in an LD<sub>50</sub> study using doses of 1500-3000 mg/kg bw and no control group (Simpson, 1972). Male New Zealand white rabbits received a single oral dose of 100, 500, or 1500 mg/kg bw of fenbutatin oxide (98.3% purity). The control group consisted of pair fed groups and an unpaired group (i.e. fed *ad libitum*). Rabbits were sacrificed and examined 21 days after treatment. Five of 8 rabbits receiving 1500 mg/kg bw and one each from the 100 and 500 mg/kg bw groups died. Two controls paired with 1500 mg/kg bw rabbits died due to dosing errors. Fenbutatin oxide-treated groups became emaciated due to markedly reduced food intake and weight gain. Body-weight of paired controls were similarly reduced except the group receiving 500 mg/kg bw had lower weights than its paired control. Fenbutatin oxide treatment produced lesions of the gastric mucosa including ulceration, erosion, and haemorrhage. Histological examination of reproductive tissues and testes weight did not reveal any significant differences between fenbutatin oxide groups and paired and unpaired controls. This study using a control group did not show male reproductive toxicity in rabbits following acutely toxic doses of fenbutatin oxide (Dix, 1981c).

### Special studies on neurotoxicity

#### Rats

Following preliminary studies to determine suitable dose levels and to confirm the induction of brain edema by triethyltin bromide, a comparative edema assay of five organotin compounds (including fenbutatin oxide, and its metabolite) for edema formation in the central nervous system of rats was undertaken. Groups of 15 male rats were intubated with corn oil (10 mg/kg bw, 40 mg/kg bw triethyltin bromide, 1000 mg/kg bw fenbutatatin oxide, 100 mg/kg bw 1,3-dihydroxy-1,1,3,3-tetrakis (2-methyl-2-phenylpropyl)-distannoxane and sizeable doses of two other tin compounds. The triethyltin bromide group showed increased brain water content after 48 h. Seven of ten brains examined at 24 and 48 h showed spongeosis of white matter in the cerebellum and sometimes of the corpus callosum and pons. No histological changes were observed in 4 rats killed after 6 h. Fenbutatin oxide

(24 and 48 h kills), and the metabolite (6, 24 and 48 h kills) did not show either increased water content or abnormal brain histology, although rats on both compounds showed signs of intoxication (Samuels & Dix, 1972).

### Dogs

Fenbutatin oxide was reported to have no effect on the EEG pattern in dogs treated with oral doses of 30 mg/kg bw/day for 14 days. Groups of 2 male and 2 female beagle dogs were used. Each dog served as its own control. EEG was performed prior to treatment, during treatment, and 7 and 14 days after treatment. During the treatment period, the dogs exhibited occasional soft/loose faeces and tremors and experienced weight loss and reduced food intake. These symptoms resolved post-exposure. Later treatment of the same dogs with nikethamide (up to 1 g intravenously), an analeptic agent, produced paroxysmal activity (Greenough, 1991).

### Observations in humans

No data available.

### COMMENTS

Fenbutatin oxide is poorly absorbed from the gastrointestinal tract. Most (more than 90%) was excreted unchanged in faeces. Less than 1% was excreted in urine.

Fenbutatin oxide has low acute oral toxicity. The World Health Organization has classified fenbutatin oxide as unlikely to present acute hazard in normal use. It is highly irritating to the skin, lungs, and gastrointestinal tract. By the oral route, bolus administration was particularly irritating. Oral administration to dogs resulted in diarrhoea and vomiting. Following gavage administration, rabbits exhibited anorexia and developed gastric mucosal lesions. An increase in SAP in a two-year study in rats may also have been related to gastrointestinal tract injury.

A seven-day feeding study in mice gave some indication that fenbutatin oxide possessed less immunotoxicity potential than other organotin compounds. However, the data were inadequate to evaluate immunotoxicity.

In a two-generation reproduction study in rats using dietary concentrations of 0, 40, 75, 250, or 500 ppm, the NOAEL was 75 ppm, equal to 6.0 mg/kg bw/day, based on reduced weight of adults and offspring at 250 ppm. Reproductive performance was unaffected.

Table 2. Results of genotoxicity assays on fenbutatin oxide

Test System	Test object	Conc. of fenbutatin oxide	Purity	Results	Reference
Ames test (1)	<i>S. typhimurium</i> TA98, TA97, TA100, TA1535	5-300 µg/plate dissolved in acetone	98 %	Negative	Arce (1987)
	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	?	?	Negative	Moriya <i>et al.</i> (1983)
<i>E. coli</i> mutation assay (1)	<i>E. coli</i> , WP2 <i>hcr</i>	?	?	Negative	Moriya <i>et al.</i> (1983)
V79/HGPRT mutation assay (1)	V79 Chinese hamster lung cells	0.3-27 µg/ml suspended in DMSO	98.8 %	Negative	Davis (1988)
CHO/HGPRT mutation assay (1)	Chinese hamster ovary cells (CHO-K1-BH4)	0.025-7.5 µg/ml dissolved in acetone	99.4 %	Negative	Stahl (1988)
Unscheduled DNA synthesis	Male rat (CrI:CD BR) primary culture hepatocytes	0.001-50 µg/ml dissolved in acetone	99.4 %	Negative	Bentley (1988)
<i>In vitro</i> cytogenetics (1)	Human lymphocytes, health male and female volunteers	0.7-5 µg/ml dissolved in acetone	99.4 %	Negative	Vlachos (1988a)
<i>In vivo</i> cytogenetics	Male and female CrI:(ICR)BR mice, bone marrow	500-5000 mg/kg bw orally X 1	99.4 %	Negative	Vlachos (1988b)

(1) Both with and without metabolic activation.

In a teratology study in rats at doses of 0, 15, 30, or 60 mg/kg bw/day, the NOAEL for maternal toxicity was 15 mg/kg bw/day based on reduced body-weight gain at 30 mg/kg bw/day. The NOAEL for embryo-fetal toxicity was 30 mg/kg bw/day based on an increase in pre-implantation loss at 60 mg/kg bw/day. In a study in rabbits at doses of 0, 1, 5, or 10 mg/kg bw/day, the NOAEL for maternal and embryofetal toxicity was 1 mg/kg bw/day based on clinical signs of toxicity and gastric lesions in does and an increase in post-implantation loss at 5 mg/kg bw/day. No teratogenic effects were found in rats or rabbits.

After reviewing the available genotoxicity data the Meeting concluded that fenbutatin oxide was not genotoxic.

The 1977 Joint Meeting reviewed two-year studies in rats and dogs in which NOAELs of 2.5 mg/kg bw/day and 15 mg/kg bw/day, respectively, were observed. A multi-generation reproduction study in rats, in which the NOAEL was 100 ppm, equivalent to 5 mg/kg bw/day, was also reviewed at that time.

The ADI of 0-0.03 mg/kg bw previously allocated in 1977 (which was based on the NOAEL of 2.5 mg/kg bw/day (50 ppm) observed in a two-year dietary study in rats in which an increase in SAP was observed at higher doses) was retained. A lower NOAEL of 1 mg/kg bw/day from a teratology study in rabbits, in which GIT irritation was observed, was considered less reflective of human exposure because of the high sensitivity of the GIT of the rabbit and the particular physiological characteristics of this species. Therefore, this study was not used as the basis of the ADI.

## TOXICOLOGICAL EVALUATION

### Level causing no toxicological effect

Rat:	50 ppm, equivalent to 2.5 mg/kg bw/day (two-year study reviewed by JMPR in 1977)
	75 ppm, equal to 6.0 mg/kg bw/day (two-generation reproduction study)
	15 mg/kg bw/day (teratology study, maternal toxicity)
Rabbit:	1 mg/kg bw/day (teratology study, maternal and embryofetal toxicity)
Dog:	15 mg/kg bw/day (two-year study reviewed by JMPR in 1977).

### 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. Adequate information on the immunotoxic potential of fenbutatin oxide.
2. Observations in humans.

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