CLOFENTEZINE

First draft prepared by
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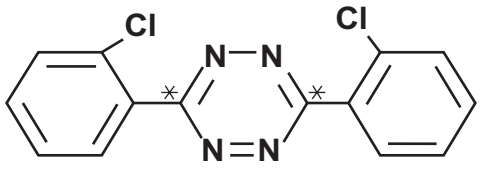
Explanation

Clofentezine is an acaricide that is used in plant protection products for the control of spider mites on a wide range of crops. It acts primarily as an ovicide, but it has some activity against early motile stages of mites. The International Union of Pure and Applied Chemistry (IUPAC) chemical name for clofentezine is 3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine (the structural formula for clofentezine is given in Figure 1). It was last evaluated by the JMPR in 1986, when an acceptable daily intake (ADI) of 0–0.02 mg/kg bw was established based on a no-observed-adverse-effect level (NOAEL) of 40 ppm (equivalent to 2 mg/kg bw per day) for hepatotoxicity in rats and a NOAEL of 50 ppm (equal to 1.72 mg/kg bw per day) for hepatotoxicity in dogs.

Clofentezine was considered by the present Meeting as part of the periodic review programme of the Codex Committee on Pesticide Residues. Some good-laboratory-practice (GLP)-compliant studies of absorption, distribution, metabolism and excretion, toxicity in dogs and effects on the rat thyroid were considered for the first time.

Figure 1. Structural formula of clofentezine



*Position of the radiolabel in the study by Campbell & Needham (1982a)

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Mice

Radiolabelled clofentezine (^{14}C in the tetrazine ring at the 3 and 6 positions) was given as a single oral dose of 10 mg/kg bw to male and female CD-1 mice. In the first 24 h, 68–93% of the administered dose had been recovered in the faeces and urine, and after 96 h, 91–95% had been recovered in excreta. Elimination of the radiolabel was mostly (about three quarters of the recovered ^{14}C) in the faeces, with the rest in urine. At 96 h after dosing, clofentezine at 0.11–0.18 ppm of equivalents was found in the liver. The concentrations in all other tissues were substantially lower than this. The principal route of excretion in the mouse is via the bile and that the main site for residues is the liver (Campbell & Needham, 1982a).

Rats

A series of studies in Sprague-Dawley rats given radiolabelled clofentezine showed that oral doses were readily absorbed from the gut lumen. Whole-body autoradiography and tissue measurements showed that the liver was the major site for the distribution of radiolabel, with high levels also being found in the kidneys. The major route for elimination was the faeces, with some excretion also occurring via the urine, especially at low doses. The radiolabel was almost completely cleared from most tissues at 96 h after treatment, but small amounts of residues persisted in the liver, fat, adrenals, and blood plasma. In different measurements, clofentezine (in unmetabolized form) was shown to have a plasma half-life of between 1.6 and 3.6 h (Campbell & Challis, 1982; Challis & Needham, 1981a, 1981b, 1981c, 1982a, 1982b, 1982c & 1983; Campbell & Needham, 1985a & 1985b; Needham, 1982).

Dermal absorption of clofentezine was low and dose-related. Mean amounts of clofentezine absorbed across the shaved back skin of male Sprague-Dawley rats over a period of 10 h were 1.0, 4.6 and 14.0 μg per rat at doses of 0.01, 0.1 and 1 mg per rat, respectively. These amounts were

respectively equal to 10%, 4.6% and 1.4% of the administered dose (Campbell & Needham, 1986; Challis, 1989)

In a study that complied with GLP, groups of Sprague-Dawley rats were given single oral doses of ^{14}C-labelled clofentezine at a dose of 10 or 1000 mg/kg bw. At 6, 24, 48, 72, 96 and 144 h after dosing, three rats of each sex per group were killed and the amounts of radiolabel in blood and tissues were measured. The half-life of total radioactive residues in the plasma was 29.1–34.1 h at 10 mg/kg bw and 40.1–46.2 h at 1000 mg/kg bw. Tissue residues were at their highest at the first time-point (6 h after treatment) and rapidly decreased over the first 24 h. Fat was the tissue containing the highest concentration of radiolabel at all time-points up to 96 h for both doses, with the concentration being especially high at 6 h after dosing (clofentezine equivalents, 177 ppm in females at 1000 mg/kg bw). Thyroid, ovaries and muscle also had high concentrations at 6 h after dosing at 1000 mg/kg bw. At 114 h, the highest concentration was in blood (1.8 ppm of clofentezine equivalents in females at 1000 mg/kg bw). At both doses, plasma, liver and kidney contained higher concentrations of residue than in other organs at all time-points up to 96 h (Needham, 1991).

In a study of tissue distribution, for which a statement of quality assurance (QA) was provided, Sprague-Dawley rats were given [^{14}C]clofentezine (radiolabelled on the tetrazine ring) at an oral dose of 20 mg/kg bw in 5% gum tragacanth twice per day for 9 days. Groups of five male and five female rats were killed at 1 or 10 days after the start of dosing. Radioactivity was measured in adrenals, heart, liver, kidneys, spleen, lung, ovaries, testes, muscle, fat, whole blood, blood plasma, eyes, brain, bone, pituitary and thyroid. The lowest group mean concentration of radioactivity at 10 days was in the brain (0.11 mg of clofentezine equivalents/kg tissue for males; 0.12 mg of clofentezine equivalents/kg tissue for females). The highest concentrations were in the liver (5.05 mg of clofentezine equivalents/kg tissue in males and 4.95 mg of clofentezine equivalents/kg tissue in females). The mean levels in thyroid were 0.95 and 1.05 mg of clofentezine equivalents/kg tissue in males and females, respectively. Concentrations of radioactivity after 10 days were 0.94 (fat of males) to 4.42 (whole blood of females) times higher than those after 1 day (Challis & Creedy, 1985).

In pregnant rats, it was shown that the placenta acts as a partial barrier to clofentezine and its metabolites. Sprague-Dawley rats were given unlabelled clofentezine at an oral dose of 3200 mg/kg bw per day on days 7–13 of gestation, unlabelled clofentezine at 320 mg/kg bw on day 14 and ^{14}C-labelled clofentezine as a single dose at 10 mg/kg bw on day 20. At 6 h after the dose of ^{14}C-labelled clofentezine, the concentrations of radiolabel in the fetuses and placenta were about five times lower than those in the maternal plasma, and lower than in most maternal tissues. Eyes, brain and spleen were exceptions to this, having similarly low levels to placenta and spleen. At 24 h after dosing, the concentrations of radiolabel in the fetuses and placenta were slightly less than in maternal plasma. These fetal and placental concentrations were similar to the levels in most maternal tissues other than liver, kidney and fat, which remained higher than in the plasma (Needham, 1981).

*Rabbit*s

Rabbits were given [^{14}C]clofentezine as a single oral dose at 10 mg/kg bw and urine and faeces were collected over the following 96 h; the animals were then killed for tissue analysis. The bulk (89.8%) of the administered dose was excreted in the first 48 h. Most of the radiolabel was excreted into the faeces (53%), but a quite large amount (35%) was in the urine. Tissue residues were highest in the liver and kidneys with mean concentrations of clofentezine equivalents of 0.24 and 0.09 mg/kg, respectively (Campbell & Needham, 1982b).

Dogs

Experiments using oral and intravenous administration of [^{14}C]clofentezine showed that the major route of elimination in the dog was the faeces. When a oral dose of 10 mg/kg bw was given, an average of 95.6% was voided within 48 h, with 94.1% of this being in the faeces. Only 1.98% of the dose was found in the urine. It was not clear whether this was owing to low absorption or high biliary excretion. A mean peak plasma residue concentration of 0.06 mg of clofentezine equivalents/l was reached at 4–8 h after dosing, and at 72 h plasma residues were undetectable. Tissue residues at 96 h after dosing were highest in the liver (0.21 mg of clofentezine equivalents/kg) and thyroid (0.16 mg of clofentezine equivalents/kg) and lowest in the bone and eye (> 0.01 mg of clofentezine equivalents/kg) (Campbell & Needham, 1982c, 1982d).

Baboons

A male and a female baboon were given [^{14}C]clofentezine as a single oral dose at 10 mg/kg bw and excretion of radioactivity was monitored over the following 96 h. About 60% of the recovered radioactivity was in the faeces and about 30% was in the urine. After 96 h, the baboons were used in a study of tissue distribution in which they were treated orally with unlabelled clofentezine at doses of up to 400 mg/kg bw, four times per day (up to 1600 mg/kg bw per day) for 56 days. On day 52 they were given [^{14}C]clofentezine at a dose of 10 mg/kg bw. On day 57, the baboons were killed for measurement of tissue residues of radiolabel. The highest concentrations were found in the adipose tissue at various sites (with tissue to plasma concentration ratios of 5.6–9.8). Liver and kidney residues were also relatively high (with tissue to plasma concentration ratios of about 4.4 and of 2.7, respectively) (Sortwell et al., 1983).

1.2 Biotransformation

In an investigation of the metabolism of clofentezine in Sprague-Dawley rats, thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) were used to analyse the metabolites in urine and faeces collected in the 24 h after a single oral dose of [^{14}C]clofentezine at 10 mg/kg bw. At least half the administered dose was found in the faeces, possibly as a result of limited absorption. In contrast, the urine contained very little unchanged clofentezine. More than 20 metabolites were separated from the faeces, most of which were minor metabolites making up less than 1% of the administered dose and none more than 5%. The urine contained more than 10 different metabolites, mostly minor. Two major pathways accounted for 70% of the radiolabel in the urine. One major pathway involved the hydroxylation of clofentezine at the 3, 4 and/or 5 positions of the phenyl ring, followed by conjugation at the 3 or 4 (or occasionally the 5) position. Both free and conjugated hydroxyl metabolites were present in urine and faeces. The other major pathway hydroxylation at the 3-phenyl position and the replacement of the chlorine atom on that phenyl ring with a methylthio group to form 3-(2'-methylthio-3'-hydroxyphenyl)-6-(2'-chlorophenyl)-1,2,4,5-tetrazine. This metabolite could then be conjugated or could appear in unconjugated form in the urine or faeces. The authors considered it likely that both the hydroxyl and the methylthio metabolites would be present in the urine and faeces in free form and as conjugates of glutathione, mercapturic acid, and cysteine (Challis & Needham, 1985).

Examination of faeces and urine from rats, mice, rabbits, dogs, calves and baboons that had been given [^{14}C]clofentezine orally showed that metabolism was qualitatively similar in all these species. In calves and baboons, the hydroxylation route was quantitatively the most important, while in rodents and rabbits the methylthiolation route was the major pathway. It was not possible to compare the urinary metabolites in dogs because of the low urinary excretion of clofentezine metabolites in this species (Challis, 1985).

The chromatographic profiles of liver extracts from rats killed at 16 h or 48 h, goats killed at 19 h, and calves killed at 12 h after oral administration of ^{14}C-labelled clofentezine were

qualitatively similar to that of rat urine. The substances identified by TLC included 3-, 4-, and 5-hydroxylated clofentezine and 3-(2'-methylthio-3'-hydroxyphenyl)-6-(2'-chlorophenyl)-1,2,4,5-tetrazine, along with their conjugates (Needham & Challis, 1985).

1.3 Effects on enzymes and other biochemical parameters

Mice

In an investigation of the effect of clofentezine on liver enzymes in the mouse, groups of 9–10 CD-1 mice were fed diets containing clofentezine at a concentration of 0, 400 or 27 000 ppm for 8 weeks. Liver weight was significantly increased ($p < 0.05$) at 27 000 ppm, but not at 400 ppm. At 27 000 ppm, clofentezine was a potent inducer of mouse hepatic microsomal mixed-function oxidases, causing three-fold increases in the activities of cytochrome P450 and cytochrome b_5, when compared with the negative control group. At 400 mg/kg (equivalent to 40 mg/kg bw per day), there was only a slight effect on these enzymes, with 20% and 6% increases in cytochrome P450 and b_5 respectively (Needham et al., 1984).

Rats

An investigation was performed into the effects of clofentezine on liver enzymes. Groups of six male Sprague-Dawley rats were given clofentezine (purity, 100.2%) at a dose of 0, 40 or 27 000 mg/kg bw per day by oral gavage for 8 weeks, after which liver enzyme activities were measured. Other groups were given 0 or 27 000 mg/kg bw per day for 10 weeks, followed by a 2-week recovery period, before measuring liver enzymes. At 40 mg/kg bw per day, there was no detectable effect on the activities of aniline hydroxylase cytochrome P_{450}, cytochrome b_5 or cytochrome P_{448} in hepatic microsomes. Dosing at 27 000 mg/kg bw per day for 8 weeks caused significant ($p < 0.05$) increases in aniline hydroxylase, cytochrome P_{450} and cytochrome b_5, but did not affect cytochrome P_{448}. Dosing at 27 000 mg/kg bw per day for 10 weeks caused significant increases ($p < 0.05$) in liver weight and in the activities of aniline hydroxylase, cytochrome P_{450} and cytochrome b_5. After the 2-week recovery period, however, the liver weight and enzyme activities had almost returned to control levels, although the activities of analine hydroxylase ($p = 0.004$) and cytochrome b_5 ($p = 0.05$) remained significantly elevated (Needham et al., 1983a and 1983b).

The effect of clofentezine on liver enzymes was studied further using groups of 10 male and 10 female Sprague-Dawley rats fed diets containing clofentezine (purity, 98.8%) at a concentration of 0, 10, 40 or 400 ppm (equivalent to 0, 1, 4 and 40 mg/kg bw per day) for 2 weeks. At 400 mg/kg bw per day, clofentezine caused significant increases ($p < 0.05$) in liver protein content, and activities of cytochrome P_{450}, aldrin epoxidase and ethoxycoumarin de-ethylase in both sexes, and cytochrome b_5 was increased in males. At 40 mg/kg bw, there was a small but significant ($p < 0.05$) increase of about 20% in the activity of ethoxycoumarin de-ethylase in males only.

The NOEL was 10 ppm (equivalent to 1 mg/kg bw per day) (Creedy et al., 1986).

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

Studies of the acute toxicity of clofentezine administered by the oral, dermal and inhalation routes are summarized in Table 1. Technical material was tested by oral and dermal routes, but a powder formulation was tested for its acute toxicity after inhalation.

Table 1. Acute toxicity of clofentezine

Species[a]	Strain	Route	Purity (%)	LD_{50} (mg/kg bw) LC_{50} (mg/l)	Reference
Mouse	CD-1	Oral	99.3	> 5200	Sharp & Harris (1986a)
Mouse	CD-1	Oral	99.1	> 3200	Crome et al. (1980a); Snowdon (1980a)
Rat	Sprague-Dawley (COBS CD)	Oral	99.3	> 5200	Sharp & Harris (1986b)
Rat	Sprague-Dawley (CFY SD)	Oral	99	> 3200	Mallyon & Sanderson (1980)
Syrian hamster	—	Oral	99.1	> 3200	Crome & Sanderson (1980); Snowdon (1980b)
Dog	Beagle	Oral	98.8 and 99.6[b]	> 2000	Chambers et al. (1981); Snowdon & Crofts (1981)
Rat	Sprague-Dawley (COBS CD)	Dermal	99.3	> 2100	Sharp & Martin (1987)
Rat	Sprague-Dawley (COBS CD)	Dermal	99.1	> 1332	Crome et al. (1980b); Snowdon (1980c)
Rat	Sprague-Dawley	Inhalation	81.1 (pre-test); 79.8 (post-test)[c]	> 0.89	Mallyon et al. (1982)

[a] Males and females were tested in all the studies.

[b] Two batches of test material were analysed.

[c] The purity of the test material was measured at the start and at the end of the study.

The acute toxicity was low by all routes in all species tested. Oral doses of up to 5200 mg/kg bw caused no treatment-related adverse effects in mice (Sharp & Harris, 1986a; Crome et al., 1980a) or rats (Sharp & Harris, 1986b; Mallyon & Sanderson, 1980). Similarly oral doses of up to 3200 mg/kg bw caused no adverse effects in hamsters (Crome & Sanderson, 1980) and up to 2000 mg/kg bw caused no treatment-related effects in dogs (Chambers et al., 1980).

Application of clofentezine as aqueous solutions at concentrations of about 300 mg/ml to the shaved intact skin of the backs of rats caused no toxicity (Sharp & Martin, 1987; Crome et al., 1980b).

Acute inhalation toxicity was investigated in dogs given nose-only exposure for 6 h to a dust containing a clofentezine-containing product (clofentezine, 9.08 mg/l) at a nominal concentration of 11.35 mg/l. Gravimetric analysis of the chamber air showed it to contain between 0.89 and 3.62 mg/l of clofentezine at various times during treatment. Particle size analyses showed the dust to have aerodynamic mass median diameters of 2 to 16 µm. The dogs were observed for 14 days after treatment and the treated animals were found to be cool to the touch and to have had pale eyes. The treatment caused no effects on mortality, body-weight gain, gross pathology or histopathology (Mallyon et al., 1982).

(b) Pharmacodynamic effects

A series of experiments were performed in various species to investigate the effects of clofentezine on the nervous, cardiovascular, respiratory and digestive systems (Morino et al., 1988).

To investigate effects on the central nervous system, groups of 10 male ddY mice or 10 male Wistar rats were given clofentezine as a single oral dose at 0, 100, 300 or 1000 mg/kg bw. Behaviour was observed continuously for the following 3 h and again after 24 h. No adverse effects were seen in the rats. The only adverse effect seen in the mice was a tendency to prone walking in one of the mice at 1000 mg/kg bw.

Effects on the autonomic nervous system were investigated in studies of the spontaneous movement of the ileum of rabbits and of guinea-pigs. Groups of three male albino Japanese rabbits were killed by exsanguination. Their ileum was resected out and hung in aerated Tryode solution containing clofentezine at a concentration of 0.001, 0.01 or 0.1 µg/ml and the muscular contractions were recorded over 10 min. No effects were seen at any dose. In a similar experiment, the resected ileum of groups of five male Hartley guinea-pigs were exposed to various contraction-inducing chemicals in the presence of clofentezine at a concentration of 0.001, 0.01 or 0.1 µg/ml. Clofentezine increased the amount of contraction caused by acetylcholine in at least some of the animals at each dose of clofentezine, but the effect was not statistically significant ($p > 0.05$). None of the doses of clofentezine affected the contractions caused by histamine or barium chloride.

In an investigation of effects on intestinal function in mice, oral doses of 100, 300 and 1000 mg/kg bw did not have any effect on the ability of the small intestine to transport charcoal.

In a test of effects on skeletal muscle function, clofentezine at a concentration of 0.001, 0.01 or 0.1 µg/ml did not affect muscle contractions caused in vitro by electrical stimulation of rat diaphragm.

In an investigation of effects on the respiratory and cardiovascular systems, groups of three male cats were given a single oral dose of 0, 100, 300 or 1000 mg/kg bw. While under anaesthesia, heart rate, blood pressure, electrocardiograph and respiratory rate were monitored. No effects were seen at any dose.

Other tests showed that oral doses of 100, 300 and 1000 mg/kg bw did not cause any effects on bleeding time in mice or on blood coagulation time in rats. Clofentezine did not cause haemolysis of rabbit blood in vitro. The haemolysis index was < 1% in all cases (Morino et al., 1988).

(c) Dermal and ocular irritation and dermal sensitization

A test for skin irritancy was performed on unformulated clofentezine using a group of six female Dunkin-Hartley guinea-pigs. Although the study appeared to be well-conducted and a statement of QA was provided, the test did not conform to the current version of OECD test guideline 404 (OECD, 2002a). Each animal received a dose of 0.2 ml of a 333 mg/ml suspension of clofentezine (purity, 99.1%) in 0.5% aqueous gum tragacanth on two absorbent lint patches applied to shaved back skin. Each animal also had an untreated patch (untreated control) and a patch containing 0.2 ml of the aqueous gum tragacanth (vehicle control) applied to its shaved back. The patches were held in place for 24 h, then removed. Observations of the areas of skin that had been covered with patches continued for 7 days after treatment, after which the animals were killed for a post-mortem examination and for histopathology on the skin. There was very slight oedema seen at the sites of 2 out of 12 treated areas. The postmortems revealed no signs of systemic toxicity, and no histopathological changes were seen in any of the samples of treated skin. The Meeting concluded that clofentezine caused negligible irritation to the skin of guinea-pigs (Crome et al., 1980c; Snowdon, 1980d).

Unformulated clofentezine (purity, 99.3%) was tested for eye irritancy in a group of six female New Zealand White rabbits. The study appeared to be well-conducted and was broadly in line with OECD test guideline 405 (OECD, 2002b) and had been performed in accordance with GLP. Seventy milligrams of clofentezine powder was placed into one eye of each animal, the other eye remaining untreated as a control. The eyes were examined at 1 h and 1, 2, 3, 4 and 7

days after instillation. A mild conjunctival reaction ("some blood vessels definitely hyperaemic (injected)") was initially seen in all treated eyes, but returned to normal within 2 days. No discharge, chemosis, corneal damage or iridial inflammation was seen. The response to treatment was interpreted as showing that clofentezine was not irritant to the eye (Liggett & Parcell, 1986).

The skin sensitization potential of unformulated clofentezine was investigated in a Magnusson & Kligman maximization test. The test appeared to be well-conducted, although the protocol did not conform to the current version of OECD test guideline 406 (OECD, 1992). A statement of QA was provided. In an attempt to induce skin sensitization, 20 female Dunkin-Hartley guinea-pigs were given intradermal injections of a saturated (0.08%) solution of technical grade clofentezine in ethanol, with and without adjuvant. This was followed by dermal application to clipped skin at the injection site of 0.5 g of clofentezine moistened with ethanol under an occlusive dressing for 48 h. The animals were challenged 14 days later on one flank by dermal application of 0.5 g of clofentezine moistened with ethanol and on the other flank they were challenged with a 50% w/v suspension of clofentezine in ethanol under an occlusive dressing for 24 h. Control animals were treated similarly except that the clofentezine was applied only at the challenge stage. Only 1 out of 20 test animals reacted when challenged with the moistened clofentezine, but none of the test animals reacted at the challenge site where the 50% solution of clofentezine was applied and none of the control animals reacted at all. The animal that reacted showed scattered mild redness at 24 h after the removal of the challenge patch, but this had disappeared by 48 h. The response in this test was classified by the authors as "Grade I: non-allergenic/weak" (Teale, 1982).

(d) Studies of toxicity after repeated doses

Mice

In a 90-day study of toxicity, groups of 20 male and 20 female CD-1 mice were fed diets containing clofentezine (purity, 99.0%) at a concentration of 0, 200, 1000 or 5000 ppm. The study appeared to be well-conducted and a QA statement was provided. Satellite groups of 10 males and 10 females were also maintained on each diet and blood samples were taken at 4 and 12 weeks for clinical chemistry and haematology.

The treatments caused no effects on mortality, body-weight gain, food consumption, clinical signs, clinical chemistry or haematology. Red stellar crystals were detected in the urine of males at the highest dose and in both sexes there was a dose-related colour change in the urine that may have been due to excretion of the (magenta-coloured) clofentezine in urine. Otherwise, no treatment-related effects were revealed by urine analysis. Examinations post mortem revealed no treatment-related gross pathology, but the relative weights of liver (in both sexes) and relative and absolute weights of thyroid (in females only) were increased in mice at doses of 1000 or 5000 ppm. Histopathology showed centilobular hepatocyte enlargement in males at the highest dose, but revealed no other lesions.

The NOAEL for this study was 200 ppm (equal to 30.3 mg/kg bw per day) on the basis of effects on organ weights (liver and thyroid) at higher doses (Hounsell et al., 1982).

Rats

In a 90-day study of toxicity, groups of 20 male and 20 female Sprague-Dawley rats were fed diets containing clofentezine (purity, about 100%) at a concentration of 0, 3000, 9000 or 27 000 mg/kg (equivalent to 0, 300, 900 and 2700 mg/kg bw per day). A statement of QA was provided. After 64 days of treatment, three to five rats of each sex from each group were killed for interim histopathological examination; 10 animals of each sex from each group were killed at the end of the 90-day treatment period; and three to five rats animals per sex per group were killed after a further 4-week recovery period.

At all doses, there were reduced intakes of feed and water, reduced body-weight gain, hair loss, reduced blood haemoglobin, increased plasma concentrations of cholesterol, triglycerides and protein, increased liver weight and centrilobular hepatocyte enlargement. The effects on the liver were not evident in the rats given a recovery period, indicating that the hepatotoxicity was reversible. A NOAEL was not identified (Ginocchio & Brooks, 1982).

In a 90-day study of toxicity, groups of 25 male and 25 female Sprague-Dawley rats were fed diets containing clofentezine (purity, > 99.1%) at a concentration of 0, 40, 400 or 4000 ppm. The study appeared to be well-conducted and a statement of QA was provided. Urine was taken for urine analysis and blood samples were taken for haematology and clinical chemistry at weeks 4, 8 and 12 of treatment and at the end of the recovery period. Most of the rats were killed at the end of the treatment period, but five animals of each sex from each group were maintained on a standard clofentezine-free diet for a recovery period of 6 weeks.

There was no treatment-related effect on mortality or on clinical signs. Towards the end of the treatment period, the mean body weight of females at the highest dose was significantly less ($p < 0.05$) than that of concurrent controls. Feed intakes of this group also tended to be relatively low with group mean food conversion being significantly less than that of controls at several times during the study. Intake of drinking water was unaffected and urine analysis revealed no treatment-related effects. Mean values for blood haemoglobin were slightly but significantly reduced ($p < 0.05$) in both sexes of the groups at 400 or 4000 ppm, in comparison with control values, at various occasions throughout the treatment period. No effect on haemoglobin was seen at the end of the recovery period. There were persistent and statistically significant increases ($p < 0.05$) in concentrations of total serum protein and serum albumin in both sexes at the intermediate and highest dose, and there was also an increase in these parameters in males at the lowest dose at week 8 only. Plasma cholesterol was raised in a dose-related manner throughout the study and the increases were significant ($p < 0.05$) in males at the highest dose and in females at the intermediate and highest dose. In the animals killed at the end of the treatment period, liver weight was increased in a dose-related and statistically significant ($p < 0.05$) manner in both sexes at the intermediate and highest dose. Less marked increases in kidney and spleen weights were also seen in these groups. Histological examination showed centrilobular hepatocyte enlargement in males at the intermediate and highest doses and in females at the highest dose. Effects on organ weights and liver histopathology were not seen in the animals that were killed at the end of the recovery period.

The NOAEL was 40 ppm, equal to 2.65 mg/kg bw per day in males and 2.96 mg/kg bw per day in females. The slight decrease in total protein and albumin in serum of the males at 40 ppm at only one time during the study was not regarded as an adverse effect (Ginocchio & Brooks, 1981; Brooks & Turnbull, 1983).

Dogs

In a 90-day study of toxicity, groups of four male and four female beagle dogs were fed diets containing clofentezine (purity, 99.7%) at a concentration of 0, 3200, 8000 or 20 000 ppm. The study appeared to be well-conducted and a statement of QA was provided. Samples of urine and blood were taken on two occasions before treatment and at intervals of approximately 30 days throughout the treatment period. Ophthalmology and electrocardiograms were performed during the final week of treatment.

There were no treatment-related effects on mortality, clinical appearance, haematology, ophthalmology or electrocardiography. Clinical chemistry showed a dose-related increase in serum alkaline phosphatase activity in female dogs (but not in males), which was statistically significant ($p < 0.05$) on days 30 and 86 of treatment (but not on day 58). Liver weight was increased in males at the lowest dose and in females at the intermediate and highest doses. No treatment-related histopathology was seen, even in animals with elevated alkaline phosphatase activity or liver weight.

A NOEL was not identified as there were treatment-related effects that might be associated with hepatotoxicity at all doses used in the study. The feed intake data from various time-points showed that the lowest dietary concentration given in the study (3200 ppm) supplied doses of 60 to 213 mg/kg bw per day to individual animals in this group (Hounsell et al., 1981).

In a 12-month study, groups of six male and six female beagle dogs were given diets containing clofentezine (purity, 98.2%) at a concentration of 0, 50, 1000 or 20 000 ppm. The study appeared to be well-conducted and a certificate was provided indicating compliance with GLP. Ophthalmoscopy was performed before treatment and in weeks 26 and 51 of treatment. Blood and urine samples were taken before treatment and during weeks 4, 8, 12, 26 and 51. Blood pressure and electrocardiography measurements were taken before treatment and in weeks 12, 24 and 50.

No unscheduled deaths occurred during the study, and no clinical signs of toxicity were recorded. The mean body-weight gains of both sexes at the highest dose were significantly ($p < 0.05$) less than concurrent control values over the first 4 weeks of treatment, but body-weight gain were similar for all groups after this. Intakes of water and feed were unaffected. There were no treatment-related effects on urine analysis, ophthalmoscopy, electrocardiography and blood pressure. In haematology, there were slight but statistically significant differences ($p < 0.05$) from the concurrent control values for various parameters related to erythrocytes at various times during the study, but the changes were inconsistent and individual values remained within the normal range. Bone-marrow smears showed no treatment-related abnormalities. From week 8 onwards, the serum alkaline phosphatase activity for male and female dogs at the highest dose was consistently greater than concurrent control values, with the difference being statistically significant ($p < 0.05$) for males at weeks 8 and 12. Serum cholesterol concentration was higher than controls throughout the study in males and females at the intermediate and highest doses, with the differences being statistically significant ($p < 0.05$) at most times for the group at the highest dose and at week 51 for the group at the intermediate dose. Significant ($p < 0.05$) increases in serum triglyceride concentration were also found in both sexes at the intermediate and highest dose at various times throughout the study. Autopsy showed that one male dog at the highest dose had a granular texture to the surface of its liver. There was a dose-related increase in mean liver weight which was statistically significant ($p < 0.01$) in females at the intermediate and highest dose and in males at the highest dose. There were also significant increases ($p < 0.05$) in the mean weights of adrenals in males at the highest dose and of the thyroid in females at the highest dose. Microscopic examination revealed hepatocellular enlargement with cytoplasmic eosinophilia in the periportal region of the liver in four out of six males (including the dog showing gross change to the liver) and four out of six females. Cytoplasmic eosinophilia of the periportal hepatocytes, in the absence of hyperplasia, was seen in two females at the intermediate dose and one female at the highest dose.

The NOEL was 50 ppm (equal to 1.72 mg/kg bw per day). Indications of hepatotoxicity were seen at doses of 1000 ppm (36.0 mg/kg bw per day) or more (Chesterman et al., 1984; Harling, 1988).

2.2 Long-term studies of toxicity and carcinogenicity

Mice

In a study of carcinogenicity, groups of 52 male and 53 female CD-1 mice were fed diets containing clofentezine (purity, 98.7%) at a concentration of 0, 50, 500 or 5000 ppm for 105 weeks. These dietary concentrations provided mean doses of 0, 5.0, 50.7 and 543.4 mg/kg bw per day for males and 0, 5.3, 56.9 and 557.1 mg/kg bw per day for females. The study was conducted in accordance with OECD test guideline 451 (OECD, 1981a). A certificate was provided, indicating compliance with GLP. Blood samples were collected during weeks 52 and 104 for haematological examination. All surviving animals were killed at the end of the treatment period.

Postmortem examinations and comprehensive histopathology were performed on all animals killed on schedule at the end of the study plus those which died prematurely or had to be euthanized.

Mortality was high in the study, ranging from 52% in females in the control group to 81% in females at the highest dose. Increased mortality (statistically significant at $p < 0.01$), was seen in females at the highest dose, as compared with controls. The authors attributed this to amyloidosis. There was also a slight but not statistically significant ($p > 0.05$) increase in the mortality of males at the highest dose, which was attributed to liver tumours. No clinical signs of toxicity were seen in any group. Body-weight gain was lower ($p < 0.05$) in males at the highest dose than in controls during the first year of treatment, but was similar to concurrent control values in the second year for this group and at all times for other groups. Food consumption was not affected by the treatment. Haematological examination of the blood samples taken in week 52 showed several statistically significant effects ($p < 0.05$): decreased erythrocyte and platelet counts in males at the highest dose; and increased mean corpuscular volume in females at the intermediate and highest dose. Total leukocyte counts were also decreased in males at the highest dose, but not significantly ($p > 0.05$). Blood samples taken at week 104 showed significantly decreased ($p < 0.05$) total leukocyte counts in males at the highest dose and lymphocyte counts in all groups of treated males (not dose-related).

Postmortem examinations showed higher incidences of liver masses in both sexes of all treatment groups in mice that died before the end of the study, but this effect was not seen in the mice that were killed on schedule at the end of the study and was not evident when all mice were considered. Liver weights were increased in both sexes at the highest dose, and the increase was statistically significant ($p < 0.05$) in females. There were also significant ($p < 0.05$) increases in heart weights in females at the highest dose and testes weights in the group at the highest dose. Histopathology revealed a slightly increased incidence in all treated groups of amyloidosis in the females that died prematurely or had to be euthanized, but the effect was not seen in males or in females that were killed on schedule. There was a dose-related increase in the incidence of foci of eosinophilic hepatocytes in the livers of all groups of males and females at 500 ppm or more. In some mice the eosinophilic hepatocytes were also vacuolated. No statistical analysis of the incidences of these non-neoplastic liver lesions was reported.

The incidences of liver cell tumours are summarized in Table 2. There were statistically significant increases in the incidences of liver cell tumours (benign plus malignant) in males at 500 ppm (incidence of 63%: $p < 0.01$) or 5000 ppm (48%: $p < 0.05$) and females at 5000 ppm (13%: $p < 0.05$), but there was a positive dose-related trend only in females ($p < 0.001$). There was no statistically significant effect on the incidences of benign and malignant liver tumours in any group when the results were analysed separately, but there was a positive, dose-related trend for benign tumours in females. The incidences of benign and malignant liver tumours in both sexes in all groups, including controls, were greater than the incidences found in historical control data (22–34% in males and 0–9% in females) from six earlier studies of carcinogenicity in mice.

Table 2. Incidence of liver-cell tumours in mice fed diets containing clofentezine

No. of mice with liver-cell tumours	Dietary concentration (ppm)							
	Males				Females			
	0	50	500	5000	0	50	500	5000
Benign only	6	7	13	8	4	3	3	7
Malignant only	14	16	24	19	0	0	0	1
Benign and/or malignant	19	20	33	25	4	3	3	7
No. of mice examined	52	52	52	52	52	52	52	52

From Lloyd et al. (1985) and Cherry et al. (1985)

Comparative analysis of the liver cell tumour data and the historical control data provided no clear evidence that treatment with clofentezine caused mouse liver tumours. The treatment had no effect on the incidences of tumours at any other site.

The significant decrease in lymphocyte counts at week 104 in blood samples from males at 50 ppm was not regarded as a treatment-related effect, as there was no dose–response relationship and no effects on other populations of leukocytes or on the total leukocyte counts for these animals. The toxicological significance of the eosinophilic foci in the livers of males in the group receiving the lowest dose is unclear (Lloyd et al., 1985; Cherry et al., 1985).

Rats

A combined long-term study of toxicity/carcinogenicity was performed in Sprague-Dawley rats. The study appeared to be well-conducted in accordance with OECD test guideline 453 (OECD, 1981b). A statement of QA was provided. Groups of rats were fed diets containing clofentezine (technical grade of unspecified purity) at a concentration of 0, 10, 40 or 400 ppm. These dietary concentrations provided mean doses of 0, 0.43, 1.72, and 17.3 mg/kg bw per day for males and 0, 0.55, 2.18 and 22.1 mg/kg bw per day for females. Supplementary groups of 20 rats of each sex from each group were killed after 12 months of treatment, while the rats comprising the main groups (50 of each sex per group) were killed after a total of 27 months (118 weeks) of treatment. Blood and urine samples were taken from the supplementary groups of rats after 6 and 12 months of treatment, and blood and urine were taken from the main group rats at 18 and 27 months. Assays for thyroid function (total triiodothyronine (T3), free T3, total thyroxine (T4), free T4, T4-binding capacity, free T4 index and thyrotropin) and levels of sex hormones (testosterone, estradiol and progesterone) were performed on the blood samples taken at 27 months. After 12 and 26 months, ophthalmological examinations were performed on the rats of the main groups. Postmortem examinations and comprehensive histopathology were performed on all animals.

There were no treatment-related effects on mortality, clinical signs, body-weight gain or consumption of feed and water. There were no treatment-related effects on haematology apart from slight statistically significant decreases ($p < 0.05$) in haemoglobin and in mean cell haemoglobin concentration in females at the highest dose at 18 and 27 months. Routine clinical chemistry parameters were unaffected by treatment with clofentezine. However, free T4 was significantly increased ($p < 0.01$) in males at the highest dose and T4 binding capacity was significantly increased in males at the intermediate dose ($p < 0.05$). Other hormone levels and thyroid function parameters were unaffected by the treatment. Urine analysis and ophthalmoscopy revealed no treatment-related effects. Relative liver weight was significantly increased ($p < 0.05$) in both sexes receiving the highest dose at the interim kill (12 months) and at the final kill (27 months); absolute liver weight was also increased ($p < 0.05$) in females at the highest dose and in all groups of males at the interim kill only. Histopathology showed an increased incidence of liver cell hypertrophy, characterized by swelling of centrilobular hepatocytes with fatty vacuoles, in the animals at the highest dose, especially the males, at the interim and final kills. There was a positive dose-related trend for this parameter in males ($p < 0.001$) and females ($p < 0.05$).

There was a significantly lower number of cutaneous and subcutaneous tissue masses at interim death for females at the highest dose ($p < 0.05$). This was reflected in a decreased incidence ($p < 0.05$) of mammary tumours in the females at the highest dose. There were also decreased numbers of animals having tumours at multiple sites in females at the highest dose at the interim kill ($p < 0.01$) and at the final kill ($p < 0.05$).

Various histopathological lesions were detected in the thyroids. Initial examination of the thyroid histopathology indicated that there was an increased incidence ($p < 0.05$) of agglomeration of colloid in the thyroids of final kill in males at the highest dose, with a positive dose-related trend in males at interim and final kill. There were also positive dose-related trends ($p < 0.05$) in the incidences of malignant thyroid follicular cell tumours, all thyroid follicular cell tumours and of thyroid hyperplasia in males at final kill, which was attributed to increased incidences of these lesions (not statistically significant: $p > 0.05$) in the males at the highest dose.

In a second examination of the thyroid histopathology by a different pathologist similar conclusions were reported (summarized in Table 3). In the males at the highest dose at interim kill and in the males at the intermediate and highest doses at final kill there was increased incidence of agglomeration of colloids in the thyroid. There was also a dose-related trend ($p < 0.05$) trend to reduction in the luminal size and in the degree of eosinophilia of the colloid in males, females and both sexes combined, although there were no significant differences from control values at any dose for either sex. The incidence of follicular cystic hyperplasia was increased in males receiving the intermediate and highest dose at the interim and final kills and also in decedent males receiving the highest dose and females at the highest dose at final kill. The increase was significant ($p < 0.05$) at the highest dose for both sexes combined and there were positive dose-related trends ($p < 0.05$) for males, females and both sexes combined. The incidences of thyroid adenomas and of adenocarcinomas were increased in males receiving the highest dose at final kill.

The Meeting considered that the increased incidences of thyroid tumours (adenomas and adenocarcinomas), when considered together with genotoxicity and the effects on thyroid hyperplasia, agglomeration of colloid and thyroid hormones, indicated that the prolonged treatment of rats with high dietary concentrations of clofentezine could produce thyroid tumours by a non-genotoxic hormone-mediated mode of action for which the mechanism was not entirely clear.

The Meeting considered that the NOAEL was 40 ppm (1.72 mg/kg bw per day) on the basis of effects on the thyroids at 400 ppm (17.3 and 22.1 mg/kg bw per day in males and females respectively) (Ginocchio & Mallyon, 1985; Saunders & Mallyon, 1986).

2.3 Genotoxicity

The results of tests for genotoxicity with clofentezine are summarized in Table 4. The results of all the tests were negative. An appropriate range of end-points was investigated, including gene mutation, clastogenicity, mutagenicity in vivo and germ-cell mutation.

The tests were well-performed by the standards at the time they were conducted. However, the protocols of some of the tests did not conform to the current recommendations of OECD test guidelines. In the assay for reverse mutation in bacteria (McConville, 1980), *Salmonella typhimurium* strain TA1538 was used, while guideline 471 (OECD, 1997a) recommends the use of *Escherichia coli* strain WP2 *uvra* or strain WP2 *uvrA* (pKM101) or *S. typhimurium* strain TA102. No independent repeat of this assay was performed to confirm the negative result. In the test for chromosome aberration in vitro (Allen, Brooker & Godfrey, 1987), the cells were incubated with the test material in the presence and absence of metabolic activation for 17 h, while guideline 473 (OECD, 1997b) recommends treatment in the presence and absence of metabolic activation for 3–6 h followed by a separate experiment using incubation in the absence of metabolic activation for about 1.5 cell cycles. In one test for micronucleus formation (Hounsell & Walker, 1982), bone marrow was harvested at only 6 h after the final dose, while guideline 474 (OECD, 1997c) recommends the use of two harvest times, at 18–24 h and 36–48 h after the last treatment. Certificates of compliance with GLP were provided for the more recent studies (after 1983) and QA certificates were provided for the older studies (1983 or earlier).

The Meeting concluded that clofentezine is unlikely to be genotoxic.

Table 3. Incidences of thyroid lesions (second examination) in mice fed diets containing clofentezine

Lesion	Dietary concentration (ppm)			
	0	10	40	400
Males at interim kill				
Slight to severe agglomeration of colloid	40% (8/20)	30% (6/20)	30% (6/20)	89% (17/19)
Follicular cystic hyperplasia	0% (0/20)	0% (0/20)	11% (2/18)	6% (1/18)
Follicular cell adenoma	0% (0/20)	5% (1/19)	0% (0/20)	0% (0/19)
Follicular cell adenocarcinoma	0% (0/20)	0% (0/19)	0% (0/20)	0% (0/19)
Females at interim kill				
Slight to severe agglomeration of colloid	5% (1/20)	10% (2/20)	5% (1/20)	15% (3/20)
Follicular cystic hyperplasia	0% (0/19)	0% (0/18)	0% (0/19)	0% (0/17)
Follicular cell adenoma	0% (0/19)	0% (0/18)	0% (0/19)	0% (0/17)
Follicular cell adenocarcinoma	0% (0/19)	0% (0/18)	0% (0/19)	0% (0/17)
Decedent males				
Slight to severe agglomeration of colloid	46% (12/26)	22% (5/23)	25% (5/20)	50% (13/26)
Follicular cystic hyperplasia	8% (2/26)	0% (0/23)	0% (0/21)	11% (3/28)
Follicular cell adenoma	0% (0/26)	0% (0/26)	0% (0/22)	0% (0/28)
Follicular cell adenocarcinoma	8% (2/26)	0% (0/26)	5% (1/22)	0% (0/28)
Decedent females				
Slight to severe agglomeration of colloid	17% (5/30)	14% (4/28)	3% (1/29)	15% (4/26)
Follicular cystic hyperplasia	7% (2/30)	0% (0/29)	7% (2/29)	0% (0/26)
Follicular cell adenoma	0% (0/30)	0% (0/29)	0% (0/29)	0% (0/26)
Follicular cell adenocarcinoma	3% (1/30)	0% (0/30)	7% (2/29)	4% (1/26)
Males at final kill				
Slight to severe agglomeration of colloid	36% (10/28)	50% (12/24)	73% (19/27)	67% (14/21)
Follicular cystic hyperplasia	4%	8%	26%	14%

Lesion	Dietary concentration (ppm)			
	0	10	40	400
Follicular cystic hyperplasia cont.	(1/24)	(2/24)	(7/27)	(3/21)
Follicular cell adenoma	0% (0/24)	4% (1/24)	0% (0/27)	14% (3/21)
Follicular cell adenocarcinoma	0% (0/24)	4% (1/24)	4% (1/27)	24% (5/21)
Females at final kill				
Slight to severe agglomeration of colloid	16% (3/19)	16% (3/19)	22% (4/18)	22% (5/23)
Follicular cystic hyperplasia	5% (1/19)	0% (0/19)	0% (0/20)	13% (3/23)
Follicular cell adenoma	0% (0/19)	5% (1/19)	0% (0/20)	0% (0/23)
Follicular cell adenocarcinoma	0% (0/19)	0% (0/19)	0% (0/19)	0% (0/23)

From Ginocchio & Mallyon (1985) and Saunders & Mallyon (1986)

2.4 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation study, groups of Sprague-Dawley rats received diets containing clofentezine (purity, 97.9–99.3%) at a concentration of 0, 4, 40 or 400 ppm. There were 30 males and 30 females in the groups at 0, 4 and 40 ppm and 40 rats of each sex in the group at 400 ppm. Treatment of the F_0 generation started at 14 days after weaning. After 74 days of treatment, the rats of the F_0 generation were paired within their groups for 20 days. After weaning the F_{1a} generation pups, the F_0 generation animals were mated once again to produce the F_{1b} generation pups, which were killed after weaning. Groups of 25 males and 25 females were selected from the F_{1a} generation pups and they were mated at 88 days after weaning to produce the F_{2a} generation.

The F_{1a} generation rats were mated again after they had weaned the F_{2a} pups, resulting in the birth of the F_{2b} generation pups. Groups of 20 male and 20 female animals were selected as the F_{2a} and F_{2b} groups, which were given the same treatments as previous generations for 82 to 84 days after weaning, at the end of which they were killed. Surplus F_{2a} pups were weighed necropsied, with organ weights being measured. Parental animals were monitored for clinical signs, behavioural changes, food consumption and body weight. Litters were examined for clinical signs, litter size, pup mortality, sex ratio and pup weight at birth and at weaning. The development of the pups was monitored by recording the times of pinna development, tooth eruption and eye opening. Postmortems and histopathology of all major organs were performed on 10 males and 10 females from each of the F_0, F_{1a} and F_{2a} groups of adults. In addition to this, the remaining F_0 animals were subjected to postmortem examination and histopathology was performed on a limited selection of organs (reproductive organs, liver and organs showing gross lesions).

Table 4. Results of studies of genotoxicity with clofentezine

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation	S. typhimurium TA1535, TA1537, TA98, TA100 and TA1538	10–3300 µg/plate in DMSO ±S9[a]	Technical-grade material	Negative	McConville (1980)
DNA damage (rec-assay)	Bacillus subtilis H17 Rec+ and M45 Rec-	156–2500 µg/disc in distilled water –S9b; 156–1250 µg/disc in distilled water +S9[b]	Technical-grade material)	Negative	Inoue & Nakajima (1986)
Mitotic recombination and gene conversion	Saccharomyces cerevisiae D-7	12.5–200 µg/ml ±S9[c]	98.4 (technical-grade material)	Negative	Riach & McGregor (1983)
Forward gene mutation	L5178Y $Tk^{+/-}$-mouse lymphoma cells	15–128 µg/ml –S9[a] and 2–128 +S9[a]	98.4	Negative	Bootman & Rees (1982)
Chromosomal aberration (metaphase analysis)	CHO (K1-BH4) cells	0.4–4 µg/ml ±S9[a]	99.6 (technical-grade material)	Negative	Allen, Brooker & Godfrey (1987)
In vivo					
Micronucleus formation	Bone-marrow polychromatic erythrocytes of male and female CD-1 mice	Single oral dose at 8000 mg/kg bw in aqueous carboxymethyl cellulose	99.6 (technical-grade material)	Negative	Allen, Pugh & Proudlock (1987)
Micronucleus formation	Bone-marrow polychromatic erythrocytes of male CD-1 mice	Two daily oral doses at 800–3200 mg/kg bw in aqueous gum tragacanth	99.6 (technical-grade material)	Negative	Hounsell & Walker (1982)
Dominant lethal mutation	Germ cells of male Sprague-Dawley rats	Dietary concentrations of 4–400 ppm (equivalent to 0.04–4 mg/kg bw per day)	≥ 98.1 (technical-grade material)	Negative	Jackson (1983)

[a] S9, 9000 × *g* supernatant from livers of male Sprague-Dawley rats pretreated by intraperitoneal injection with Aroclor 1254

[b] S9, 9000 × *g* supernatant from livers of male Sprague-Dawley rats pretreated by intraperitoneal injection with phenobarbitone and 5,6-benzoflavone

[c] S9, 9000 × *g* supernatant from livers of male Fisher 344 rats pretreated by intraperitoneal injection with Aroclor 1254

The conduct of the study was in line with contemporary requirements set out in OECD test guideline 416 (OECD, 1983), but it did not investigate all the parameters recommended in the updated OECD guideline 416 (OECD, 2001a). A statement of QA was provided.

An increased incidence of centrilobular hepatocellular enlargement was observed in adult F_{1a} males at the highest dose. No other treatment-related pathological effects on the reproductive or other organs were seen. Liver weights for the F_1 females were lower than those of controls at all doses, but this was unlikely to be a treatment-related effect as there was no dose–response relationship and there was an unusually high control value. Sporadic differences from control values for the weights of various other organs (kidneys, heart and ovaries) were not associated with any gross pathology or histopathology. The treatment had no effect on pup weight at birth, but the pup body-weight gains for the F_{2a} generation were low in the groups at 40 or 400 ppm in

the first few weeks of life (Table 5). The mean weights of the surplus F_{2a} pups that were killed after weaning were significantly decreased ($p < 0.05$) in males at 40 ppm and in both sexes given 400 ppm (Table 6). There was no effect on the pup weights at weaning for the F_{1a}, F_{1b} or F_{2b} generations. The pup weights were low at the end of the first week after weaning in the 40 and 400 ppm groups of the F_2 males and the F_1 males at 400 ppm. Low body weights, as compared to controls, were maintained in highest dose group male and female F_2 animals during the weeks after weaning (statistically significant in post-weaning weeks 1–6 in males and 2–3 in females). There were no effects seen on any of the other indicators of reproductive toxicity that were measured in this study.

(b) Developmental toxicity

Rats

Groups of 34–35 pregnant Sprague-Dawley rats were given clofentezine at daily doses of 0, 320, 1280 or 3200 mg/kg bw by oral gavage on days 7–21 post-coitum in an aqueous solution of carboxymethylcellulose at 50 mg/l. The clofentezine had been supplied in crystalline form and was claimed to be 100% pure, although no analysis was performed to confirm this. All of the pregnant rats were killed on day 21 post-coitum. The contents of their uteri were examined and their livers were weighed and examined microscopically. Half the fetuses from each litter were examined for skeletal abnormalities; the rest were examined for visceral abnormalities. The study was in line with the current requirements of OECD guideline 414 (OECD, 2001b) and a statement of QA was provided.

Pregnant rats at the highest dose had a lower rate of body-weight gain than did the controls. There was a dose-related increase in liver weight at the intermediate and highest doses. At the highest dose there was an increased incidence of a histological change to the liver, which was characterized by enlargement of the centrilobular hepatocytes with altered staining properties x

Table 5. Effect on mean weights of F_{2a} pups (both sexes combined)

Dietary concentration (ppm)	Postnatal day				
	1	4	10	14	21
0	5.5	7.4	14.3	21.4	34.0
4	5.5	7.3	14.2	20.3	32.0
40	5.4	7.2	14.0	19.9	30.2*
400	5.4	6.9	13.0*	18.3**	28.1**

From Jackson & Chambers (1984) and Jackson & Turnbull (1986)

* Statistically significantly different ($p < 0.05$) from controls

** Statistically significantly different ($p < 0.01$) from controls

Table 6. Effect on body weight in weanling F_{2a} animals

Dietary concentration (ppm)	Mean body weight (g)	
	Males	Females
0	31	30
4	33	31
40	28*	28
400	28*	27*

From Jackson & Chambers (1984) and Jackson & Turnbull (1986)

* Statistically significantly different ($p < 0.05$) from controls

(described as "slight differential staining"). Mean fetal weight was increased at the highest dose, but there were no treatment-related effects on litter size, litter weight, placental weight or postimplantation loss. There was also no treatment-related effect on the incidence of any skeletal or visceral abnormality.

The NOAEL was 320 mg/kg bw per day on the basis of maternal toxicity. There was no evidence of any effects on the development of the embryo or fetus, even at maternally toxic doses (Jackson, 1982).

Rabbits

Groups of 14 or 15 pregnant New Zealand White rabbits were given technical grade clofentezine (purity, 98.5%) at a dose of 0, 250, 1000 or 3000 mg/kg bw per day by oral gavage in aqueous solution of sodium carboxymethylcellulose on days 7 to 28 of gestation. The pregnant rabbits were killed on day 29 of gestation and the uterine contents were examined. All fetuses were examined externally, dissected to look for visceral abnormalities and then cleared and stained for skeletal examination. The conduct of the study was in line with the current requirements of OECD guideline 414 (OECD, 2001b). A statement of QA was provided.

Body-weight gain was retarded in does at the intermediate and highest dose, and feed intake was decreased at the highest dose. One doe at the highest dose was euthenased after showing anorexia and body-weight loss. Mean fetal body weight was lower than control values at the highest dose. Pregnancy rate, litter size, preimplantation loss and postimplantation loss were not affected by the treatment. At all doses, gross, visceral and skeletal abnormalities were comparable with control values.

The NOAEL was 250 mg/kg bw per day on the basis of maternal toxicity (impaired body-weight gain). The low fetal body weight at the highest dose may have been secondary to the maternal toxicity and reduced feed intake of the does. There was no evidence of embryotoxicity or teratogenicity, even at maternally toxic doses (Cozens et al., 1983).

2.5 Special studies: thyroid function

Rodents

A study of tissue distribution in rats showed that [^{14}C]clofentezine was distributed to a wide range of tissues, including the thyroid. Details of the study are given in section 1.1. The amount going to the thyroid was unremarkable. There was no preferential accumulation of the radiolabel in the thyroid (Challis & Creedy, 1985).

The effect of a high dose of clofentezine on T4 clearance was investigated in male Sprague-Dawley rats. Two groups of 10 rats were given known amounts of [^{125}I]thyroxine intravenously and the amount of radioactivity in the blood was measured at various time-points in order to calculate the blood half-life of thyroxine before treatment. The groups were then fed diets containing clofentezine at a concentration of 0 or 30 000 ppm for 4 weeks. At the end of this treatment period the rats were again given [^{125}I]thyroxine intravenously for the estimation of the blood half-life of thyroxine after treatment. Before treatment, the mean thyroxine half-lives of control and treated groups were 16.70 ± 1.09 h and 17.05 ± 1.08 h. After treatment the half-lives were 17.61 ± 1.62 h and 16.42 ± 1.44 h, respectively. Thus there had been a slight increase in the blood half-life of thyroxine in controls and a slight decrease in the half-life in treated rats. However, the differences between control and treated groups were too small to be statistically significant ($p > 0.05$) (Challis & Creedy, 1985).

The effect of a high dose of clofentezine on the uptake of iodine by the thyroid was investigated in Sprague-Dawley rats and CD-1 mice. For each species, groups of 20 males and 10 females were maintained on diets containing clofentezine at either 0 or 30 000 ppm for 4 weeks.

After this time, they were dosed intraperitoneally with ^{131}I-labelled sodium iodide. At 6 h and 24 h after the iodide injection, 10 animals of each sex were anaesthetized with ether and exsanguinated. The thyroid glands were removed. Radioiodine concentrations were measured in samples of blood and thyroid. The results of the study (Table 7) show that there was a clear increase in the uptake of iodine from the blood and into the thyroid in rats; a similar but less marked effect was seen in mice.

In a study of effects on the thyroid, groups of 10 male and 10 female Sprague-Dawley rats were fed diets containing technical clofentezine (purity not stated) at a concentration of 0, 400 or 30 000 ppm for 6 weeks. At the end of the treatment period, the animals were all exsanguinated and killed. Blood samples were examined for total T3, T4, T4-binding capacity, free T4 index, thyrotropin (TSH), testosterone, estradiol, progesterone and dehydroepiandrosterone sulfate. Postmortem examinations were performed and livers were weighed. Pituitary and thyroid glands of all groups were examined by electron microscopy and the thyroids of the females in the control group and at the highest dose were examined morphometrically by light microscopy.

A statement of QA was supplied with the electron microscopy report, but not with the reports of other aspects of the study.

No unscheduled deaths, clinical signs or gross pathological lesions were seen. At 30 000 ppm, liver weight and blood levels of T4, free T4 index, TSH, progesterone and dehydroepiandrosterone sulfate were significantly increased ($p < 0.05$) in both sexes. In addition to this, total T3 was significantly increased in males at the highest dose, and terminal body weight and T4 binding capacity were significantly decreased in females at the highest dose. At 400 ppm, T4 and liver weight were significantly increased in males and dehydroepiandrosterone sulfate was significantly decreased in females. Electron microscopy revealed hypertrophy of TSH-producing cells of the anterior pituitary and dilation of the rough endoplasmic reticulum in these cells in all males that were examined in the group receiving the highest dose (five out of five) and in one of four males from group receiving the lowest dose.

The cisternae of the rough endoplasmic reticulum were dilated and filled with an amorphous material. Secretory granules were seen in the rough endoplasmic reticulum of the TSH-producing cells in four out of five male rats examined. No ultra-structural differences (when

Table 7. Mean levels[a] of ^{125}I in thyroids and blood of rats and mice fed diets containing clofentezine

Species	Dietary concentration (ppm)	Sex	Thyroid		Blood	
			6 h	24 h	6 h	24 h
Rat	0 (control)	Male	113 400	163 500	13 300	5 500
	0 (control)	Female	114 000	144 900	15 900	4 800
	30 000	Male	189 600[b]	210 200	10 900[b]	4 600[b]
	30 000	Female	301 700[b]	27 7100[b]	12 800[b]	3 900[b]
Mice	0 (control)	Male	77 600	85 900	1 550	350
	0 (control)	Female	67 900	69 400	17 00	350
	30 000	Male	73 000	129 600[b]	800[b]	400
	30 000	Female	60 100	83 400	950[b]	350

From Challis & Creedy (1985)

[a] The units are counts per minute (cpm)/thyroid for the thyroid measurements, cpm/ml for blood levels in rats and cpm/20 µl for blood levels in mice.

[b] Statistically significantly different from the value for the controls of the same species and same sex at a confidence level of $p = 0.05$ or less.

compared with controls) were seen at any dose in the pituitaries from females or in the thyroids of either sex. Some treatment-related histomorphological changes were seen in the thyroids of rats at 30 000 ppm (females at 400 ppm and all males were not examined): the total area of thyroid was significantly increased ($p < 0.05$) when compared with controls; the total number of follicular cells was significantly increased ($p < 0.01$); and there was a non-significant increase in the number of follicles. The results of this study suggested that high oral doses of clofentezine could cause increased synthesis of TSH in the pituitary and enlargement of the thyroid (Braybrook et al., 1986; Saunders & Mallyon, 1986; Yarwood & Gopinath, 1989).

A special study was performed to investigate the effect of clofentezine on thyroid hormones and histopathology to provide information on the mechanism by which thyroid tumours were produced in male rats in the long-term study of carcinogenicity/toxicity that had been performed earlier (section 2.3). A statement of GLP compliance was provided with the report of the study. Groups of 60 male Sprague-Dawley rats were fed diets containing clofentezine (purity, 97% w/w) at a concentration of 0, 10, 40, 400 or 30 000 ppm for 13 weeks. These concentrations provided clofentezine at mean doses of 0, 0.71, 2.88, 28.9 and 2250 mg/kg bw per day. Blood samples were taken after 4, 8 or 13 weeks of treatment, and routine clinical chemistry was performed along with measurements of blood levels of thyrotropin (TSH), total T4, free T4, total T3, free T3 and reverse T3 and measurement of liver microsomal uridine diphosphoglucuronyl transferase (UDPGT). Twenty rats from each group were killed after 4, 8 and 13 weeks. Necropsies were performed on five rats per group from the animals killed at 4 or 13 weeks. The pituitaries and thyroids from the animals necropsied after 4 weeks were examined microscopically.

There were no treatment-related effects on mortality, clinical signs, food consumption or gross pathology. Body-weight gain was about 10% less than control values throughout the study. At 4, 8 and 13 weeks in the group at 30 000 ppm, there were statistically significant ($p < 0.05$) increases in serum levels of cholesterol, total protein, and globulin and corresponding decreases in the albumin : globulin ratio. At the same times, there were significant ($p < 0.05$) increases in liver microsomal UDPGT in the groups at 400 and 30 000 ppm.

The results of hormone measurements are summarized in Table 8. They showed significant increases in TSH at each time-point for the group at 30 000 ppm. Total T4 was significantly increased at 30 000 ppm at 8 and 13 weeks. Free T4 was significantly increased at 400 ppm at 4 weeks and at 400 and 30 000 ppm at 13 weeks. Free T3 was slightly but significantly decreased in the group at 30 000 ppm at 4 weeks.

Relative liver weights were significantly increased ($p < 0.05$) in the groups at 400 and 30 000 ppm at 4 and 13 weeks. Thyroid weight was significantly increased ($p < 0.05$) at 30 000 ppm at 13 weeks. Light microscopy showed moderate to severe thyroid follicular cell hypertrophy with colloid depletion in the follicles at 30 000 ppm. These thyroid lesions were also seen at lower doses and in controls, but in these groups the effects were described as minimal or slight. In the pituitary there was focal hypertrophy of TSH-producing cells in seven out of ten rats examined in the group at 30 000 ppm, in contrast to two out of ten controls having similar pituitary hypertrophy.

The NOAEL was 40 ppm (equal to 2.88 mg/kg bw per day), with effects on blood levels of thyroid hormones, liver activity of microsomal UDPGT, thyroid pathology and increased liver weights at doses of 400 ppm (28.9 mg/kg bw per day) or more (Mallyon, 1990).

A GLP-compliant study was performed to investigate the time course of the thyroid changes seen at high doses of clofentezine. Groups of 50 male Sprague-Dawley rats were fed diets containing technical crystalline clofentezine of unstated purity at a concentration of 0 or 30 000 ppm (equal to 1915 mg/kg bw per day) for up to 2 weeks. After 1, 2, 4, 7 and 14 days of treatment, 10 rats from each group were killed by exsanguination. The concentrations of TSH, total T4 and total T3 were measured in the blood samples. Necropsies were performed and livers

Table 8. Measurements of hormones and UDPGT in male rats given diets containing clofentezine

Dietary concentration (ppm)	TSH	Total T4	Free T4	Total T3	Free T3	Reverse T3	UDPGT
4 weeks							
0	NS	NS	NS	NS	NS	NS	NS
10	NS	NS	NS	NS	NS	NS	NS
40	NS	NS	NS	NS	NS	NS	NS
400	NS	NS	↑	NS	NS	NS	↑
30 000	↑	NS	NS	NS	↓	NS	↑
8 weeks							
0	NS	NS	NS	NS	NS	NS	NS
10	NS	NS	NS	NS	NS	NS	NS
40	NS	NS	NS	NS	NS	NS	NS
400	NS	NS	↑	NS	NS	NS	↑
30 000	↑	↑	↑	NS	NS	NS	↑
13 weeks							
0	NS	NS	NS	NS	NS	NS	NS
10	NS	NS	NS	NS	NS	NS	NS
40	NS	NS	NS	NS	NS	NS	NS
400	NS	NS	NS	NS	NS	NS	↑
30 000	↑	↑	NS	NS	NS	NS	↑

From Mallyon (1990)

NS, not statistically significantly different ($p > 0.05$) from the concurrent control value; T3, triiodothyronine; T4, thyroxine; TSH, thyrotropin; UDPGT, uridine diphosphoglucuronyl transferase

↑ statistically significantly greater ($p < 0.05$) than the concurrent control value.

↓ statistically significantly less ($p < 0.05$) than the concurrent control value.

and thyroids were weighed. Thyroids and livers were fixed, but only the thyroids were examined microscopically.

No adverse effects were seen on mortality, clinical signs or gross pathology. Food intake and body-weight gain of the treated rats were initially less than in the controls, but there was no effect after day 4. Livers were enlarged by an average of 17% at 2 days, rising to 60% after 4 days and remaining elevated. There was no effect on thyroid weight on most days but it was significantly raised ($p < 0.05$) on day 3. Total T3 was significantly lower than control values ($p < 0.05$) on days 2, 4 and 7 and TSH was significantly elevated ($p < 0.05$) on days 4, 7 and 14. Microscopic examination of thyroids showed that, after 7 days, there was colloid depletion in the follicles, pronounced hypertrophy and increased mitotic activity in the follicular cells and some rats showed follicular cell hyperplasia. At 14 days the increased mitosis was less pronounced but the other effects were more severe than at 7 days (Markham & Mallyon, 1988).

A study was performed to investigate the temporal changes to the thyroid after administration of clofentezine at a range of doses. No QA statement or GLP certificate was provided. Groups of 80 male Sprague-Dawley rats were fed diets containing technical crystalline clofentezine of unstated purity at a concentration of 0, 10, 400, 3000 or 30 000 ppm (equal to average doses of 0, 0.58, 22.69, 169.4 and 1635 mg/kg bw per day) for up to 4 weeks. After 4, 7, 14 and 28 days of treatment, 20 rats from each group were killed by exsanguination. Necropsies

were performed. Livers were weighed fresh; thyroids were fixed and then weighed. The activity of microsomal UDPGT was measured in a sample of each liver, the rest being fixed for possible histopathological examination in the future. Thyroids were examined microscopically. No adverse effects were seen on mortality, clinical signs or gross pathology. Body-weight gain and food consumption were reduced at all time points in rats given the highest dose. At all time-points, relative and absolute liver weights were significantly increased ($p < 0.001$) at the highest dose and liver weight was increased after 14 days of treatment in the group at 400 ppm. Hepatic microsomal UDPGT was significantly ($p < 0.001$) and consistently raised at dietary concentrations of 400 ppm or more throughout the study. There was also a significant increase ($p < 0.05$) in UDPGT at 10 ppm after 5 and 14 days of treatment. There were at least some significant increases ($p < 0.05$) in thyroid weight at all time-points for doses of 400 ppm or more. Histopathological changes to the thyroid were seen at 400 ppm or more. These changes included colloid depletion, follicular cell hypertrophy and hyperplasia, and increased mitotic activity of follicular cells.

The Meeting considered the NAOEL to be 10 ppm (0.58 mg/kg bw per day) on the basis of effects on the liver and thyroid at 400 ppm (22.69 mg/kg bw per day). The occasional slight effects seen on liver microsomal UDPGT at 10 ppm were considered to be of little toxicological relevance in the absence of other effects (Mallyon, 1989).

In a GLP-compliant study on the effect of clofentezine on biliary excretion of T4, groups of six male Sprague-Dawley rats were fed diets containing clofentezine (purity, 99.3%) 0 or 30 000 ppm for 2 to 3 weeks and then were given an intravenous injection of radiolabelled T4 (L-[^{125}I]-thyroxine). The rats were kept under pentobarbitone anaesthesia throughout the study. Bile was collected from cannulae in the rats and analysed for T3 and T4 by TLC. Blood was collected at 15, 30, 45, 60, 90, 120, 150, 180 and 240 min after dosing. Blood and bile were analysed for ^{125}I by direct gamma counting. The clearance of ^{125}I from the blood, the excretion of ^{125}I into bile, the biliary flow rate and the amounts of ^{125}I present in bile as L-[^{125}I]-thyroxine and as L-[^{125}I]-thyroxine glucuronide were estimated. The results showed that dosing with clofentezine at 30 000 ppm resulted in a doubling of the flow rate of bile and a 1.6-fold increase in the biliary excretion of the ^{125}I radiolabel. Less of the ^{125}I was excreted into the bile in the form of thyroxine (free T4) and thyroxine glucuronide in the clofentezine-treated animals than in controls (Dawson & Needham, 1988).

The effect of 5 weeks of dietary administration on T4 excretion was investigated in a study that complied with GLP. Groups of five male Sprague-Dawley rats were fed clofentezine (purity, 99.3%) at a concentration of 0 or 30 000 ppm for 5 weeks. The rats were then given an intravenous injection of radiolabelled T4 (L-[^{125}I]-thyroxine) and transferred to metabolism cages. Urine and faeces were collected at frequent intervals for 72 h, after which the animals were bled and killed. The gastrointestinal tract was removed from each rat and the amounts of ^{125}I in urine, faeces and the gastrointestinal tract were measured.

The results showed that in clofentezine-treated rats there was more ^{125}I in the faeces and blood, and less in the urine than in controls. Urinary and biliary ^{125}I were at similar concentrations in controls, but after 72 h faecal ^{125}I in the clofentezine-treated rats was 2.3 times greater than the level in urine. The blood concentration of ^{125}I was double that of controls in clofentezine-treated rats. The amount of ^{125}I remaining in the gut was similar to the control value. It was concluded that clofentezine slowed down the clearance of T4 and caused a shift from urinary excretion to faecal excretion. The decreased clearance of T4 may have been caused by the biliary excretion rate being slower than urinary excretion or due to reabsorption of T4 from the lower gut after biliary excretion (Needham, 1987).

A study was performed to investigate the effects of clofentezine on thyroid function and morphology in rabbits. No QA statement or GLP certificate was provided. Groups of five male

and five female New Zealand White rabbits were fed diets containing clofentezine at a concentration of 0 or 8000 ppm for 13 weeks. Blood samples were then taken for analysis of serum concentrations of T3, T4 and T4-binding capacity. All animals were killed. Their liver and thyroids were examined and weighed. Thyroids were examined by light microscopy.

No effects were seen on mortality, clinical signs, body weight, thyroid hormone parameters, macroscopic appearance or microscopic appearance of the thyroid. The absolute liver weight was significantly increased ($p < 0.05$) by 25% in females given clofentezine, but not in males. There was no evidence to suggest that clofentezine could cause thyroid changes in rabbits (Mallyon et al., 1986).

2.6 Studies with metabolites

No toxicological studies have been performed on metabolites of clofentezine.

3. Observations in humans

Workers at a plant manufacturing clofentezine have been examined medically. Manufacturing personnel, including formulators, packers and maintenance staff, were routinely examined before starting employment and thereafter annually or whenever an adverse work-related health effect was reported. The medical examination included an extensive physical examination (including height, body weight, skin, ear, nose and throat, and respiratory, nervous, alimentary, reticulo-endothelial and cardiovascular systems, and locomotion) and specific tests for blood pressure and vision. The examination also included tests for hearing, lung function, urine analysis and blood tests when these were considered appropriate. Examinations of staff between 1985 and 2004 covered 153.5 man-hours of work. Operator activities most likely to involve worker exposure were identified as "kegging off" of the active ingredient, formulation, packing and maintenance work. Occupational hygiene monitoring showed air concentrations of active ingredient to range between < 0.1 mg/m^3 and 29 mg/m^3 (< 0.1 and 29 µg/l air) during synthesis and between < 0.1 mg/m^3 and 0.8 mg/m^3 (< 0.1 and 0.8 µg/l air) during formulation. There was considered to be only a small risk of skin exposure during packing. The risk of operator exposure was minimized by control measures including local exhaust ventilation and use of personal protection equipment (filter face piece respirator and rubber gloves). No adverse health effects attributable to clofentezine were detected during medical surveillance between 1985 and 2004 (Makhteshim Agan Industries Ltd, 2004).

Comments

Biochemical aspects

Pharmacokinetic studies in laboratory animals showed that oral doses of clofentezine were quickly absorbed from the gut lumen, with peak concentrations occurring in the plasma after a maximum of 4–6 h in rats. At least half the administered oral dose was absorbed. The liver was the major site for distribution in all species investigated, with high concentrations of radiolabel also being found in the kidneys. Residues were persistent in several tissues, with low concentrations of radiolabel still being present in the liver and adipose tissue of rats at 25 days after the last dose of radiolabelled clofentezine. Radiolabel from orally administered [^{14}C]clofentezine crossed the placental barrier of rats to reach the fetuses of pregnant rats, but concentrations of radiolabel in the fetuses were about five times lower than in the mothers.

Primary metabolism occurred by two major pathways:

— hydroxylation of the phenyl ring at the 3, 4 and/or 5 position;

— hydroxylation at the 3-phenyl position and replacement of the chlorine atom on the same phenyl ring with a methylthio group.

The relative importance of the two pathways differed from species to species, with hydroxylation being the main route in calves and baboon, but methylthiolation being more important in rodents and rabbits. The primary metabolites could be conjugated with glutathione, mercapturic acid or cysteine before excretion in the bile or urine.

Clofentezine and/or its metabolites were found in the urine and faeces of treated animals with up to about three-quarters of an oral dose being voided in the faeces. About 50% of the radiolabel was associated with unchanged clofentezine. The chemical identity of the rest of the radioactivity in the faeces was not investigated. The possible occurrence of enterohepatic circulation was not investigated.

Studies of the effects of oral doses on liver enzymes showed that clofentezine is a potent inducer of several enzymes, including UDPGT in rats and cytochrome P450 in mice and rats. The NOEL for effects on these enzymes in rats was 1 mg/kg bw per day.

Toxicological data

Clofentezine has low acute oral toxicity in all species tested (mouse, rat, Syrian hamster and dog), causing no serious adverse effects at any dose tested (up to 5200 mg/kg bw in mice and rats). It also has low acute toxicity in rats exposed dermally (LD_{50} > 2100 mg/kg bw) or by inhalation (LD_{50} > 0.89 mg/l).

Clofentezine was not an irritant to the skin of guinea-pigs or the eyes of rabbits. It gave a negative result in a Magnusson & Kligman maximization test for skin sensitization in guinea-pigs.

The main toxicological effects seen in short-term studies in mice, rats or dogs given repeated doses of clofentezine in the diet were hepatotoxicity (changes in histopathology and clinical chemistry) and changes to the thyroid, including follicular hyperplasia. The lowest NOAEL identified from short-term feeding studies was 40 ppm (equal to 2.65 mg/kg bw per day) for effects on the liver in a 90-day study of toxicity in rats. In mice, the NOAEL was 200 ppm (equal to 30.3 mg/kg bw per day) for increased weights of the thyroid and the liver. In dogs, the lowest NOAEL identified was 50 ppm (equal to 1.72 mg/kg bw per day) for hepatotoxicity in a 12-month feeding study.

In a study of carcinogenicity in mice, non-neoplastic changes to the liver included vacuolation and eosinophilia of the hepatocytes. There were no consistent or dose-dependent effects on any tumour type.

The Meeting concluded that there was no evidence of a tumourigenic response in mice.

In the long-term study of toxicity/carcinogenicity in rats, there was limited evidence to suggest that prolonged high doses of clofentezine could cause thyroid follicular cell adenomas and carcinomas in this species. A marginal increase in the incidence of these tumours was seen only in the males at the highest dietary concentration (400 ppm), and was only slightly greater than the incidence in control male rats in a different long-term study of toxicity/carcinogenicity performed in the same laboratory. No changes in the thyroid were seen in the long-term study of toxicity/carcinogenicity in rats at 40 ppm (equal to 1.72 mg/kg bw per day). The results of studies of effects on hormones, enzymes and morphological changes associated with thyroid homeostasis did not clearly establish a mode of action for the development of thyroid tumours.

The Meeting concluded that there was no risk of thyroid tumours developing in rats given oral doses of 1.72 mg/kg bw per day or less.

Clofentezine gave negative results in an adequate range of tests for genotoxicity in vitro and in vivo.

The Meeting concluded that clofentezine is unlikely to be genotoxic.

Noting the absence of genotoxicity, the Meeting concluded that the marginal increase in incidence of thyroid follicular cell tumours in males at the highest dose did not indicate a carcinogenic risk to humans at the levels of exposure likely to be experienced by consumers or workers.

The results of a two-generation study of reproduction in rats showed that exposure to clofentezine at a dietary concentration of 400 ppm caused decreased body-weight gains in pups during lactation, resulting in low body-weights of pups in the weeks following lactation. A transient marginal decrease in pup weight of the F_2 generation males at 40 ppm at 1 week after weaning was not considered to be toxicologically significant. The NOAEL for the study was 40 ppm (equivalent to 2.7 mg/kg bw per day) on the basis of decreased pup weight.

Studies of developmental toxicity in rats and rabbits treated by gavage showed that clofentezine was neither teratogenic nor embryotoxic. The only indication of fetotoxicity was low fetal body weight in rats at maternally toxic doses. The NOAEL for maternal toxicity in these studies was 320 mg/kg bw per day in rats and 250 mg/kg bw per day in rabbits.

No evidence of neurotoxicity was apparent from the available studies of toxicity.

Routine monitoring of workers in a factory producing clofentezine has shown no adverse effects attributable to exposure to clofentezine.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.02 mg/kg bw based on the NOAEL of 1.72 mg/kg bw per day for thyroid changes in a long-term study of toxicity/carcinogenicity in rats and also for hepatotoxicity in a 12-month study in dogs, and using a safety factor of 100.

The Meeting concluded that it was not necessary to set an ARfD for clofentezine, since clofentezine has low acute toxicity and does not cause developmental toxicity or any other toxicological effect that would be elicited by a single exposure.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Carcinogenicity	Carcinogenicity	500 ppm (51 mg/kg bw per day)[a]	—
Rat	90-day study of toxicity	Liver enlargement	40 ppm (2.65 mg/kg bw per day)	400 ppm (26.2 mg/kg bw per day)
	Long-term study of toxicity/carcinogenicity	Thyroid changes including tumours	40 ppm (1.72 mg/kg bw per day)	400 ppm (17.3 mg/kg bw per day)
	Two-generation study	Decreased weights of pups of the F_2 generation	40 ppm (2.7 mg/kg bw per day)	400 ppm (27 mg/kg bw per day)
	Developmental toxicity [b]	Maternal toxicity (hepatotoxicity)	320 mg/kg bw per day	1280 mg/kg bw per day
Dog	12-month study of toxicity	Hepatotoxicity	50 ppm (1.72 mg/kg bw per day)	1000 ppm (36.0 mg/kg bw per day)
Rabbit	Developmental toxicity [b]	Maternal toxicity (reduced body-weight gain)	250 mg/kg bw per day	1000 mg/kg bw per day

[a] Highest dose tested

[b] Oral gavage administration

Estimate of acceptable daily intake for humans

0–0.02 mg/kg bw

Estimate of acute reference dose

Unnecessary

Information that would be useful to the continued evaluation of the compound

Results from epidemiological, occupational health and other observational studies of human exposures

Critical end-points for setting guidance values for exposure to clofentezine

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid with peak levels at 4–6 h after dosing. At least half of an oral dose was absorbed.
Distribution	Extensive. Radiolabel crossed the placental barrier. Radiolabel was persisted in liver and fat for 25 days.
Potential for accumulation	Low
Rate and extent of excretion	In the urine and faeces, with about three-quarters of an oral dose being voided in the faeces.
Metabolism in mammals	By hydroxylation and methylthiolation plus conjugation.
Toxicologically significant compounds (animals, plants and environment)	Clofentezine
Acute toxicity	
Rat LD_{50} oral,	> 3200 mg/kg bw
Rat LD_{50} dermal	> 2100 mg/kg bw
Rat LC_{50} inhalation	> 0.89 mg/l
Guinea-pig, skin irritation	Non-irritant
Rabbit, eye irritation	Non-irritant
Skin sensitization (test method used)	No skin sensitization potential in guinea-pigs (Magnusson & Kligman test)
Short-term studies of toxicity	
Target/critical effects	Hepatotoxicity
Lowest relevant oral NOAEL	1.72 mg/kg bw per day (12-month study in dogs)
Genotoxicity	
	Not genotoxic
Long-term studies of toxicity and carcinogenicity	
Target/critical effects	Hepatotoxicity in mouse. Changes to thyroid of rat.
Lowest relevant oral NOAEL	1.72 mg/kg bw per day (rats)
Carcinogenicity	Thyroid tumours in rats possible at high doses. Non-genotoxic mechanisms are likely. Unlikely to pose a risk to humans.
Reproductive toxicity	
Reproduction target/critical effect	Decreased body weights of pups of the F_2 generation
Lowest relevant reproductive NOAEL	2.7 mg/kg bw per day

Developmental target/critical effects	Not embryotoxic. Not directly fetotoxic. Not teratogenic.
NOAEL for maternal toxicity	250 mg/kg bw per day (reduced body-weight gain in rabbits)
Lowest relevant developmental NOAEL	3000 mg/kg bw per day (highest dose tested in rabbits)
Special studies	
Effects on enzymes	Mouse liver enzymes induced at 40 mg/kg bw per day or more (no NOEL identified). NOEL for induction of rat liver enzymes was 1 mg/kg bw per day.
Medical data	
Health monitoring of workers	No adverse effects reported in production workers

Summary

	Value	Study	Safety factor
ADI	0–0.02 mg/kg bw	Rat, long-term study of toxicity/carcinogenicity study; dog, 12-month study	100
ARfD	Unnecessary	—	—

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