HALOXYFOP (INCLUDING HALOXYFOP-R AND HALOXYFOP-R METHYL ESTER)

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Explanation

Haloxyfop is the International Organization of Standardization (ISO) approved name for (*R/S*)-2-[4-(3-chloro-5-trifluoromethyl-2-pyridyloxy)phenoxy]propionic acid. It is a substituted phenoxypropionic acid derivative that was developed as a selective herbicide for control of grass weeds in broadleaf crops. In the first formulations produced, the active substance was either racemic haloxyfop ethoxyethyl ester or the racemic methyl ester. As it has been demonstrated that haloxyfop-R is the herbicidally active isomer, and essentially no activity is associated with the *S* isomer, a resolved methyl ester has been developed which is approximately 98% *R* isomer. When applied to plants, the esters are rapidly hydrolysed to the acid.

Haloxyfop (racemic), its sodium salt and its esters (racemic haloxyfop ethoxyethyl ester and racemic haloxyfop methyl ester) were first evaluated by the JMPR in 1995, when the Meeting established an acceptable daily intake (ADI) of 0–0.0003 mg/kg bw based on a no-observed-adverse-effect level (NOAEL) of 0.03 mg/kg bw per day for liver tumours in a 2-year study in mice. New toxicological data had been made available since this date. Haloxyfop was re-evaluated at the request of the Codex Committee on Pesticide residues (CCPR). New data on pharmacokinetics, dermal toxicity, genotoxicity and special studies of hepatocellular peroxisome proliferation had become available since the last evaluation.

All studies with haloxyfop-R methyl ester and pivotal studies with haloxyfop were certified as being compliant with good laboratory practice (GLP). Other studies were carried out before the Organisation for Economic Co-operation and Development (OECD) guidelines on GLP were promulgated. The quality of these studies was considered to be acceptable.

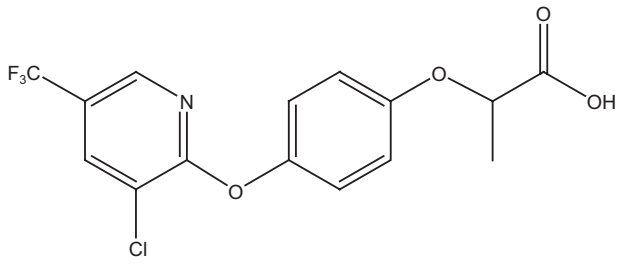
Numerous studies of the pharmacology and toxicology of various chemical forms of haloxyfop were available. Investigations with haloxyfop-R and its methyl ester were limited to studies of absorption, distribution, metabolism and excretion, acute toxicity, short-term studies of toxicity, and genotoxicity.

Haloxyfop-R methyl ester and haloxyfop-R are used as active ingredients in herbicide products used on crops, including carrots, fodder legumes, rapeseed, soya bean and sugar beet. The *R*-isomer is the herbicidally-active form of the chemical. Haloxyfop-R methyl ester and haloxyfop-R are the only forms of haloxyfop that are now manufactured and registered globally, although racemic forms and the ethoxyethyl ester have been used in the past. Commercially produced haloxyfop-R methyl ester contains a minimum of 98% of *R*-isomer and a maximum of 2% *S*-enantiomer.

The CAS Nos for these substances are: haloxyfop methyl ester, 69806-40-2; haloxyfop-R methyl ester, 072619-32-0; haloxyfop acid, 69806-34-4; and haloxyfop ethoxyethyl ester (haloxyfop-etotyl), 87237-48-7. The structural formula of haloxyfop acid is shown in Figure 1.

Chemical names (IUPAC) for these substances are as follows: racemic haloxyfop acid: (*RS*)-2-[4-[3-chloro-5-trifluoromethyl-2-pyriyloxy)phenoxy]propionic acid; haloxyfop-R methyl ester, methyl (*R*)-2-[4-[3-chloro-5-trifluoromethyl-2-pyriyloxy)phenoxy]propionate; racemic haloxyfop ethoxyethyl ester, ethoxyethyl (*RS*)-2-[4-[3-chloro-5-trifluoromethyl-2-pyriyloxy)phenoxy]propionate.

Figure 1. Structural formula of haloxyfop acid



Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Mice

In a study of pharmacokinetics that complied with GLP, groups of 21 male and 21 female B6C3F_1 mice were given the sodium salt of racemic haloxyfop as a single oral dose at 5 mg/kg bw. Groups of three mice of each sex were killed at 6, 12, 24, 48, 72, 96 and 168 h after dosing for measurement of the radioactivity in the plasma, kidney and liver. Urine and faeces were collected at 24-h intervals for measurement of radioactivity. Peak plasma concentrations of 23 (males) and 24 mg eq/g (females) were attained at 6 h after dosing. The half-life for apparent first-order absorption into the plasma was 1.5 h for males and 1.9 h for females. Disappearance of radiolabel from the plasma appeared to follow first-order kinetics with half-lives of 1.7 and 1.9 days in males and females respectively and volumes of distribution (V_d) of 196 and 194 ml/kg. The half-lives for removal from the liver and kidneys were respectively 1.7 and 1.8 days in males and 1.9 and 2.0 days in females. At 168 h after dosing, 18% (males) and 24% (females) of the administered radiolabel had been recovered in urine and 66% (males) and 60% (females) had been recovered in faeces. The proportion of the excreted radiolabel that was found in the faeces was 79% for males and 71% for females (Smith et al., 1984).

Rats

In a dose range-finding study, groups of Fischer 344 rats were given racemic haloxyfop methyl ester (radiochemical purity > 98%) that was labelled with ^{14}C on the phenyl ring. Males were given single intravenous or oral doses at either 0.5 mg/kg bw or 50 mg/kg bw, while females were received doses of 10 mg/kg bw orally or intravenously. Urine and faeces were collected at frequent intervals throughout the study. Three rats from each group were killed at 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, 96 and 120 h after dosing in order to obtain samples of plasma. The oral doses were rapidly absorbed and there was little difference between the pharmacokinetic behaviours of oral and intravenous doses. The clearance of radioactivity from the plasma seemed to follow first-order kinetics. Pharmacokinetics results for appearance and disappearance of radioactivity in plasma are summarized in Table 1. Most of the radioactivity remaining in the bodies of male rats at 5 days after dosing was in the carcass (19.3–25.0% of the administered dose), liver (9.8–18.0%) and skin (10.3–14.6%). The proportion of the excreted radiolabel that was found in the faeces was 63–73% for males and in females the proportion in urine was 68–78% (Smith et al., 1982).

Table 1. Pharmacokinetics in a dose range-finding study in mice given a single dose of radiolabelled racemic haloxyfop methyl ester

Parameter	Males		Females		Males	
	0.5 mg/kg bw, oral	0.5 mg/kg bw, IV	10 mg/kg bw, oral	10 mg/kg bw, IV	50 mg/kg bw, oral	50 mg/kg bw IV
Absorption half-life (h)	1.8	—	1.7	—	3.0	—
Plasma clearance half-life (days)	3.6	3.9	1.1	1.0	2.8	4.5
Volume of distribution (ml/kg bw)	—	236	194	199	—	179
Peak plasma concentration (µg/g)	1.13	2.22	50	50	213	299

Recovery of radioactivity in urine (%)						
3 days after dosing	—	—	45.1	57.0	—	—
5 days after dosing	13.4	11.1	—	—	16.3	15.1
Recovery of radioactivity in faeces (%)						
3 days after dosing	—	—	21.4	15.7	—	—
5 days after dosing	29.0	30.2	—	—	27.7	27.4

From Smith et al. (1982)
IV, intravenous.

In a study that complied with GLP, Fischer 344 rats were given racemic haloxyfop methyl ester labelled with ^{14}C on the phenyl ring (radiochemical purity, > 98%) as a single oral dose at 0.1 mg/kg bw by gavage. Urine and faeces were collected daily throughout the study. Groups of three males were killed for blood sampling at 1, 4, 7, 10, 13, 16, 21 and 24 days after dosing and groups of three females were killed for sampling of liver, kidney, skin, fat, brain, heart, muscle and spleen at 1, 2, 3, 4, 5, 10, 16 and 21 days. One day after dosing, mean concentrations of radioactivity (expressed as equivalents to haloxyfop methyl ester) in plasma were 0.60 µg/g in males and 0.33 µg/g in females. The absorption half-lives were 4.6 h in males and 2.7 h in females. The volumes of distribution (V_d) were 176 ml/kg in males and 251 ml/kg in females. At 1 day after dosing, concentrations of radioactivity were found in the following tissues in decreasing order: liver (0.608 µg eq/g), plasma (0.597 µg eq/g), kidney (0.345 µg eq/g), erythrocytes (0.128 µg eq/g), heart (0.101 µg eq/g), skin (0.081 µg eq/g) and spleen (0.063 µg eq/g), with the relative concentrations remaining in this order throughout the study. The half-lives for clearance of radiolabel from these tissues are shown in Table 2. It was not possible to determine half-lives for skin and spleen in females as the residue concentrations were below the limit of detection after the first day (Smith et al., 1982).

Table 2. Clearance of ^{14}C-radiolabel from tissues of rats given radiolabelled racemic haloxyfop methyl ester as a single dose by gavage

Tissue	Half-life (days)	
	Males	Females
Liver	5.87	1.55
Plasma	5.55	1.59
Kidney	6.13	1.34
Erythrocytes	5.82	1.23
Heart	6.73	—
Skin	7.30	—
Spleen	5.78	—

From Smith et al. (1982)

A series of GLP-compliant experiments was performed to determine the pharmacokinetics of haloxyfop methyl ester (purity, 99.6%) in Fischer 344 rats.

Firstly, two males and two females were given racemic [^{14}C]haloxyfop methyl ester (uniformly labelled on the phenyl ring; radiochemical purity, > 99%) as an oral dose at 2.5 mg/kg bw to determine the time-plasma concentration profiles of radiolabel, haloxyfop methyl ester and haloxyfop at 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing. Urine and faeces were collected at 12 h and 24 h. Haloxyfop methyl ester was not found in any of the samples of blood plasma, while haloxyfop was found at concentrations that matched the amounts of radiolabel found. The Meeting concluded that all of the radiolabel in plasma was in the form of haloxyfop. Peak plasma concentrations of haloxyfop occurred at 8 h after dosing in males and females, with a half-life for absorption of 4.5 h. The haloxyfop was

excreted into urine with an elimination half-life of 23.3 h. The V_d was 185 ml/kg. Urinary excretion occurred more rapidly in females than in males, with 23% of the administered dose being excreted into the urine in the 24 h after dosing by females and only 1.4% in males. Faecal excretion was erratic, with no discernible difference between the sexes.

Secondly, 12 male and 12 female rats were given racemic ^{14}C-haloxyfop methyl ester as a single oral gavage dose at 0.104 mg/kg bw (equimolar to a dose of 0.1 mg/kg bw of haloxyfop) to investigate the routes and rates of excretion, tissue distribution and time-plasma concentration of ^{14}C profiles over the 15 days after dosing. Groups of three males were killed on days 1, 5, 10 and 15 for sampling of plasma, erythrocytes, kidney, liver and adipose tissue for analysis of radioactivity. Similar samples were taken from groups of three females on days 1, 3, 5 and 7. Urine and faeces were collected each day. Plasma concentrations of ^{14}C peaked on day 1 at 0.44 µg eq/g in males and 0.40 µg eq/g in females. Plasma ^{14}C decreased inline with first-order kinetics, but at different rates for males (half-life, 1 day) and females (half-life, 5.5 days). The highest concentration of ^{14}C was found in the plasma, followed by liver, kidney, erythrocytes and adipose tissue in that order, with the amount in adipose tissue being 5–30 times less than in plasma, representing less than 0.2% of the administered material. The plasma clearance half-life was 6.41 days in males and 1.21 days in females and the clearance half-lives for liver and kidney were similar to these values. Urinary and faecal excretion half-lives were 4.8 days and 5.8 days in males and 1.1 days and 1.3 days in females. Faecal excretion was the major route of excretion in males with 59.6% of the administered dose recovered in faeces and 18.6% in urine. In females, the urinary route of excretion was predominant, with 23.5% in faeces and 66.5% in urine. The proportion of the excreted radiolabel that was found in the faeces was 76% for males and for females the proportion found in the urine was 74% (Waechter et al., 1982).

The pharmacokinetics of haloxyfop-R methyl ester in rats given repeated doses was investigated in a GLP-compliant study. Groups of four male and four female Fischer 344 rats were given unlabelled haloxyfop-R methyl ester (purity, 98.6%) as 14 daily oral doses at 0.1 mg/kg bw by gavage, followed by a single oral dose of ^{14}C-labelled haloxyfop-R methyl ester (radiochemical purity, 97.9%) at 0.1 mg/kg bw. During the 7 days after treatment, urine and faeces were collected. At 7 days after dosing, the rats were killed and samples of blood were taken. Autopsies were performed and samples of kidneys, liver, skeletal muscle, skin, and perirenal fat were taken. The amounts of radiolabel in the urine, faeces, plasma, tissues and residual carcass were measured. In addition, urine and faeces from selected intervals, liver, kidney and terminal plasma were analysed for metabolites by high-performance liquid chromatography (HPLC). The results showed that 93% (males) and 89% (females) of the administered radiolabel had been absorbed from the gut. The tissues with the highest concentrations of radiolabel were liver and kidney. The faecal route of elimination predominated in males, while in females the urine was the major route of excretion. In males at 7 days after dosing, 12.4% of the administered dose was excreted in the urine and 41.5% was recovered in the faeces. In females, 74.4% was in the urine and 21.6% in the faeces. Both urinary and faecal excretion of radiolabel occurred more rapidly in females than in males. The half-life for urinary excretion and for faecal excretion was 3.9 days for males; while in females the half-life for urinary excretion was 1.0 days and that for faecal excretion was 1.8 days. The proportion of the excreted radiolabel that was found in the faeces was 77% for males and for females the proportion in the urine was 78% (Mendrala & Hansen, 2001).

The aspects of this study relating to metabolism are summarized in section 1.2.

The dermal absorption of racemic haloxyfop methyl ester by Fischer 344 rats was investigated in a GLP-compliant study. A solution of racemic [^{14}C]haloxyfop methyl ester (purity, 99.0%; uniformly labelled on the phenyl ring) was prepared in acetone and was applied to the clipped but unabraded

skin of nine male and nine female rats. When the acetone had evaporated, the application site was covered with Saran film and an occlusive bandage. Urine and faeces were collected. Groups of three rats were killed for sampling of blood and liver at days 0.5, 2 and 5 for males and days 0.5, 1 and 2 for females. The test material was readily absorbed, with 16.7% and 27.7% of the administered radioactivity being absorbed by males and females over 2 days and 22.5% by males over 5 days. The radiolabel was detected in all samples of blood plasma, but haloxyfop methyl ester was absent from all samples of plasma. It was presumed that the ^{14}C was in the chemical form of [^{14}C]haloxyfop (as seen in the studies using oral administration), but this was not measured. In males, the major route of excretion was the faeces, with 0.5% of the administered dose recovered in the urine and 0.8% in faeces by 2 days after dosing and 1.7% and 6.0% respectively after 5 days. In females, the urine was the major route with 8.3% of the administered dose in the urine and 2.0% in the faeces at 2 days after dosing. The Meeting concluded that haloxyfop methyl ester can be absorbed across the skin and that the kinetics of the absorbed material are similar to the kinetics of orally administered doses. The proportion of the excreted radiolabel that was found in the faeces was 62% for males and for females the proportion in the urine was 81% (Ramsey et al., 1983).

Dogs

In an attempt to explain why dogs appear to be less sensitive to the effects of haloxyfop than do rats and mice, a study of pharmacokinetics was performed in beagle dogs. The study complied with GLP. Two male dogs were given a single oral gavage dose of 2.4 mg/kg bw of racemic haloxyfop acid (purity = 98.8%) that was radiolabelled with ^{14}C uniformly on the phenyl ring (radiochemical purity > 99%). Samples of urine and faeces were collected daily, and blood was collected at 0.5, 1, 2, 4, 6, 8, 12, 31, 48, 72, 144 and 192 h after dosing. The dogs were killed after 8 days and samples of liver, kidney, perirenal fat and bile were taken. The various samples were analysed for radioactivity (^{14}C) and by HPLC and gas chromatography (GC) for metabolites. [^{14}C]Haloxyfop was rapidly and completely absorbed into the blood plasma with a half-life of 9 min. A peak plasma concentration of 23 µg eq/g was reached at 0.5 h after dosing. Average concentrations of radioactivity in samples taken at termination were: bile, 8.49 µg eq/g; liver, 0.51 µg eq/g; plasma, 0.20 µg eq/g; kidney, 0.18 µg eq/g; and perirenal fat, 0.14 µg eq/g. Plasma clearance appear to be biphasic with an initial phase lasting 6–8 h with a half-life of 1–2 h and the second phase having a longer half-life of 34 h. After 8 days, 76.9% of the administered radioactivity had been found in the faeces and 10% in the urine. Of the excreted radiolabel, 88% was in the faeces (Nolan et al., 1987).

The results of the chemical analyses of the samples of organs and excreta that were taken in this study are described in section 1.2.

Monkeys

In a GLP-compliant study, cynomolgus monkeys were given the sodium salt of racemic haloxyfop acid (purity, 99.6%), either unlabelled or labelled with ^{14}C (radiochemical purity, 98.5%), as a single dose at 1 mg/kg bw administered by nasogastric intubation, a second single dose being administered 10 weeks later. The results indicated that the test materials were rapidly absorbed from the gastrointestinal tract with a half-life of less than 30 min. The concentrations of haloxyfop and radioactivity in the plasma and urine were similar at various time-points after dosing. This similarity was taken by the authors to indicate that most of the radioactivity was associated with haloxyfop that had either not been metabolized or conjugates that had become uncoupled during sample preparation. Peak plasma concentrations of 10 µg/g were observed 1–2 h after dosing at 1 mg/kg bw. Haloxyfop was excreted, mainly (84%) in the urine, in a bi-exponential manner; the half-life for the initial phase was 2.5 h and that of the slower second phase was 3 days. Most (95%) urinary excretion occurred in the 24 h after dosing and concentrations were below the limit of quantification (150 ng/g) by 96 h. Only 0.5% of the administered dose of radiolabelled material was

found in the faeces over the 6 days after dosing. Thus more than 99% of the excreted radiolabel was in the urine (Gerbig et al., 1985).

Humans

A GLP-compliant study of pharmacokinetics was performed in three segments.

The first segment was a pilot study in which one male volunteer was given the sodium salt of racemic haloxyfop acid as a single oral dose at 0.2 mg/kg bw. Samples of blood and urine were collected at frequent intervals up to 528 h after dosing. Faeceal samples were also collected, but were not analysed.

The second segment was performed in three male volunteers who were given the sodium salt of racemic haloxyfop as a single oral dose at 0.2 mg/kg bw. Samples of blood and urine were collected at frequent intervals up to 20 days after dosing.

The third segment was performed in four male volunteers who were given racemic haloxyfop methyl ester as a single dermal dose at 0.8 mg/kg bw. Samples of blood and urine were collected at frequent intervals up to 20 days after dosing.

Samples of blood and urine were subjected to acid hydrolysis to convert all forms of haloxyfop into the acid form. Then the samples were analysed by GC with an electron-capture detector.

No adverse effects on health were reported in any segment of the study.

Orally administered racemic haloxyfop sodium salt was rapidly absorbed with a half-life of 0.9 h. Peak plasma concentrations (C_{max}) of haloxyfop of 1.4–2.2 µg/g were detected at 4–12 h after dosing. The removal from plasma into urine had a half-life of 6.3 days. Based on the amount of haloxyfop detected in urine, the volunteer in the pilot study absorbed and excreted 100% of the oral dose into the urine and the three volunteers in the second segment excreted 65–74% of the oral dose into the urine.

Racemic haloxyfop methyl ester was absorbed across skin slowly with a half-life of 36.5 h and peak plasma concentrations of haloxyfop of 0.1–0.2 µg/g were detected at 3 days after dosing. The haloxyfop was removed from plasma into the urine with a half-life of 5.8 days. An average of 2.4% of the haloxyfop applied to the skin was recovered in the urine.

The Meeting concluded that oral doses of haloxyfop methyl ester are rapidly and extensively absorbed. In contrast, dermal uptake of haloxyfop sodium is limited and slow. Urine was the principal route of excretion (65–100% of the excreted radiolabel) (Nolan et al., 1985).

A study in human volunteers was performed with Verdict Herbicide (a water-emulsifiable concentrate containing 25.7% haloxyfop-R methyl ester as its active ingredient). Detectable levels (limit of detection, LOD, and tissue not stated) were identified in only three out of eight volunteers after field application according to label instructions: diluting 5 US quarts (2.4 l) of the Verdict concentrate (containing 2.5 lbs [1.1 kg] of haloxyfop-R methyl ester) with 100 US gallons (378.5 l) of water and spraying 400 US gallons (1514 l) of diluted herbicide onto 20 acres of cropland using an open cab self-propelled or tractor-drawn boom spray. The skin exposure was assessed from patches placed under the clothing of the applicators. Inhalation exposure was estimated from air sampling. All of the urine passed in the 2 days after spraying was collected for biomonitoring analysis. Average skin exposure during mixing was estimated to be 959 µg per lb (2114 µg/kg) of active ingredient handled and during spraying it was 30 µg per lb (66 mg/kg) of active ingredient. The average inhaled dose of active ingredient during mixing was 9.1 µg/m^3 and during spraying it was 2.1 µg/m^3. The average absorbed dose of haloxyfop was 0.31 µg/kg bw, based on biomonitoring by urinary excretion. Detectable absorbed doses of haloxyfop were found in only three of the eight applicators with estimated absorbed doses ranging from < 0.04 to 1.4 µg/kg bw. No adverse health effects were reported in any of the applicators (Scortichini et al., 1987; Nolan et al., 1991)

1.2 Biotransformation

Mice

In a GLP-compliant study, groups of three male and three female mice were given radiolabelled (^{14}C label on the phenyl ring) racemic haloxyfop sodium salt as a single oral dose at approximately 10 (males) or 11 (females) mg/kg bw administered by gavage and samples of plasma were collected 6 h after dosing for HPLC profiling. Groups of three male and three female mice were given 18 (males) and 21 mg/kg bw (females) and urine was collected 24 h after dosing and bile was collected at 28 h after dosing for HPLC profiling. Haloxyfop was present in all samples analysed and was the only chemical species seen in plasma. The HPLC results for bile from males and females showed three major peaks. Three similar major peaks were seen in the HPLC results for urine from females (haloxyfop and two other substances), but only two peaks (including haloxyfop) were seen in males. Acid hydrolysis of the urine caused an increase in the haloxyfop peak, a reduction in the peaks for the other two substances and the appearance of a new more polar peak. The peak that was seen only in females corresponded to the glucuronide and disappeared completely after the acid hydrolysis. The Meeting concluded that, in the mouse, excretion of haloxyfop is in the form of unchanged haloxyfop and conjugates. Haloxyfop glucuronide appeared only in the urine of females. In addition, males and females eliminated another more polar conjugate in urine and bile (Smith et al., 1984).

Rats

As haloxyfop is chemically similar to 2-aryl-propionates that are known to undergo stereochemical inversion from *S*- to *R*-forms in several animal species, a study was conducted in rats to find out whether haloxyfop undergoes similar inversion. In a GLP-compliant study, groups of four male and four female Fischer 344 rats were given racemic [^{14}C]haloxyfop acid (purity, 98.7%; labelled in the phenyl moiety; radiochemical purity, 99.1%) as a single oral dose at 11 mg/kg bw by gavage. Urine and faeces were collected daily for 10 days after dosing. Analysis of urine and faeces showed that in females 79.4% of the radiolabel was excreted in urine and 10.5% in faeces within 10 days. In males, 16.9% was in the urine and 54.6% in the faeces. The *S*-haloxyfop underwent rapid and nearly complete inversion to the *R*-enantiomer in urine and faeces from males and females, with each daily collection of urine or faeces containing 0.2% or less of the administered dose in the form of the *S*-enatiomers. In males, the recovery of *S*-haloxyfop over the whole 10-day collection period was 0.3% of the administered dose in urine and 0.2% in faeces; while in females it was 0.1% in urine and 0.7% in faeces. The Meeting concluded that *S*-enantiomers of haloxyfop underwent rapid and almost complete stereochemical inversion to *R*-forms after oral dosing. (Bartels & Smith, 1988)

In a GLP-compliant study, groups of bile-duct-cannulated male and female Sprague-Dawley rats (numbers not stated) were given either ^{14}C-labelled racemic haloxyfop methyl ester (radiochemical purity, > 98%) or ^{14}C-labelled racemic haloxyfop acid (radiochemical purity, > 99%) as a single oral dose at 2.5 mg/kg bw by gavage. Both test materials were radiolabelled on the phenyl ring. Urine and bile were collected for about 48 h after dosing and plasma, erythrocytes and liver were sampled at 48 h. The samples were analysed by reverse-phase HPLC to determine whether haloxyfop methyl ester could be detected. For rats given haloxyfop methyl ester or haloxyfop acid, more than 97% of the radioactivity in plasma, erythrocytes and liver was found at the peak corresponding to haloxyfop acid and no haloxyfop methyl ester was detected in these samples. In urine of rats given haloxyfop methyl ester, 60% (males) and 89% (females) of the radioactivity resolved as haloxyfop acid, while the urine of those given haloxyfop acid had 74% (males) and 94% (females) of the radioactivity in the form of haloxyfop acid. The remainder of the radiolabel existed as unidentified metabolites

(one major and two minor). The amounts of haloxyfop acid in bile were 56% (males) and 48% (females) for those given haloxyfop methyl ester and 51% (males) and 55% (females) for those given haloxyfop acid. Only the major unidentified metabolite was detected in bile. Acid hydrolysis of the major unidentified metabolite in urine showed a 90% reversion to haloxyfop acid. It was also observed that, on standing urine or bile at room temperature for 24 h, the three unidentified metabolites were partly converted to haloxyfop acid. It is possible that the major metabolite was a conjugate of haloxyfop acid but attempts to identify it as a sulfate or glucuronide were inconclusive (Smith et al., 1982).

Samples of excreta and tissues were taken in the study of pharmacokinetics in rats given repeated doses of [^{14}C]haloxyfop-R methyl ester, as described in section 1.1. The amounts of radiolabel in the urine, faeces, plasma, tissues and residual carcass were measured. In addition, urine and faeces from selected intervals after dosing, liver, kidney and terminal plasma were analysed for metabolites by HPLC. The results showed that haloxyfop methyl ester was not present in the urine, plasma or tissues, but was present in faeces; 3.7% (males) and 8.2% (females) of the administered dose, being recovered in faeces as unchanged haloxyfop methyl ester (possibly representing unabsorbed material). Haloxyfop acid was the major metabolite found in urine (8.9% and 67.3% of administered dose in males and females, respectively), and it was the only metabolite found in plasma, liver and kidneys. Haloxyfop glucuronide was identified in the urine of females at 6.4% of the administered dose. In faeces, haloxyfop acid was the major metabolite, at 37.9% (males) and 13.4% (females) of the administered dose (Mendrala & Hansen, 2001).

In a GLP-compliant study that is described more fully in section 2.2, male Fischer rats were given diets containing racemic haloxyfop acid (purity, 99.5%) for a 4-week challenge period, followed by a 6-week recovery period. The doses given were 0 (control), 0.1 and 1.0 mg/kg bw per day. At 2-week intervals throughout the challenge and recovery periods, 7–10 rats from each group were killed, then blood was collected and pooled serum for each group was analysed for haloxyfop. Serum concentrations of haloxyfop at each sample time were approximately proportionate to the doses given. The plasma clearance half-life was 8 days for both doses (Herman et al., 1983).

Dogs

The analysis by GC and HPLC of the samples of tissues and excreta taken in the study of pharmacokinetics in dogs given an oral dose of racemic ^{14}C-haloxyfop acid (described in section 1.1) showed that the concentrations of haloxyfop in the plasma completely accounted for the amount of radiolabel that had been found in plasma. This suggested that unchanged haloxyfop was the main excretory product in dogs. Other metabolites appeared to be present in excreta. In faeces, most of the excretion was as haloxyfop, but there was a second unidentified metabolite that accounted for 8% of the radioactivity. In urine, haloxyfop was present, but a second major metabolite accounted for 50–68% of the radioactivity and was unaffected by acid hydrolysis. As most of the excretion was via the faeces, it might be concluded that haloxyfop was the major excretion product in the dog. However the presence of a second metabolite in urine is noted (Nolan et al., 1987).

Monkeys

The results of the study in cynomolgus monkeys that is described in section 1.1 suggested that oral doses of haloxyfop acid undergo little primary metabolism in cynomolgus monkeys (Gerbig et al., 1985).

2. Toxicological studies

2.1 Acute toxicity

(a) Systemic toxicity

(i) Oral administration

Mice

In a GLP-compliant study of acute oral toxicity, groups of five male and five female B6C3F$_1$ mice were given haloxyfop-R methyl ester (purity, 98.6%) as a single oral dose at 100, 500 or 1000 mg/kg bw by gavage in corn oil. The mice were observed for 15 days after dosing and were then killed for autopsy. The entire group of mice at the highest dose died within 3 days of treatment, after showing laboured breathing, lethargy, eyelid closure and perineal soiling. No gross pathology was seen in these mice at autopsy. In the group at the intermediate dose, all males and three of the females had thickened forestomachs (possibly due to irritancy), and all mices in this group gained little or no body weight. No treatment-related effects were seen in the mice given 100 mg/kg bw. The NOAEL was 100 mg/kg bw. The oral median lethal dose (LD_{50}) was 707 mg/kg bw (Mizell & Lomax, 1989a).

Rats

In a GLP-compliant study of acute oral toxicity, groups of five male and five female Fischer 344 rats were given haloxyfop-R methyl ester (purity, 98.6%) as a single oral dose at 100, 500 or 1000 mg/kg bw by gavage in corn oil. The rats were observed for 15 days after dosing and were then killed for autopsy. The entire group of rats at the highest dose and four males and one female from the group at the intermediate dose died within 8 days of treatment after showing lethargy and perineal soiling. At autopsy, several rats in the group at the highest dose showed signs of gastric irritation and had dark-red coloured urine in the bladder. No treatment-related effects were seen at 100 mg/kg bw. The NOAEL was 100 mg/kg bw. The oral LD_{50} was 300 mg/kg bw for males and 623 mg/kg bw for females (Mizell & Lomax, 1989b).

In a non-GLP-compliant study of acute oral toxicity, groups of six male and six female (except where indicated) Fischer 344 rats were given racemic haloxyfop acid (purity not reported) as a single oral dose at 250, 320 (males only), 400 (males only), 500, 1000 or 2000 mg/kg bw dissolved in a mixture of acetone and corn oil by gavage. The rats were observed for 15 days after dosing and were then killed for autopsy. All rats at 1000 or 2000 mg/kg bw died within 2 days and several rats from all of the groups at 320 mg/kg bw or more died during the 15-day observation period. The reported signs of toxicity were lethargy, rough coat, body tremors, hypersensitivity to stimuli, decreased appetite and dark exudate-staining around the eyes. Autopsies showed swollen livers with an accentuated lobular pattern in some rats. No treatment-related effects were seen in the rats at 250 mg/kg bw. The NOAEL was 250 mg/kg bw. The oral LD_{50} was 337 mg/kg bw for male rats and 545 mg/kg bw for females (Carreon et al., 1980).

Monkeys

In a GLP-compliant study of the acute oral toxicity of the sodium salt of racemic haloxyfop (purity, 99.6%), single doses at 40, 80 or 120 mg/kg bw or five daily doses at 20, 40 or 60 mg/kg bw per day were given to individual male cynomolgus monkeys by nasogastric intubation. The single dose of 40 mg/kg bw per day was well tolerated. Food consumption was decreased in the monkeys at 80 or 120 mg/kg bw, but returned to normal by 72 h after dosing. The monkey at 120 mg/kg bw also showed decreased physical activity for the 4 h after dosing. The repeated dose of 20 mg/kg bw per day

was well tolerated for the 5 days of treatment. The monkey at 40 mg/kg bw per day for 5 days showed a slight reduction in food consumption for 8 days and a concomitant reduction in body weight, but no effect was seen on physical activity or appearance. The treatment of the monkey at 60 mg/kg bw per day was stopped after 2 days, due to marked inappetance and emesis. This monkey continued to have decreased food consumption and had a concomitant loss of body weight; it showed general weakness, decreased activity and trembling with a progressive deterioration in physical condition. Gross and histopathological findings for the monkey given repeated doses of 60 mg/kg bw per day revealed lesions that were compatible with an infectious process and the pathology report attributed the progressive physical deterioration to an encephalopathy of unknown infectious etiology (Gerbig et al., 1985).

(ii) Dermal administration

Rats

The acute percutaneous toxicity of haloxyfop-R methyl ester was evaluated in a GLP-compliant study. Groups of five male and five female Fischer 344 rats were given a single dermal exposure at 2000 mg/kg bw on their clipped backs. The application site was kept under occlusive dressing for 24 h and was then washed. The rats were observed in the days after treatment and were killed at 15 days after treatment. No adverse effects were seen on mortality or body-weight gain. The only effects seen were lethargy and eyelid closure that were reported in two of the five males, but not in any females (Mizell et al., 1989).

(b) Dermal and ocular irritation

In a GLP-compliant study, 0.5 ml of undiluted haloxyfop-R methyl ester (purity, 95.7%) was applied to the clipped skin of three male and three female New Zealand White rabbits. The application site was kept under occlusive dressing for 4 h and was then wiped clean. Observations over the following 72 h indicated that haloxyfop-R methyl ester is not an irritant to skin (Mizell, 1989a).

The potential of undiluted haloxyfop-R methyl ester (purity, 98.6%) to cause eye irritation was investigated in a GLP-compliant study in three male and three female New Zealand White rabbits. Haloxyfop-R methyl ester did not cause eye irritation in this test (Mizell, 1989b)

(c) Dermal sensitization

The skin sensitization potential of haloxyfop-R methyl ester (purity, 98.6%) was tested in a GLP-compliant Buehler test in male Hartley guinea-pigs. The study used 20 guinea-pigs (10 receiving haloxyfop-R methyl ester and 10 positive controls. Haloxyfop-R methyl ester did not induce skin sensitization in this test. (Mizell, 1989c)

Undiluted haloxyfop-R methyl ester (purity not reported) was tested for skin sensitization potential in a Magnusson & Kligman maximization test, which was performed in accordance with GLP. Female Hartley guinea-pigs were used (10 receiving haloxyfop-R methyl ester and 5 controls). Haloxyfop-R methyl ester showed no skin sensitization potential in this test (Jones, 1994)

2.2 Short-term studies of toxicity

Mice

A 13-week feeding study studying mice was performed in accordance with GLP. Groups of 10 male and 10 female B6C3F_1 mice were fed diets containing racemic haloxyfop acid (purity,

96%) at a concentration designed to give doses of 0.002, 0.02, 0.2 and 2 mg/kg bw per day. A control group of 15 males and 15 females received basal diet. All mice were killed at the end of the treatment period and autopsied. Weights of brain, heart, liver, kidneys and testes were recorded. Terminal samples of blood were taken for haematology and clinical chemistry. A wide range of tissues from controls and mice at the highest dose were examined microscopically. For the other treatment groups, only the liver, gall bladder and kidneys were examined microscopically. There were no treatment-related deaths during the study and no abnormal behaviour or signs of toxicity were observed. There was reduced body weight in the females at 2 mg/kg bw per day when compared with concurrent controls from the sixth day of treatment onwards, but the body weights of all groups of males were unaffected. Food consumption was unaffected by treatment. There were no treatment-related effects on haematological parameters. Clinical chemistry results showed a statistically significant increase ($p < 0.05$) in serum alkaline phosphatase (AP) activity in the group of males at the highest dose (2 mg/kg bw per day) (22% greater than control values) and a small non-significant increase in this parameter in females (8% greater than control values). Absolute and relative weights of liver were also increased in male and female mice in the group at the highest dose and the livers appeared slightly enlarged and dark at autopsy. Microscopically, the centrilobular hepatocytes appeared enlarged in all males and 8 out of 10 females in the group at the highest dose and the cytoplasm of these cells was more eosinophilic and appeared more homogeneous than in controls. It is likely that the hepatocellular hyperplasia seen in this study was caused by a mode of action involving hepatocellular peroxisome proliferation that is not relevant to humans. The NOAEL was 0.2 mg/kg bw per day, on the basis of increased serum AP activity at 2 mg/kg bw per day (Gorzinski et al., 1982a).

In a 36-week feeding study that was performed in accordance with GLP, groups of 12 male and 12 female $B6C3F_1$ mice were fed diets containing racemic haloxyfop acid (purity, 96%) at a concentration designed to give dosaes of 0 (control group) or 2 mg/kg bw per day. An additional group of 10 males and 10 females was given a dose of 0.02 mg/kg bw per day. All mice were killed at the end of the treatment period and autopsied. Weights of brain, heart, liver, kidneys and testes were recorded. Terminal samples of blood were taken for haematology and clinical chemistry. Only the liver, gall bladder and kidneys were examined microscopically. Two males and one female from the group at the highest dose and one female from the group at the lowest dose died during the treatment period. No abnormal behaviour or signs of toxicity were observed and body weights and food consumption were unaffected by treatment. Haematology showed significantly decreased erythrocyte count in females at the highest dose but increased erythrocyte count and decreased erythrocyte volume fraction in the group at the lowest dose. These changes were not thought to be treatment-related. Clinical chemistry results showed increases in serum AP activity in males at the highest dose (105% greater than control values and statistically significant) and in females (8% greater than control values and non-significant). Absolute and relative weights of liver and kidney were increased in male and female mice in the group at the highest dose and the livers appeared slightly enlarged and dark at autopsy. Microscopically, the centrilobular hepatocytes appeared enlarged in all males and in 8 out of 10 females in the group at the highest dose. Also, the cytoplasm of these cells was more eosinophilic and appeared more homogeneous than in controls. One male mouse at the highest dose had a small focus of basophilic hepatocytes which the authors of the study suggested was consistent with the hepatocellular adenoma or type A nodule that is frequently seen in $B6C3F_1$ mice. It is likely that the hepatocellular hyperplasia seen in this study was caused by a mode of action involving hepatocellular peroxisome proliferation that is not relevant to humans. The kidneys of the males at the highest dose had decreased cytoplasmic vacuolation of the proximal convoluted tubule cells. The NOAEL was 0.02 mg/kg bw per day on the basis of increased serum AP activity and effects on the renal proximal convoluted tubules seen at 2 mg/kg bw per day (Gorzinski et al., 1982a).

Rats

In a 4-week probe study that was performed to GLP standards, groups of five male and five female Fischer 344 rats were given diets containing purified (purity, 99.99%) or technical-grade (purity, 99%) racemic haloxyfop acid at concentrations that gave doses of 0.01, 0.1, 1 or 10 mg/kg bw per day. A group of 10 males and 10 females was kept as controls. At the end of the treatment period, urine was collected for urine analysis (specific gravity, pH, glucose, protein, ketones, occult blood, bilirubin and urobilinogen) and blood was taken for haematology (total leukocyte count, differential leukocyte count, erythrocyte count, erythrocyte volume fraction, haemoglobin, platelets and erythrocyte morphology) and clinical chemistry (AP, alanine aminotransferase [ALT], glucose, blood urea nitrogen [BUN], total protein, albumin and globulin). All rats were then killed for autopsy. Weights of brain, liver, kidneys, heart, thymus and testes were recorded. A wide range of tissues from each rat were fixed, but only liver, kidneys, testes and epididymes were examined microscopically. The treatment did not affect mortality, behaviour, clinical signs and body weight or food consumption. Haematology showed that males at the highest dose, given either purified or technical-grade material, had statistically significant ($p < 0.05$) decreases in erythrocyte count, haemoglobin and erythrocyte volume fraction. Clinical chemistry showed statistically significant ($p < 0.05$) increases in serum AP activity, serum glucose and serum albumin in males given either technical or purified compound. Urine analysis parameters were unaffected by treatment. At autopsy, visibly enlarged livers were seen in all groups at the highest dose and in males at 1 mg/kg bw per day. Statistically significant increases ($p < 0.05$) in relative liver weight were seen in all groups at 10 mg/kg bw per day, and the males given a dose of 1 mg/kg bw per day of either test material showed a smaller increase that was not statistically significant. Microscopic examination of the liver showed swollen hepatocytes with eosinophilic cytoplasm that involved the entire hepatic lobule in males and females given 10 mg/kg bw per day of either test material, but was confined to the centrilobular area in males given 1 mg/kg bw per day of either material. Some of the males given 10 mg/kg bw per day of either material showed a slight decrease in the number of mature spermatozoa in the testes and epididymes. It was concluded that there was no difference in toxicity between the technical racemic haloxyfop acid and purified racemic haloxyfop acid. The NOAEL was 0.1 mg/kg bw per day on the basis of hepatic changes in males that were of no significance to humans. The NOAEL for effects that were relevant to the assessment of risk to humans was 1 mg/kg bw per day on the basis of changes in haematological and clinical chemistry parameters at 10 mg/kg bw per day (Gorzinski et al., 1982b).

In a GLP-compliant study, groups of 80 male Fischer 344 rats were given diets containing racemic haloxyfop acid (purity, 99.5%) for a 4-week challenge period, followed by a 6-week recovery period during which all rats received control diet. The doses given were 0 (control), 0.1 and 1.0 mg/kg bw per day. At 2-week intervals throughout the challenge and recovery periods, 7–10 rats from each group were killed, blood was collected and pooled serum for each group was analysed for haloxyfop. All rats were autopsied and liver weights were recorded. A wide range of tissues from each rat were fixed, but only liver was examined microscopically. The treatment had no effect on mortality, behaviour, clinical signs and body weight or food consumption. Serum concentrations of haloxyfop at each sample time were approximately proportionate to the doses given. The plasma clearance half-life was 8 days for both doses. Visibly enlarged livers were seen in the group of rats at the highest dose at both sacrifices during the challenge period, but no such effect was seen at any of the sacrifices during the recovery period (i.e. 2 weeks or more after dosing). Statistically significant increases in absolute and relative weights of liver were observed at both doses at the 2-week challenge sacrifice and in the group at the highest dose at the 4-week challenge sacrifice. Histopathological changes were seen in the livers of the group of rats at the highest dose at both challenge sacrifices, but no effects were seen at the lowest dose or in any group during the recovery period. The hepatic change reported

was centrilobular hepatocellular hyperplasia, accompanied by increased cytoplasmic eosinophilia. As the statistically significant increase in liver weight seen in the group at the lowest dose was not associated with any gross pathology or histopathology, this finding was regarded as fortuitous.

The NOAEL was 0.1 mg/kg bw per day on the basis of hepatocellular hyperplasia at 1.0 mg/kg bw per day. This effect was likely to be produced by a mode of action involving hepatocellular peroxisome proliferation and is of no relevance to humans. The NOAEL for effects of relevance to humans was the highest dose tested, 1 mg/kg bw per day (Herman et al., 1983).

In a GLP-compliant feeding study, groups of 10 male and 10 female Fischer 344 rats were fed diets containing haloxyfop-R acid (purity, 99.4%), receiving doses of 0 (control), 0.065, 0.2 or 2 mg/kg bw per day for 16 weeks. Additional groups of 10 males and females received doses of 0 or 2 mg/kg bw per day for 16 weeks, followed by a 4-week recovery period during which all rats received control diet. At the end of the study period for each group (with or without recovery), blood was collected from each rat for haematology (total leukocyte count, differential leukocyte count, erythrocyte count, erythrocyte volume fraction, haemoglobin and platelets) and clinical chemistry (glucose, BUN, creatinine, calcium, phosphorus, triglycerides, total protein, albumin, globulin, AP, ALT and aspartate aminotransferase (AST). Urine was collected at termination for urine analysis (specific gravity, pH, glucose, protein, ketones, occult blood, bilirubin and urobilinogen). All rats were killed for autopsy and weights of adrenals, brain, liver, kidneys, heart, thymus and testes or ovaries were recorded. A wide range of tissues from the groups of control rats and rats at the highest dose that were killed at the end of the challenge period were examined microscopically. For the other doses and the recovery groups, only a selection of tissues was examined, including liver, kidneys and lungs. The treatment had no effect on mortality, behaviour, clinical signs and body weight or food consumption. Small but statistically significant ($p < 0.05$) decreases in erythrocyte count, haemoglobin, and erythrocyte volume fraction were seen in males at the highest dose at the end of the treatment period and after the recovery period, but these effects were too small to be regarded as toxicologically significant. No adverse effects were seen in bone marrow. Clinical chemistry showed statistically significant ($p < 0.05$) increases in serum AP activity in males and females of the group at the highest dose after 16 weeks of treatment, but the effect was not seen after the 4-week recovery period. Serum cholesterol was significantly ($p < 0.05$) decreased at the end of the treatment period in males at 0.2 or 2 mg/kg bw per day and a small but non-significant decrease was seen in females at 2 mg/kg bw per day. The decreases in serum cholesterol were too small to be regarded as being toxicologically significant. By the end of the recovery period, serum cholesterol concentrations were back to normal. There were no effects on urine analysis parameters.

The treatment caused no gross lesions. Statistically significant ($p < 0.05$) increases in absolute and relative liver weights were seen in males and females of the group at the highest dose at the end of the treatment period and significant increases ($p < 0.05$) were also seen males given 0.2 mg/kg bw per day at the end of treatment and in males at the highest dose after the recovery period. Absolute and relative weights of testes were significantly ($p < 0.05$) decreased in males at the highest dose at the end of treatment and after the recovery period, but no histopathology was seen in the testes. In the livers of males and females of rats killed directly after treatment at 2 mg/kg bw per day, there was a slight hypertrophy of the centrilobular hepatocytes, accompanied by an increased eosinophilia of these cells. These hepatic changes were not apparent in rats killed after the recovery period. The hepatic changes were likely to be associated with a hepatocellular hypertrophy mediated by peroxisome proliferation and were not regarded as relevant to humans.

The NOAEL was 0.065 mg/kg bw per day on the basis of an increase in liver weight in males at 0.2 mg/kg bw per day. The NOAEL for effects relevant to humans was the highest dose tested, 2 mg/kg bw per day (Barna-Lloyd et al., 1989).

In a GLP-compliant study, groups of 15 male and 15 female Fischer 344 rats were fed diets containing racemic haloxyfop acid (purity, 96%), receiving doses of 0 (control), 0.002, 0.02, 0.2 or 2.0 mg/kg bw per day for 16 weeks. Blood samples were taken from 10 males and 10 females per group after 84, 99 and 106/7 days on test and haematology (total leukocyte count, differential leukocyte count, erythrocyte count, erythrocyte volume fraction and haemoglobin) was performed. Urine analysis (specific gravity, pH, glucose, protein, ketones, occult blood, bilirubin and urobilinogen) was performed at the end of the treatment period. All rats were killed for autopsy at the end of the treatment period and the following organs were weighed: brain, liver, kidneys, heart, thymus and testes. Clinical chemistry (glucose, AP, ALT, BUN, total protein, albumin and globulin) was performed on serum from blood taken at autopsy. Histopathology was performed on a wide range of tissues from 10 males and 10 females of the control group and group at the highest dose. For the other treatment groups, only the liver was examined microscopically.

The treatment had no effect on mortality, behaviour, clinical signs and body weight or food consumption. However, the growth of several of the rats in all groups was adversely affected by a viral infection. There were no treatment-related effects on haematological parameters. Serum AP activity was significantly ($p < 0.05$) increased by 24% in males given 2.0 mg/kg bw per day. Urine analysis showed no treatment-related effects. Absolute and relative liver weights were significantly ($p < 0.05$) increased in the group of females at the highest dose. In males, there were dose-related increases in absolute and relative liver weights, with the absolute weight being significant ($p < 0.05$) for the groups given 0.2 or 2.0 mg/kg bw per day and the increase in relative liver weight being significant ($p < 0.05$) at all doses tested. Small but significant ($p < 0.05$) increases in the relative weights of heart and kidneys were seen in females at 0.02 or 2.0 mg/kg bw per day. At autopsy, all males at the highest dose and two of the males at 0.2 mg/kg bw per day had visibly enlarged livers and several of the rats of either sex at 0.2 or 2.0 mg/kg bw per day had discoloured kidneys (darkened or greenish appearance). Microscopic examination showed hepatocellular hypertrophy in males at 0.2 or 2.0 mg/kg bw per day, with the effect being more severe at the highest dose. The hepatocytes of males and females at the highest dose had an increased degree of cytoplasmic homogeneity. No renal histopathology was seen.

The NOAEL was 0.02 mg/kg bw per day on the basis of hepatocellular hypertrophy (not relevant to humans) seen at 0.2 mg/kg bw per day or more. The small but statistically significant increases in the weights of liver, heart and kidneys that were seen at 0.02 mg/kg bw per day or less were not considered to be toxicologically significant as they were not associated with any effects on clinical chemistry or histopathology. The NOAEL for effects of relevance to humans was 0.2 mg/kg bw per day on the basis of the slightly increased serum AP activity at 2 mg/kg bw per day (Gorzinski et al., 1982c).

In a GLP-compliant study, groups of 12 male and 12 female Fischer 344 rats were fed diets providing racemic haloxyfop acid (purity, 96%) at a dose of 0 (control), 0.02 or 2.0 mg/kg bw per day for 37 weeks. Blood samples were taken from eight males and eight females per group after 258 days and haematology was performed (total leukocyte count, differential leukocyte count, erythrocyte count, erythrocyte volume fraction and haemoglobin). Urine analysis (specific gravity, pH, glucose, protein, ketones, occult blood, bilirubin and urobilinogen) was performed at the end of the treatment period. All rats were killed for autopsy at the end of the treatment period and the following organs were weighed: brain, liver, kidneys, heart, thymus and testes. Clinical chemistry (glucose, AP, ALT, BUN, total protein, albumin and globulin) was performed on serum from blood taken at autopsy. The liver and kidneys of all rats were examined microscopically. In addition, sections of kidney from five males in the control group and the group at the highest dose were treated with special stains (Zeihl Nielson acid fast, PAS, Gomori iron, Von Kossa calcium, Hall bilirubin and Dahl calcium) and were examined by microscope.

The treatment had no effect on mortality, behaviour, clinical signs and body weight or food consumption. However, the growth of several of the rats in all groups was adversely affected by a viral infection. There were no treatment-related effects on haematological parameters. Serum AP activity was significantly ($p < 0.05$) increased in males and females at 2.0 mg/kg bw per day. Urine analysis showed no treatment-related effects. Absolute and relative liver weights were significantly ($p < 0.05$) increased in groups of males and females at the highest dose. At autopsy, all males at the highest dose had enlarged livers and most (13 out of 15) also had darkened kidneys. Microscopy showed hepatocellular hyperplasia with cytoplasm of homogeneous appearance in males and females at the highest dose. In addition, there was a moderate increase in the amount of lipofuscin in renal tubular epithelial cells of males and (to a lesser extent) females at the highest dose. It was noted that this pigment can occur naturally in this strain of rat but has also been associated with exposure to peroxisome proliferators.

The NOAEL was 0.02 mg/kg bw per day on the basis of hepatocellular hypertrophy seen at 2.0 mg/kg bw per day. This effect was likely to have been produced by a mode of action involving hepatocellular hypertrophy that is not relevant to humans. The NOAEL for effects of relevance to humans was 0.02 mg/kg bw per day for increased serum AP activity at 2 mg/kg bw per day (Gorzinski et al., 1982c).

The dermal toxicity of the formulation EF-1400 has been evaluated in a GLP-compliant study in Fischer 344 rats. EF-1400 contained haloxyfop-R methyl ester at a concentration of 111 g/l. Groups of 10 males and 10 females were given EF-1400 as dermal doses (semi-occluded) at 0, 40, 200 or 1000 mg/kg bw per application, 6 h per day and 5 days per week for 13 weeks. The highest dose caused slight erythema, oedema, and/or scaling of the skin with very slight to slight hyperkeratosis and acanthosis. Dose-related decreases in erythrocyte count, haemoglobin and erythrocyte volume fraction were seen in males at 200 or 1000 mg/kg bw, and erythrocyte count was also decreased in males at 40 mg/kg bw. A treatment-related increase in the amount of hyaline droplets in the renal tubular epithelium was seen in males at all doses. Increased liver weight, increased serum AP activity and hepatocellular hypertrophy were seen in males at all doses and in females at the highest dose. Ultrastructural examination by electron microscope of centrilobular hepatocytes from rats at the highest dose showed increased numbers of peroxisomes in males but not females. No NOAEL was identified in this study (Stebbins et al., 2001).

Dogs

In a 5-week pilot feeding study, groups of two male and two female beagle dogs were given diets providing racemic haloxyfop acid (purity, 99.6%) at a dose of 0, 5, 15, or 45 mg/kg bw per day. The treatment at the highest dose caused gastroduodenal ulcers (males only), body-weight loss, decreased food consumption, decreases in serum cholesterol, erythrocyte volume fraction, erythrocyte count, haemoglobin and platelet count and increases in serum ALT, AP, bromosulfthalein retention and weights of liver and kidneys. At the intermediate dose there was reduced body-weight gain (males only), decreased serum cholesterol and increased liver weight (males only). No treatment-related effects were seen at the lowest dose. The NOAEL was 5 mg/kg bw per day (Barna-Lloyd et al., 1984).

In a GLP-compliant 13-week study, groups of four male and four female beagle dogs were given diets providing racemic haloxyfop acid (purity, 99.8%) at a dose of 0, 2, 5 or 20 mg/kg bw per day. Ophthalmology was performed on all dogs before treatment and at the end of the treatment period. Blood was taken for haematology (total leukocyte count, differential leukocyte count, erythrocyte count, erythrocyte volume fraction, haemoglobin and platelets) and clinical chemistry (glucose, AP, ALT, AST, BUN, total protein, albumin, globulin, Ca, Na, K, PO_4 and Cl)

before treatment and after 1, 2 and 3 months of treatment. The concentrations of several thyroid hormones (free-T3, free-T4, total-T3 and total-T4) were measured in the serum taken at 3 months. Urine was taken at 3 months for urine analysis (specific gravity, pH, glucose, protein, ketones, occult blood, bilirubin, urobilinogen, colour and microscopy of sediment). All dogs were killed at the end of the treatment period and autopsied and the weights of adrenals, brain, kidneys, liver, pituitary, thyroid (including parathyroid), ovaries and testes were recorded. An extensive range of tissues from all dogs in the control group and the group at the highest dose was examined by light microscopy, but the histopathology performed on the other dogs was limited to the following tissues: adrenals, epididymes, kidneys, liver, thyroid, parathyroid, pituitary, prostate, skin, testes, thymus and tonsils. Samples of liver were taken for measurement of hepatic fatty acid beta-oxidation (FAOX; by measuring cyanide-insensitive beta-oxidation of palmitoyl-CoA) in vitro and for electron microscopy.

No dogs died during the treatment period and no treatment-related clinical signs were observed. Body weights of males and females in the group at the highest dose became statistically significantly lower ($p < 0.05$) than the concurrent body weights of controls, but there was no effect on food consumption. No treatment-related effects were revealed by ophthalmoscopy. Small but statistically significant ($p < 0.05$) haematological changes were seen in the groups of dogs at the highest dose, for males and females: decreases in erythrocyte count, haemoglobin, erythrocyte volume fraction and platelet count. Clinical chemistry results showed that there were small dose-related decreases in serum cholesterol in males and females throughout the study that were statistically significant ($p < 0.05$) in all treated groups of males and in females at 5 and 20 mg/kg bw per day. The reduction in serum cholesterol concentrations was considered to be too small to be of toxicological significance. In addition, significant ($p < 0.05$) decreases in the serum concentrations of total triiodothyronine (T3), free T3 and total thyroxin (T4) were found in male and female dogs at 5 or 20 mg/kg bw per day. Absolute and relative weight of the thyroid/parathyroid was significantly ($p < 0.05$) decreased in males and females at 5 and 20 mg/kg bw per day and was associated with slight decreases in follicular size and hypertrophy of follicular epithelial cells. Relative kidney weights were increased in males and females in the group at the highest dose, but no renal pathology was seen and this effect was thought to be due to the loss of body fat by this group (as reflected in their lower body weight). Absolute liver weight, relative to controls, was significantly ($p < 0.05$) increased in males at 5 or 20 mg/kg bw per day and in females at 20 mg/kg bw per day; relative liver weight was significantly increased in males and females at 20 mg/kg bw per day. Light microscopy showed slight hepatocellular hypertrophy in males and females at 20 mg/kg bw per day and in females at 5 mg/kg bw per day that was shown to be due to increased stores of glycogen (vacuoles stained positive for glycogen but negative for fat). Electron microscopy showed no treatment-related changes. Hepatic activity of fatty acid beta-oxidation (FAOX) significantly increased in males and females at 5 and 20 mg/kg bw per day, but not at 2 mg/kg bw per day.

The NOAEL was 2 mg/kg bw per day on the basis of various effects seen at 5 and 20 mg/kg bw per day, including decreased serum thyroid hormone levels with associated morphological changes to the thyroid and induction of hepatic FAOX (Dietz et al., 1987).

In a GLP-compliant study, groups of six male and six female beagle dogs were given diets providing racemic haloxyfop acid (purity, 99.6%) at a dose of 0, 0.05, 0.5 or 5.0 mg/kg bw per day for 12 months. Blood was taken before treatment and after 1, 3, 6, 9 and 12 months of treatment for haematology (total leukocyte count, differential leukocyte count, erythrocyte count, erythrocyte volume fraction, haemoglobin, platelets, prothrombin time and reticulocyte count) and clinical chemistry (glucose, ALT, AST, AP, BUN, cholesterol, total protein, albumin, globulin, total bilirubin and triglycerides). In addition, liver function was tested by bromosulfthalein retention measurements at 6 and 12 months. Urine was collected for urine analysis before treatment and at 6 and 12 months.

All dogs were killed for autopsy at the end of the treatment period. The following organs were weighed: brain, heart, liver, kidneys, adrenals and ovaries or testes. Histopathology was performed on a wide range of tissues from dogs in the control group and the group at the highest dose. For the dogs in other treatment groups, histopathology was performed on only liver, kidney, bone marrow and bone.

The treatment had no effect on mortality, clinical signs, body-weight gain or food intake. Some of the measurements of erythrocyte volume fraction, erythrocyte count and haemoglobin were statistically significantly lower than concurrent control values, but comparison with historical control values showed that the concurrent control values were unusually high, so the effect was not considered to be treatment-related. Serum ALT and AST activities were significantly increased in the group of females receiving the highest dose at 12 months and ALT was also high in this group at 9 months. Serum cholesterol concentration was significantly decreased in males at the highest dose at 1, 6, 9 and 12 months. The reductions in concentrations of serum cholesterol and the increases in serum AST and ALT were too small to be of toxicological significance. Urine analysis parameters were unaffected by treatment. No treatment-related gross pathology was noted at autopsy and there was no effect on organ weights or histopathology. Bone marrow from all dogs appeared normal. The NOEL was 0.5 mg/kg bw per day (Barna-Lloyd et al., 1983 & 1984).

Liver samples from dogs in this study were later examined by electron microscopy. The results are summarized in section 2.7 (Barnard et al., 1986); no adverse effects of treatment were observed.

Monkeys

In a GLP-compliant 4-week study, groups of two male cynomolgus monkeys were given the sodium salt of racemic haloxyfop (purity, 99.6%) at a dose of 0, 5 or 20 mg/kg bw per day in distilled water by nasogastric intubation. An additional two monkeys were given a dose of 20 mg/kg bw per day for 4 weeks, followed by a 4-week recovery period. Data were collected on clinical appearance, behaviour, body weights, food consumption, haematology and clinical chemistry. At the end of the study, all monkeys were killed, autopsies were performed, organs were weighed and histopathological examinations were made of the major organs. Samples of liver and kidney were prepared for examination by electron microscopy, but only the liver samples were examined. The electron micrographs of the centrilobular area of livers were examined at 8000 × magnification for peroxisomal volume density, which was expressed as a percentage of cytoplasmic volume. The results showed no effects on mortality, clinical signs, body weight, food consumption, haematology, organ weights, gross pathology or histopathology. The only clinical chemistry parameter affected was serum cholesterol, which was slightly reduced at 20 mg/kg bw per day at the end of treatment, but was similar to control values in the monkeys that had a recovery period after treatment. In the absence of other effects, the reversible reduction in plasma cholesterol was not regarded as an adverse effect. Electron microscopy showed no effects on the appearance of liver tissue or on hepatocellular peroxisomal volume density. The NOAEL was the highest dose, 20 mg/kg bw per day (Gerbig et al., 1985).

The investigation of peroxisome proliferation in this study is also mentioned in section 2.8.

In a GLP-compliant 13-week study, groups of four male and four female cynomolgus monkeys were given racemic haloxyfop acid (purity, 99.8%) at a dose of 0, 2, 10 or 30 mg/kg bw per day dissolved in buffered water by nasogastric intubation. The monkeys were observed for clinical signs daily, body weights were measured weekly and eyes were examined with an ophthalmoscope at the beginning and end of the study. Blood samples were collected for haematology and clinical chemistry before the start of treatment (days −27 and −6) and during the treatment period (days 37, 66 and 92). Urine samples were collected for analysis before treatment (days −12 and −13) and towards the end of treatment (day 86). The monkeys were killed for autopsy at the end of 92 days of treatment.

Organs were weighed and histopathology was performed on major organs. Livers from the control group and the group at the highest dose were evaluated for electron microscopic and peroxisomal fatty acid beta-oxidation (FAOX) enzyme changes (by measuring beta-oxidation of palmitoyl-CoA in the presence of cyanide).

There was no mortality in the study and no treatment-related clinical signs were seen. The body weights of the groups of males and females at the highest dose were higher than those of the controls at the start of the treatment period and remained so throughout the study, but there was no effect on body-weight gain. Small but statistically significant decreases in erythrocyte count, haemoglobin and erythrocyte volume fraction were seen in males and females at 30 mg/kg bw per day, but smears of peripheral blood and bone marrow appeared normal, with differential cell counts similar to those for controls. Clinical chemistry showed statistically significant decreases in concentrations of serum triglycerides in males and females at all doses, but the values were not dose-related and were all within the range of values for historical controls. Urine analysis and ophthalmology revealed no adverse effects. Organ weights of liver and kidney were significantly increased in males and females of the group at the highest dose and kidney weight was also increased in males at 10 mg/kg bw per day. Thyroid weights were significantly decreased in males and females at the highest dose. At autopsy, thyroids were visibly smaller in the group of monkeys at the highest dose than in controls. The livers of the females at 10 mg/kg bw per day and males and females at 30 mg/kg bw per day had a pale appearance with an accentuated lobular pattern. Light microscopy showed slight hepatocellular hypertrophy with increased numbers of fatty vacuoles in monkeys at 10 or 30 mg/kg bw per day. In the thyroid there was decreased follicular size and hypertrophy of follicular epithelial cells in males and females at the highest dose and one female in the group at 10 mg/kg bw per day was similarly affected. No histopathological changes were seen in the kidneys. Ultrastructural evaluation by electron microscopy of centrilobular hepatocytes showed the livers of monkeys at the highest dose to have a slightly higher cytoplasmic lipid volume density and larger numbers of small fat droplets than controls. Peroxisomal volume densities were similar for controls and monkeys at the highest dose. Hepatic peroxisomal FAOX enzyme activity was not significantly different from that in controls at 30 mg/kg bw per day for males and females. The NOAEL was 2 mg/kg bw per day on the basis of changes to the liver and thyroid at 10 mg/kg bw per day or more (Yano et al., 1987).

The investigation of peroxisome proliferation in this study is also mentioned in section 2.7.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a GLP-compliant long-term study of combined toxicity and carcinogenicity, groups of 70 male and 70 female B6C3F_1 mice were fed diets providing racemic haloxyfop acid (purity, 99.6%) at a dose of 0, 0.03, 0.065 or 0.6 mg/kg bw per day. Satellite groups of 10 males and 10 females were killed at 6 and 12 months and the remaining 50 male and 50 female mice per dose were killed after 104 weeks of treatment. Terminal samples of blood were collected from mice in the main group and satellite groups for haematology and clinical chemistry, and the mice were autopsied with organ weights being recorded and microscopic examinations being performed.

There was no treatment-related effect on mortality, with 84–90% of males and 78–80% of females (non-satellite groups) surviving the scheduled 104 weeks. Body weights and feed consumption were unaffected by the treatment. No adverse haematological effects were reported at 6, 12 or 24 months. Clinical chemistry showed significantly elevated serum AP activity (16% increase relative to controls) in the group of males at the highest dose at the 12-month interim killing, but this parameter was not affected in females or in males at other time-points and there was no dose–response relationship. Other clinical chemistry parameters were unaffected. Also in males at the highest dose at 12 months, there was a statistically significant ($p < 0.05$) increase in relative liver weight of 14% compared

with that of controls (but no significant increase in absolute liver weight). Females of the group at the intermediate dose (0.065 mg/kg bw per day) had significantly reduced kidney weights (7% less than those of controls). Gross pathology on the mice killed at 24 months, showed a dose–response relationship to the number of mice with one or more liver masses/nodules, with incidences of 22, 30, 32 and 34% in males and of 12, 14, 26 and 26% in females at 0, 0.03, 0.065 and 0.6 mg/kg bw per day, respectively. Microscopic examinations of tissues showed a number of treatment-related lesions in the liver. The livers of the groups of males and females at the highest dose killed after 6, 12 or 24 months showed a slight increase in eosinophilia of the cytoplasm of hepatocytes in the centilobular region and a concomitant decrease in the amount of cytoplasmic vacuolation. Histopathological examination of the livers of the main groups of mice, that had been scheduled to be killed after 24 months, showed that there were some significant increases in the incidence of certain tumours in the group at the highest dose (see Table 3). The incidences of males with adenomas, males with adenomas or carcinomas and of females with carcinomas were all significantly increased, in comparison with controls. In addition, there was a significant positive dose-related trend towards increased incidence of adenomas or carcinomas. Only the increased incidence of carcinomas in females at the highest dose was above the range of data for historical controls from 11 earlier studies in the same strain of mice.

The NOAEL was 0.065 mg/kg bw per day on the basis of neoplastic and non-neoplastic hepatic changes at 0.6 mg/kg bw per day. The Meeting concluded that the results of this study indicated that high doses of haloxyfop could be tumorigenic to mice. The NOAEL for effects of relevance to humans was the highest dose tested, 0.6 mg/kg bw per day (Tollett et al., 1985).

Table 3. Liver tumours in mice in the main groups scheduled to be killed after 2 months

Finding	Dose (mg/kg bw per day)							
	Males				Females			
	0	0.03	0.065	0.6	0	0.03	0.065	0.6
Group size	50	50	50	50	50	50	50	50
Adenoma	8	10	15	19*	6	6	12	10
Carcinoma	5	8	7	9	1	3	2	8*
Adenoma or carcinoma	13	15	20	27*	7**	9	13	15

From Tollett et al. (1985)

* Value that is significantly different from the control value ($p < 0.05$).

** Significant dose–response relationship ($p < 0.05$).

Rats

In a GLP-compliant long-term study of combined toxicity and carcinogenicity, groups of 90 male and 90 female Fischer 344 rats were fed diets providing racemic haloxyfop acid (purity, 99.6%) at a dose of 0, 0.01, 0.03, 0.065 or 0.1 mg/kg bw per day for males, and 0, 0.01, 0.03, 0.065 or 1.0 mg/kg bw per day for females. Satellite groups of 20 males and females were killed at 6 and 12 months and the remaining 50 rats of each sex per dose were killed after 24 months of treatment. Data on clinical appearance, behaviour, body weights and feed consumption were recorded throughout the study. Haematology, clinical chemistry and urine analysis were conducted at 6 and 12 months for the satellite groups. For the main group, samples of blood and urine were taken for analysis at 18 and 24 months. Gross pathology, organ weights and histopathology were conducted at termination (6, 12 and 24 months).

At 2 years, survival of the various groups was 70–84% for males and 76–88% for females, with no dose–response relationship. Clinical signs, body weight and feed consumption were unaffected by

treatment. At 6 and 12 months, there were small and transient but statistically significant differences in some haematological parameters (erythrocyte count, haemoglobin, and erythrocyte volume fraction) from control values in various treated groups. The effects were not dose-related and there were no effects on these parameters seen at 18 and 24 months, so the haematological effects were not considered to be due to the treatment. Clinical chemistry showed significant increases in serum ALT in males at 0.065 and 0.1 mg/kg bw per day at 6 and 12 months. There were also significant decreases in serum globulin concentrations in males at 0.1 mg/kg bw per day at 6 months and in females at 1.0 mg/kg bw per day at 12 months. There was no effect on urine analysis parameters. No effects on organ weights were seen in the main groups of rats (24-month kill) or at the 12-month interim kill, but relative liver weights were increased at the 6-month interim kill in males at 0.1 mg/kg bw per day and males and females at 0.065 mg/kg bw per day.

The only treatment-related effect reported at autopsy was a dark or greenish discoloration of the renal cortex in the groups at the highest dose for males and females (0.1 mg/kg bw per day for males; 1.0 mg/kg bw per day for females). Histopathology revealed hepatocellular hypertrophy and cytoplasmic eosinophilia in the centrilobular region of the liver of the males and females at the highest dose at 6 months and in the females at the highest dose at 12 months. From 6 months onwards, an iron-containing pigment, that was suspected to be lipofuscin, was found in the proximal convoluted tubules of the kidneys of the group of rats at the highest dose. In the pancreas, adjacent to the islets of Langerhans of one male at the highest dose (0.1 mg/kg bw per day) and two females at the highest dose (1.0 mg/kg bw per day), there were focal accumulations of cells that appeared to be normal hepatocytes. The biological significance of this "ectopic hepatocellular tissue" is unclear. There was no evidence of an increased incidence of any benign or malignant neoplasm at any site.

The NOAEL was 0.065 mg/kg bw per day on the basis of hepatocellular hypertrophy in males at 0.1 mg/kg bw per day and in females at 1.0 mg/kg bw per day. The hepatocellular hypertrophy was likely to have been caused by a mode of action involving hepatocellular peroxisome proliferation that is not relevant to humans. The NOAEL for effect of relevance to humans was 0.1 mg/kg bw per day in males and 1 mg/kg bw per day in females, the highest doses tested (Tollett et al., 1983; Yano et al., 1984).

2.4 Genotoxicity

Haloxyfop-R methyl ester was tested for genotoxicity in a bacterial reverse mutation assay (Mecchi, 1999) and a mammalian cell cytogenetics assay (Linscombe et al., 1999). Haloxyfop-R acid was tested in assays for reverse mutation in bacteria (Gollapudi & Samson, 1989), cytogenetics in mammalian cells in vitro (Gollapudi & Linscombe, 1989) unscheduled DNA synthesis in vitro (McClintock & Gollapudi, 1989). Racemic haloxyfop acid was tested in assays for reverse mutation in bacteria (Domoradzki, 1981), gene mutation in mammalian cells in vitro (Myhr, 1982), cytogenetics in mammalian cells in vitro (Bootman et al., 1986), unscheduled DNA synthesis in vitro (Domoradzki, 1980) and micronucleus formation in rat bone marrow in vivo (Gollapudi et al., 1983). All of the tests gave negative results. Details are given in Table 4. Certificates of compliance with GLP were provided for all tests.

The Meeting concluded that haloxyfop-R methyl ester and haloxyfop were not genotoxic.

Table 4. Results of studies of genotoxicity with haloxyfop-R methyl ester and haloxyfop-R acid

End-point	Test object	Concentration/dose	Result	Reference
In vitro				
Haloxyfop-R methyl ester				
Reverse mutation	*Salmonella typhimurium* TA1535, TA1537, TA98 & TA100; *Escherichia coli* WP2*uvr*A	6.67–5000 µg/plate	Negative (±S9)	Mecchi (1999)
Cytogenetics	Rat lymphocytes	33.3–1000 µg/ml	Negative (± S9)	Linscombe et al. (1999)
Haloxyfop-R acid				
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA98 & TA100	5.0–5000 µg/plate	Negative (±S9)	Gollapudi & Samson (1989)
Cytogenetics	Rat lymphocytes	50–5000 µg/ml	Negative (±S9)	Gollapudi & Linscombe (1989)
Unscheduled DNA synthesis	Primary rat hepatocyte culture	5–5000 µg/ml	Negative	McClintock & Gollapudi (1989)
Haloxyfop acid				
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98 & TA100	5.0–5000 µg/plate	Negative (±S9)	Domoradzki (1981)
Gene mutation	Chinese hamster ovary cells (CHO-K1), *Hprt* locus	25–2000 µg/ml	Negative (±S9)	Myhr (1982)
Cytogenetics	Human lymphocytes	100–1000 µg/ml	Negative (±S9)	Bootman et al. (1986)
Unscheduled DNA synthesis	Primary rat hepatocyte culture	0.3×10^{-9}–0.3×10^{-3} mol/l	Negative	Domoradzki (1980)
In vivo				
Haloxyfop acid				
Micronucleus formation	Sprague-Dawley rats (bone marrow polychromatic erythrocytes)	0, 30, 100 or 300 mg/kg bw as single oral dose	Negative (30 and 40 h harvests of bone marrow)	Gollapudi et al. (1983)

2.5 Reproductive toxicity

(a) Multigeneration studies

Racemic haloxyfop acid has been tested for effects on reproduction in a two-generation study and a three-generation study.

The two-generation study was performed in accordance with GLP. For the F_0 generation, groups of 30 male and 30 female Sprague-Dawley rats were given diets providing racemic haloxyfop acid (purity, 99.4%) at a dose of 0, 0.01, 0.065 or 1.0 mg/kg bw per day. After 8 weeks on the test diets, pairs of F_0 rats were mated to produce the F_{1a} generation. After weaning the F_{1a} litters, the F_0 rats were mated again to produce the F_{1b} generation. Groups of 30 F_{1b} weanling rats of each sex per dose were randomly chosen from the litters, and while being maintained on the same test diets as their parents they were mated twice to produce two further generations of rats: F_{2a} and F_{2b}. The rats were observed for general condition and behaviour and measurements were made of food intake and body weights. Data on reproductive parameters were collected, including duration of pregnancy, litter size, litter weight, pup weights and any gross abnormalities in the pups. All adults and 10 randomly selected weanlings of each sex from each group of the F_{1b} and F_{2b} generations were killed for autopsy. Tissues were stored for possible microscopic examination but were not looked at. The results showed no treatment-related effects on mortality, clinical signs, feed intake or body weights of the F_0 or F_1

adults. Some of the livers of the F_0 adults at 1.0 mg/kg bw per day appeared to be slightly enlarged, but the livers were not weighed or examined histopathologically, and the effect was not seen in the F_1 generation. Pup weights of the F_{1a} and F_{1b} rats in the group at 1.0 mg/kg bw per day were statistically significantly lower than those of the controls at age 14 and 21 days, and the body weights of the F_{1b} rats remained consistently less than hose of the controls as they were raised to adulthood. The body weights of the F_{2a} neonates were also decreased at age 21 days. Other litter parameters were not affected by the treatment and no grossly abnormal pups were seen. The NOAEL was 0.065 mg/kg bw per day on the basis of low pup body weights at 1.0 mg/kg bw per day (Nitschke et al., 1985).

The three-generation study was performed in accordance with GLP. For the F_0 generation, groups of 30 male and 30 female Fischer 344 rats were fed diets designed to provide racemic haloxyfop acid (purity, 99.6%) at a dose of 0, 0.005, 0.05 or 1.0 mg/kg bw per day. After 102 days on the test diets, the rats were mated to produce F_{1a} litters, which were killed for autopsy after weaning. The F_0 rats were rested for 1 week after weaning of their litters and then mated again to produce the F_{1b} litters. After all the F_{1b} litters were weaned, the F_0 rats were killed for autopsy. For each sex at each dose, 30 rats of the F_{1b} generation were randomly selected for mating (after 137 days on test diets) to produce three litters of F_2 generation rats (F_{2a}, F_{2b} and F_{2c}). After weaning of the F_{2c} litters, all of the F_{1b} parent rats were killed for autopsy and histopathology. For males and females of each test group, 10 of the surplus F_{2b} rats that were not chosen for mating were killed for autopsy, and five of each sex per group of these were used for histopathology. The first litter from the F_{1a} matings (ie. the F_{2a} litter) was weaned and then killed at day 28 of life, with autopsies being performed on 10 rats of each sex per group. For each sex at each dose, 30 rats of the F_{2b} generation were randomly selected for mating with one another (after 64 days on test diets) to produce two litters of F_3 generation rats (F_{3a} and F_{3b}). All the F_{2b} adults were killed for necropsy (no histology) after the F_{3b} litters were weaned. The F_{3b} pups were killed at day 28 of life and 30 males and 30 females per group were randomly chosen for necropsy (histopathology on five of these); the F_{3a} pups were not autopsied. From the F_{2c} rats, 30 males and 30 females per group were fed the test diets for 61 days and then were mated with untreated males and untreated females. The mated females (treated F_{2c} females that had been mated with untreated males and untreated females that had been mated with treated F_{2c} males) were killed at mid-gestation and their uterine contents were examined.

There were no treatment-related effects on mortality, clinical signs, body weight or feed consumption for any of the generations of adult rats. Most of the indices of reproductive performance, including number of live pups born, gestational survival index and sex ratio of pups, were unaffected by treatment at all generations. The fertility index of the treated rats was less than the control value for the second and third matings of the F_{1b} adults (see Table 5). Although the effect was not dose-related, it was statistically significant for the groups at the intermediate (0.05 mg/kg bw per day) and highest (1.0 mg/kg bw per day) dose of the second mating of the F_{1b} rats (to produce the F_{2b} pups) and group at the highest dose (1.0 mg/kg bw per day) for the third mating of the F_{1b} rats (to produce the F_{2c} pups). The authors of the study did not consider this effect to be treatment-related as it was not dose-related and not seen in the other two generations. Although there was no treatment-related effect on pup birth weight, the body weights of F_{1b} and F_{2a} pups of the group at the highest dose (1.0 mg/kg bw per day) were significantly ($p < 0.05$) less than concurrent control values on most days during weaning and up to termination at 28 days. No such effect on pup body weight was seen for the F_{1a}, F_{2b} or F_{2c} pups. Autopsies on the adults showed some changes in organ weights at the highest dose of 1 mg/kg bw per day: liver weights were increased while kidney and testes weights were decreased. Histopathology showed some changes to the liver (hepatocellular hypertrophy with centrilobular eosinophilia and decreased intracellular lipid) and kidneys (pigment granules in the epithelium of proximal convoluted tubules), but no adverse histopathology was seen in the testes. The authors considered the effect on the liver to be a treatment-related adverse effect, but not the effects on the kidneys and testes. Autopsies on pups showed increased liver weight in F_{1b}, F_{2a} and F_{2b} pups, but with no corresponding histopathology. The Meeting concluded that there was no convincing evidence that

doses of up to 1.0 mg/kg bw per day caused any adverse effect on reproduction in Fischer 344 rats. The NOAEL was 0.05 mg/kg bw per day on the basis of hepatocellular hypertrophy at 1.0 mg/kg bw per day. This effect was not regarded as being relevant to humans. The NOAEL for effects of relevance to humans was the highest dose tested, 1 mg/kg bw per day (Jeffries et al., 1985).

Table 5: Fertility index of F344 rats fed diets containing racemic haloxyfop acid

Generation		Fertility index (%)			
Adults mated	Pups produced	0 mg/kg bw per day	0.005 mg/kg bw per day	0.05 mg/kg bw per day	1.0 mg/kg bw per day
F_0	F_{1a}	83	93	93	97
F_0	F_{1b}	87	87	97	93
F_{1b}	F_{2a}	63	57	67	53
F_{1b}	F_{2b}	73	50	47*	47*
F_{1b}	F_{2c}	70	43	50	40*
F_{2b}	F_{3a}	53	63	53	67
F_{2b}	F_{3b}	77	80	83	90
F_{2c}	Cross-mating	90	90	83	93

From Jeffries et al. (1985)

* Statistically significantly different ($p < 0.05$) from the value for untreated controls (0 mg/kg bw per day).

(b) Developmental toxicity

Rats

In a GLP-compliant study of developmental toxicity, groups of 30 mated female Fischer 344 rats were given oral doses of the sodium salt of racemic haloxyfop (purity, 99.7%) at 0, 0.1, 1.0 or 7.5 mg/kg bw per day by gavage on days 6 to 15 of gestation. On day 21 of gestation, all surviving dams were killed and autopsied. Livers were weighed and liver plus other tissues were fixed, but histopathology was not performed. Uteri were examined for number of live and dead fetuses, position of fetuses, number and position of resorption sites, number of corpora lutea, sex, body weight and length of fetuses, and any overt fetal malformations. Apparent non-pregnancy was confirmed by staining the uterus with sodium sulfide. At least half the fetuses from each litter were examined by microdissection for evidence of visceral malformations. The remaining fetuses were examined for skeletal malformations. No maternal deaths occurred during the study and no clinical signs were seen. At the highest dose (7.5 mg/kg bw per day), there was decreased feed intake and decreased body-weight gain on days 6 to 8 of gestation, low body weight when compared with that of controls on days 16 to 21, and increased intake of water on days 12 to 20. There were no effects on mortality, clinical signs, or liver weight of the dams. Pregnancy rate was not affected. Litter data showed no effects on number of resorbed implantations, pre-implantation loss, litter size, sex ratio, fetal weight or fetal length. There was no effect of treatment on the numbers of any visceral malformations in fetuses. The only skeletal effect was a significantly increased incidence ($p < 0.05$) of fetuses with incompletely ossified centra of thoracic vertebrae in the group at the highest dose (6% fetuses; 33% litters) when compared with the rate in controls (fetuses, 1%; litters, 8%). The Meeting concluded that no embryotoxicity or teratogenicity was seen in this study, and that the NOAEL was 1.0 mg/kg bw per day on the basis of delayed ossification in fetuses at 7.5 mg/kg bw per day (Hayes et al., 1983).

Rabbits

In a GLP-compliant study of developmental toxicity, groups of 30 mated female New Zealand White rabbits were given the sodium salt of racemic haloxyfop (purity, 99.7%) as an oral dose at 0,

1.0, 7.5 or 20 mg/kg bw per day by gavage on days 6 to 18 of gestation. On day 29 of gestation, all surviving dams were killed and autopsied. Livers were weighed and liver plus other tissues were fixed but histopathology was not performed. Uteri were examined for number of live and dead fetuses, position of fetuses, number and position of resorption sites, number of corpora lutea, sex, body weight and length of fetuses, and any overt fetal malformations. Apparent non-pregnancy was confirmed by staining the uterus with sodium sulfide. At least half the fetuses from each litter were examined by microdissection for evidence of visceral malformations. The remaining fetuses were examined for skeletal malformations. Seven maternal deaths occurred during the study: one in the group at the lowest dose, one in the group at the intermediate dose and five in the group at the highest dose. Autopsies showed the rabbit at the intermediate dose died of acute pneumonia that may have been due to an intubation error, pleuritis and constrictive pericarditis were seen in the rabbit at the lowest dose and pleuritis and pneumonia were seen in one of the rabbits at the highest dose. The cause of death was not identified for the other four rabbits at the highest dose. No clinical signs were reported. Body weight and liver weight were unaffected by treatment. Pregnancy rate was not affected. There was a significant ($p < 0.05$) increase in the number of resorbed implantations in the group at the highest dose (22% of implantations, affecting 79% of litters) compared with controls (9% of implantations, affecting 30% of litters). Other litter parameters were unaffected by treatment. Atypically, no external or soft tissue malformations were seen in fetuses from the control group, and although a few external and soft tissue malformations were recorded for the treated groups, the incidences were mostly well within the range of incidences among historical controls for the laboratory. The exceptions were the findings of one fetus at the intermediate dose and one fetus at the highest dose with microphthalmia, for which there had been no reports in the data for historical controls. As only single fetuses were affected at these doses, the authors suggested that this finding was incidental. Skeletal findings showed a significantly ($p <0.05$) increased number of litters containing fetuses with a crooked hyoid bone in the group at the highest dose (19%) compared with controls (4%). The finding was not significant when calculated on the basis of the number of fetuses affected rather than the number of litters. The NOAEL was 7.5 mg/kg bw per day on the basis of fetotoxicity (crooked hyoid bone) and unexplained maternal mortality at 20 mg/kg bw per day (Hayes et al., 1983).

As a result of concern over the relevance of the microphthalmia that was seen in some test groups in the earlier study of developmental toxicity in rabbits, a repeat study was performed. The same batch of sodium salt of racemic haloxyfop was used. Analysis of this test material showed it to be 99.6% pure. The study was GLP-compliant. Groups of 25–30 mated female New Zealand White rabbits were given the test material as an oral dose at 0, 3.0, 7.5 or 15 mg/kg bw per day by gavage on days 6 to 18 of gestation. On day 29 of gestation, all surviving dams were killed and autopsied. Livers were weighed and liver plus other tissues were fixed but histopathology was not performed. Uteri were examined for number of live and dead fetuses, position of fetuses, number and position of resorption sites, number of corpora lutea, sex, body weight and length of fetuses, and any overt fetal malformations. Apparent non-pregnancy was confirmed by staining the uterus with sodium sulfide. At least half the fetuses from each litter were examined by microdissection for evidence of visceral malformations. The remaining fetuses were examined for skeletal malformations. Five maternal deaths occurred during the study: one in the group at the lowest dose and four in the group at the highest dose. Two of the deaths in the group at the highest dose appeared to be due to intubation error, but the cause of death of the other rabbits was not clear. No clinical signs were reported. Body weight and liver weight were unaffected by treatment. Pregnancy rate was not affected. There was a statistically significantly increased rate of resorptions in the group at the lowest dose (32% of implantations affecting 78% of litters) compared with controls (10% of implantations; 63% of litters), but no effect was apparent at higher doses so this was considered to be an incidental finding. Other litter parameters were unaffected. Examinations of the fetuses showed no cases of microphthalmia. There was no significant effect on the incidence of crooked hyoid bone at any dose and the incidence of fetuses with

delayed ossification in the hyoid bone was significantly decreased in the group at the lowest (29%) and the intermediate (27%) dose compared with controls (46%). There were no treatment-related adverse effects on the incidences of any external, soft tissue or skeletal malformations.

The NOAEL was 7.5 mg/kg bw per day on the basis of the unexplained maternal mortality at 15 mg/kg bw per day. The Meeting concluded that no developmental toxicity was seen at any dose tested (Hanley et al., 1985).

The increased incidence of litters with crooked hyoid bone that was found in the initial study of developmental toxicity in rabbits was not found in the repeat study. As the effect was not reflected in an increased incidence of fetuses with crooked hyoid, and the effect was seen at only one maternally toxic dose and as crooked hyoid is regarded as a minor variation rather than a more serious effect, the Meeting concluded that there was no convincing evidence that the sodium salt of racemic haloxyfop could cause developmental toxicity (including fetotoxicity) in rabbits.

2.6 Special studies: hepatic peroxisome proliferation

In addition to observations on the effects on hepatic peroxisomes incorporated into some of the conventional studies of toxicity in non-rodents that are mentioned above, several separate investigations of effects on hepatocellular peroxisomes and the reversibility of these effects have been conducted in vitro and in rodents, dogs, and monkeys.

The effects of racemic haloxyfop acid (purity, 98.8%) on peroxisome proliferation, replicative DNA synthesis at the S-phase of the cell cycle and apoptosis were investigated in a primary culture of hepatocytes derived from young male $B6C3F_1$ mice. The study was not GLP-compliant, as the laboratory was not GLP-certified, but the study was performed in accordance with most of the concepts and principles of GLP. Haloxyfop acid was used at a concentration of 0, 10, 30, 100, 300 and 1000 µmol/l dissolved in DMSO. The positive controls used were: epidermal growth factor (EGF) at 25 ng/ml, for replicative DNA synthesis; transforming growth factor beta-1 ($TGF\beta_1$) at 5 ng/ml, for apoptosis; and WY14,643 at 100 µmol/l, for replicative DNA synthesis, apoptosis and peroxisome proliferation. The amount of peroxisome proliferation was estimated by measuring the amount of cyanide-insensitive oxidation of palmitoyl CoA (FAOX). The results showed highly significant ($p < 0.001$) increases in the rate of oxidation of palmitoyl CoA at doses of 30 µmol/l or more (indicating peroxisome proliferation), significantly decreased ($p < 0.05$) replicative DNA synthesis at 30 µmol/l or more, and significantly increased ($p < 0.05$) numbers of hepatocytes undergoing apoptosis at doses of 10 µmol/l or more. Most of the positive controls gave the expected results, except that the increase in apoptosis with WY14,643 was not statistically significant. The Meeting concluded that racemic haloxyfop acid caused an increase in peroxisome proliferation, a decrease in replicative DNA synthesis and an increase in apoptosis in cultured mouse hepatocytes. This was not the anticipated result, as the authors had expected to find increases in all three parameters (Elcombe, 2002a).

A study with similar protocol to the studies in mouse or human hepatocytes in vitro was performed in guinea-pig hepatocytes using the same concentrations of the same batch of racemic haloxyfop acid. In this study there were significant ($p < 0.01$) increases in peroxisome proliferation at concentrations of 10, 100, 300 and 1000 µmol/l, highly significant ($p < 0.001$) dose-related decreases in replicative DNA synthesis at all doses (10 µmol/l or more), and highly significant ($p < 0.001$) increases in the number of cells undergoing apoptosis at all doses (10 µmol/l or more). This time the material used as a positive control WY14,643 did not give significant changes in either replicative DNA synthesis or apoptosis, although the other positive controls gave the expected significant increases in the corresponding parameters. The Meeting concluded that racemic haloxyfop acid caused an

increase in peroxisome proliferation, a decrease in replicative DNA synthesis and an increase in apoptosis in cultured guinea-pig hepatocytes (Elcombe, 2002b).

A study with similar protocol to the studies in mouse and guinea-pig hepatocytes in vitro was performed in human hepatocytes using the same batch of racemic haloxyfop acid. Concentrations of 0, 30, 300 and 1000 μmol/l were used. Samples of human liver were obtained from three patients undergoing resections for the removal of tumours. The liver cultures from each donor were tested separately. This time, WY14,643 was used as a positive control for induction of peroxisome proliferation only and not for replicative DNA synthesis or apoptosis. The results showed no consistent effect of racemic haloxyfop acid on peroxisome proliferation, although there was a significant increase for one donor at 30 μmol/l and a significant decrease for another donor at 100 μmol/l. The positive control (WY14,643 at 100 μmol/l) had no effect on peroxisome proliferation in cells from any of the donors, suggesting that human hepatocytes are not responsive (or, at least, not as responsive as mouse or guinea-pig hepatocytes) to peroxisome proliferators. There was a dose-related decrease in replicative DNA synthesis that was significant ($p < 0.05$) in all three sets of cells from different donors at 300 μmol/l or more, and at 30 μmol/l there was a significant decrease with the cell culture from one of the donors. The positive control (EGF at 25 ng/ml) caused a highly significant ($p < 0.001$) increase in replicative DNA synthesis in all three sets of cells. There were significant ($p < 0.05$) increases in apoptosis in all three donors at concentrations of 300 μmol/l or more and in two of the donors at 30 μmol/l, and significant increases were seen in cells from all three donors in response to the positive-control material ($TGF\beta_1$ at 5 ng/ml) (Elcombe, 2002c).

The results of in-vitro studies in hepatocytes from different species showed that human hepatocytes responded to racemic haloxyfop acid in a different way to hepatocytes from the other two species, in that they did not show the increase in peroxisome proliferation that was seen in mice and guinea-pigs (Table 6). The hepatocytes from all three species showed a decrease in replicative DNA synthesis and an increase in the number of cells undergoing apoptosis. The direction of the effect on replicative DNA synthesis was opposite to the direction of the response to the positive control (EGF). The finding of decreased replicative DNA synthesis at S-phase was unexpected and no explanation for it was available.

Table 6. Effects of haloxyfop acid on hepatocytes from different species in vitro

Effect	Species		
	Mouse	Guinea-pig	Human
Haloxyfop acid			
Peroxisome proliferation	+	+	0
Replicative DNA synthesis	−	−	−
Number of cells undergoing apoptosis	+	+	+
Positive controls			
Peroxisome proliferation (WY14,643)	+	+	0
Replicative DNA synthesis (EGF)	+	+	+
Number of cells undergoing apoptosis ($TGF\beta_1$)	+	+	+

From Elcombe (2002a), (2002b) & (2002c)

EGF, epidermal growth factor; $TGF\beta_1$, transforming growth factor beta-1.

\+ Increase in this parameter when compared with untreated controls.

0 No consistent increase or decrease (similar to untreated control).

− Decrease in this parameter when compared with untreated controls.

Mice

In a GLP-compliant study, groups of 25 male and 25 female B6C3F$_1$ mice were given diets providing racemic haloxyfop acid (purity, > 99%) at a dose of 0, 0.1, 0.5, 1.0 or 10 mg/kg bw per day for 4 weeks. This was followed by a 2-week or 4-week recovery period. Possible effects that might have been related to peroxisome proliferation were monitored at the end of the 4-week treatment period and after the 2-week and 4-week recovery periods. These possible effects were monitored by measuring liver weights, light and electron microscopy of the liver, and hepatic enzyme activities of FAOX, catalase, glycerophosphate dehydrogenase (GPD) and carnitine acyl transferase (CAT). The treatment groups were divided into subgroups of three males and three females for light and electron microscopy and of eight (end of treatment) or three to four (recovery periods) males and females for assays for enzyme activity.

No clinical signs or effects on body weight were reported for any group.

Immediately after the treatment period, liver weights were greatly increased (75–90%) in males and females at 10 mg/kg bw per day. Light microscopy showed changes in all treated groups, including hepatocellular hypertrophy and/or increased cytoplasmic eosinophilia. The changes were seen throughout the liver. Electron microscopy showed increased peroxisome volume densities at 10 mg/kg bw per day in males (8-fold) and females (14-fold). Both the size and number of peroxisomes had increased. Induction of FAOX, relative to controls, was seen at 10 mg/kg bw per day in males (237%) and females (157%). Catalase was induced in males and females at 10 mg/kg bw per day (106%). CAT was induced in males and females at 1.0 (230%) and 10 mg/kg bw per day (1400%) and in females at 0.5 mg/kg bw per day (70%). Male and female mice at 10 mg/kg bw per day had a 19–38% increased activity of GPD, relative to controls.

After the recovery periods, the effect on liver weights was less marked with significant increases seen only at the highest dose for males and females at 2 and 4 weeks after dosing. Hepatocellular hypertrophy and eosinophilia were seen only at 10 mg/kg bw per day at 2 weeks recovery (males and females), and were not seen in any group after 4 weeks recovery. Electron microscopy was not performed on the mice allowed a recovery period. FAOX and CAT were induced (up to 200%) only in the group at the highest dose (both sexes) at the 2-week recovery. No induction of GPD or catalase was seen in any group after the 2-week or 4-week recovery periods.

A NOAEL was not identified for this study as hepatocellular hypertrophy was seen at all doses tested. Hepatocellular peroxisome proliferation was demonstrated at 10 mg/kg bw per day. Biochemical changes associated with the peroxisome proliferation were seen at doses of 0.5 mg/kg bw per day or greater (Stott et al., 1985a & 1985b)

In a study that complied with GLP, groups of 20 female B6C3F$_1$ mice were given oral doses of haloxyfop acid (presumably racemic; purity, > 98.5%) at a dose of 0 (vehicle control) or 0.6 mg/kg bw per day by gavage for 14 days. A group of five mice serving as positive controls was given clofibric acid at a dose of 200 mg/kg bw per day for 14 days. All rats were killed at the end of the treatment period. The livers from the mice were weighed and analysed for enzyme activity of peroxisome acyl-CoA (ACO). RNA was isolated and pooled RNA from five mice per group was used for measurement of gene expression levels for selected genes (ACO, CYP4A10, CTE-I, Bcl-2, Bak-1 and *c-myc*), using real-time polymerase chain reaction (RT-PCR) analysis. Treatment with haloxyfop acid caused significant increases in absolute and relative liver weights, a 3-fold increase in ACO activity and increased expression of the ACO (2.4-fold), CYP4A10 (13-fold) and CTE-I (15-fold) genes. These up-regulated genes had previously been shown to be associated with peroxisome proliferation. A slight increase was seen in transcription of *c-myc* (1.5-fold). There was no clear effect on markers of apoptosis, as increased transcription was seen for both the anti-apoptotic marker Bcl-2 (2.2-fold) and the pro-apoptotic marker Bak-1 (1.4-fold). A similar pattern of induced gene expression (although a smaller response) was seen in the positive controls that were given clofibric acid at 200 mg/kg bw

per day. The Meeting concluded that treatment with haloxyfop acid at 0.6 mg/kg bw per day for 14 days caused biochemical changes that are associated with peroxisome proliferation in the livers of the treated mice (Charles et al., 2002).

Rats

In a study that complied with GLP, groups of 25 male and 25 female Fischer 344 rats were given diets containing racemic haloxyfop acid (purity, > 99%) at a dose of 0, 0.1, 0.5, 1.0 or 10 mg/kg bw per day for 4 weeks. This was followed by a 2-week or 4-week recovery period. Possible effects that might be related to peroxisome proliferation were monitored at the end of the 4-week treatment period and after the 2-week and 4-week recovery periods. The parameters related to peroxisome proliferation that were measured were liver weights, light and electron microscopy of the liver, and enzyme activities of FAOX, catalase, GPD and CAT. The treatment groups were divided into subgroups of three males and three females for light and electron microscopy and of eight (end of treatment) or three to four (recovery periods) of each sex for enzyme assays.

No clinical signs were reported for any group. In the males at 10 mg/kg bw per day, body weight was reduced by 4% relative to controls.

Immediately after the treatment period, relative liver weights were significantly increased in males at 1.0 or 10 mg/kg bw per day, but were significantly decreased in females at 10 mg/kg bw per day, relative to controls. Light microscopy showed changes in all treated groups, including hepatocellular hypertrophy and/or increased cytoplasmic eosinophilia. The changes were seen throughout the liver in males at the highest dose, but were restricted to the centrilobular area in females at all doses and in males at doses of up to 1.0 mg/kg bw per day. Electron microscopy showed increased peroxisome volume density at 10 mg/kg bw per day in males (12-fold), but not in females. Both the size and number of peroxisomes had increased. Induction of FAOX, relative to controls, was seen at 10 mg/kg bw per day in males (393%) and females (109%). Smaller increases were seen in males at 0.5 (60%) and 1.0 mg/kg bw per day (194%). Catalase was induced in males at 1.0 (45%) and 10 mg/kg bw per day (74%), but catalase activity was only slightly increased (34%) at 10 mg/kg bw per day in females. CAT was induced in males at 0.5 (260%), 1.0 (360%) and 10 mg/kg bw per day (2200%) and in females at 10 mg/kg bw per day (450%). In male rats at 10 mg/kg bw per day, hepatic GPD was statistically significantly increased, with an activity that was 52% greater than that in males in the control group. Hepatic GPD was not significantly increased in females and there was no dose–response relationship, although hepatic GPD activities in groups treated with haloxyfop were 19–36% greater than those in females in the control group.

After the recovery periods, the effect on liver weights was less marked, with significant increases seen only in the males at the highest dose at 2 and 4 weeks after dosing. Hepatocellular hypertrophy and eosinophilia were seen only in males at 1.0 or 10 mg/kg bw per day at 2 weeks recovery and only in males at the highest dose (10 mg/kg bw per day) at 4 weeks recovery. Electron microscopy was not performed for rats allowed a recovery period. Induction of FAOX (up to 200%), CAT (up to 200%) and GPD (71%) was seen only in the group of males at the highest dose after a 2-week recovery period. No induction of catalase was seen in any group after the 2-week or 4-week recovery periods.

A NOAEL was not identified for this study as hepatocellular hypertrophy was seen at all doses tested. Hepatocellular peroxisome proliferation was demonstrated at 10 mg/kg bw per day. Biochemical changes associated with peroxisome proliferation were seen at doses of 0.5 mg/kg bw per day or greater (Stott et al., 1985a & 1985b).

A 13-week study of dermal toxicity with the formulation EF-1400 (haloxyfop-R methyl ester at 111 g/l) was performed in Fischer 344 rats at doses of 0, 40, 200 or 1000 mg/kg bw per application, 6 h per day and 5 days per week. The study is described more fully in Section 2.3. Increased liver weight, increased serum AP activity and hepatocellular hypertrophy were seen in males at all doses

and in females at the highest dose. Ultrastructural examination by electron microscope of centrilobular hepatocytes from rats at the highest dose showed increased numbers of peroxisomes in males but not females. No NOAEL was identified for this study (Stebbins et al., 2001).

Guinea-pigs

Groups of five Dunkin-Hartley guinea-pigs were given racemic haloxyfop acid (purity, 98.8%) at a dose of 0, 3, 10 and 30 mg/kg bw per day in corn oil by oral gavage for 7 days. The study did not comply with GLP, as the laboratory was not GLP-certified, but the study was performed in accordance with most of the concepts and principles of GLP. All guinea-pigs were implanted with osmotic pumps containing bromodeoxyuridine (BrdU) at 15 mg/ml on the first day of treatment. After 7 days of treatment, the guinea-pigs were killed by CO_2 asphyxiation and the gall bladders and livers were removed for investigations, including histopathology, activity of acyl CoA oxidase, mean cell replication index (BrdU incorporation) and the number of apoptotic cells (identified by TUNEL staining). There was a dose-dependent increase in body-weight gain during treatment, with the body-weight gain of the group at the highest dose being 358% of control value. There were small dose-related increases in both absolute and relative liver weight, which were statistically significant ($p < 0.05$) at the highest dose only (absolute, 131% of control value; relative, 120%). Acyl CoA oxidase activity was raised (184% of control value) in the group at the highest dose only. No treatment-related liver histopathology was seen at any dose. Some guinea-pigs in the group at 30 mg/kg bw per day showed small increases in cell replication index and apoptotic index, but the group mean effects were not statistically different from the control values. The Meeting concluded that the NOAEL was 10 mg/kg bw per day on the basis of small increases in liver weight and peroxisomal enzyme (acyl CoA oxidase) activity at 30 mg/kg bw per day (Elcombe, 2002d).

Dogs

The 13-week study in dogs given racemic haloxyfop acid (purity, 99.8%), described more fully in section 2.2, incorporated an investigation of effects on hepatic peroxisome proliferation using light microscopy and electron microscopy on samples of liver. Groups of four male and four female beagle dogs had been given oral doses at 0, 2, 5 or 20 mg/kg bw per day. Absolute liver weight, relative to that of controls, was significantly ($p < 0.05$) increased in males at 5 or 20 mg/kg bw per day and in females at 20 mg/kg bw per day; relative liver weight was significantly increased in males and females at 20 mg/kg bw per day. Light microscopy showed slight hepatocellular hypertrophy in males and females at 20 mg/kg bw per day and in females at 5 mg/kg bw per day that was due to increased stores of glycogen (vacuoles stained positive for glycogen but negative for fat). Electron microscopy showed no treatment-related changes. Hepatic activity of cyanide-insensitive beta-oxidation of palmitoyl-CoA (FAOX) significantly increased in males and females at 5 and 20 mg/kg bw per day, but not at 2 mg/kg bw per day. The NOAEL for hepatic effects was 2 mg/kg bw per day on the basis of induction of FAOX at 5 and 20 mg/kg bw per day (Dietz et al., 1987).

In a 12-month study that did not comply with GLP and that is described more fully in section 2.2 (Barna-Lloyd et al., 1984), groups of six male and six female beagle dogs were given diets containing racemic haloxyfop acid (purity, 99.6%) at amounts that provided doses of 0, 0.05, 0.5 or 5 mg/kg bw per day. At the end of the study, livers were weighed and examined by light microscopy, but no adverse effects were seen. Liver samples from three males and three females from each group were prepared for electron microscopy. The resultant micrographs of the centrilobular area at 8000 × magnification were examined for peroxisomal volume density, which was expressed as a percentage of cytoplasmic volume. There were no effects on liver weight, liver histopathology or on peroxisomal volume density. The NOAEL for peroxisome proliferation was the highest dose tested, 5 mg/kg bw per day (Barnard et al., 1986).

Monkeys

In a GLP-compliant 4-week study that is described more fully in section 2.2, groups of two male cynomolgus monkeys were given the sodium salt of racemic haloxyfop acid (purity, 99.6%) in distilled water by nasogastric intubation at dosages of 0, 5 or 20 mg/kg bw per day. An additional two monkeys were given a dose of 20 mg/kg bw per day for 4 weeks, followed by a 4-week recovery period. At the end of the study, livers were weighed and examined by light microscopy and electron microscopy. The electron-micrographs of the centrilobular area at 8000 × magnification were examined for peroxisomal volume density, which was expressed as a percentage of cytoplasmic volume. No effects were seen on liver weight, liver histopathology or on peroxisomal volume density. The morphology of peroxisomes and other organelles in the hepatocytes was unaffected by treatment. The NOAEL for peroxisome proliferation was the highest dose tested, 20 mg/kg bw per day (Gerbig et al., 1985).

In a GLP-compliant 13-week study that is described more fully in section 2.2, groups of four male and four female cynomolgus monkeys were given racemic haloxyfop acid (purity, 99.8%) at a dose of 0, 2, 10 or 30 mg/kg bw per day by nasogastric intubation. The monkeys were killed at the end of the 13 weeks of treatment, autopsied and histopathology was performed on major organs. Livers from the controls and from the group at the highest dose were evaluated by electron microscopy and for enzyme changes associated with peroxisomal fatty acid beta-oxidation (FAOX). The absolute and relative weights of the liver were significantly ($p < 0.05$) increased, relative to those of controls, in males and females of the group at the highest dose. At autopsy, the livers of the females at 10 mg/kg bw per day and males and females at 30 mg/kg bw per day had a pale appearance with an accentuated lobular pattern. Light microscopy showed slight hepatocellular hypertrophy with increased numbers of fatty vacuoles in monkeys at 10 or 30 mg/kg bw per day. Ultrastructural evaluation by electron microscopy of centrilobular hepatocytes showed the livers of monkeys at the highest dose to have a slightly higher cytoplasmic lipid volume density and larger numbers of small fat droplets than controls. Peroxisomal volume densities were similar for the monkeys in the control group and for the group at the highest dose. Hepatic peroxisomal FAOX enzyme activity was not significantly different from that of controls at 30 mg/kg bw per day for males and females. The Meeting concluded that there was no effect on peroxisome proliferation in the livers of cynomolgus monkeys at doses of up to 30 mg/kg bw per day. It was noted, however, that doses of 10 mg/kg bw per day or more caused fatty change in hepatocytes, with a NOAEL of 2 mg/kg bw per day for this effect (Yano et al., 1987).

3. Observations in humans

3.1 Studies in volunteers

Studies of pharmacokinetics in humans given the sodium salt of racemic haloxyfop by oral administration and racemic haloxyfop methyl ester by dermal application are covered in section 1.1, together with the results of a study of people using a formulated product (containing haloxyfop-R methyl ester) as recommended. No adverse health effects were reported in any of the studies (Scortichini et al., 1987).

3.2 Observations in potentially exposed humans

Haloxyfop-R methyl ester had been manufactured by Dow AgroSciences at Drusenheim, France since 1994. There had been no reported incidents of employee exposure since 1995. A review of medical surveillance data for 50 plant employees, including 38 with potential exposure, showed no health effects that could be attributed to exposure to haloxyfop (Dow AgroSciences, 2002).

Between January 1999 and January 2002 in China, there were 82 reported incidents of alleged human health effects associated with an herbicidal product that contained haloxyfop-R methyl ester. Of these incidents, 42 involved oral exposure (22 intentional), 23 involved skin exposure, 5 involved eye exposure and 13 involved inhalation during spraying. In the intentional oral exposures, the signs of toxicity included drowsiness, lethargy, seizures, coma and death. These non-specific signs can be attributed either to the petroleum distillate solvent used as coformulant or to haloxyfop, or both, since they are both contained at high concentrations (about 20% and 10%, respectively) in the commercial preparation. The majority of the non-intentional oral exposures were small and resulted in minor symptoms (Dow AgroSciences, 2002).

Comments

Biochemical aspects

Studies of pharmacokinetics and metabolism were conducted with racemic haloxyfop and haloxyfop-R methyl ester. Oral doses of haloxyfop-R methyl ester or haloxyfop were rapidly and extensively absorbed in all laboratory species tested (mice, rats, dogs and cynomolgus monkeys) and in humans (absorption half-life in men, 0.9 h). Irrespective of whether haloxyfop or haloxyfop-R methyl ester was administered, haloxyfop was the only substance detected in the plasma. *S*-Isomeric forms of haloxyfop underwent rapid and almost complete inversion to *R*-forms in rats, and it was assumed that this also occurred in other species. The highest concentrations of residue were in the liver and kidneys. Biphasic elimination was seen in dogs and cynomolgus monkeys, with a rapid initial phase (half-lives, 1–2 h in dogs and 2.5 h in monkeys) followed by a slow second phase (half-lives, 34 h in dogs and 3 days in monkeys). There was little primary metabolism of haloxyfop in any species tested, but there was some conjugation. Glucuronidation occurred in mice and rats. The major route of elimination was the faeces in mice (recovered radiolabel: males, 79%; and females, 71%), male rats (55–77%) and male dogs (88%), but the urine was the major route of excretion in female rats (68–81%) and cynomolgus monkeys (99%). Men excreted 65–100% of a single oral dose in the urine.

The pharmacokinetics and metabolic data on haloxyfop-R methyl ester and haloxyfop suggest that the results of studies of oral toxicity with racemic haloxyfop methyl ester, haloxyfop or haloxyfop sodium salt should be relevant to the investigation of the toxicity of haloxyfop–R methyl ester and haloxyfop-R, as all stereoisomeric forms and esterified forms end up as the de-esterified *R* enantiomer.

Toxicological data

Haloxyfop administered orally was of moderate acute toxicity in mice, rats and cynomolgus monkeys, with apparently higher toxicity in male rats than in female rats (LD_{50} for haloxyfop-R methyl ester: males, 337 mg/kg bw; females, 545 mg/kg bw). Signs of gastric irritation were seen at high oral doses (1000 mg/kg bw) in one study in rats and signs of hepatotoxicity were seen in another study of acute toxicity in rats. Haloxyfop-R methyl ester was not an irritant to the skin or eyes of rabbits and was not a skin sensitizer in guinea-pigs (Magnusson & Kligman and Buehler tests).

The toxicity of haloxyfop has been investigated in short-term studies in mice, rats, dogs and monkeys. In all these species, haloxyfop caused hepatocellular hypertrophy, which was often associated with increased eosinophilia of the cytoplasm and a more homogeneous appearance to the cytoplasm than in controls. The lowest NOEL for this effect was 0.02 mg/kg bw per day in short-term studies of toxicity in mice and rats. A modest but consistent increase in serum AP activity was seen in mice and rats at 2 mg/kg bw per day, with a NOAEL of 0.2 mg/kg bw per day. Hepatocellular hypertrophy was seen at 5 mg/kg bw per day or greater in a study in dogs, and at 10 mg/kg bw per day or greater in a study in monkeys. In addition to these effects on the liver, thyroid changes were

seen and a NOAEL of 0.2 mg/kg bw per day was identified in the studies in dogs and monkeys. Small decreases in serum cholesterol concentrations and small increases in haematological parameters were measured in dogs receiving haloxyfop at doses below that producing hepatocellular hypertrophy, but were not regarded as toxicologically significant.

Repeated dermal doses of 40 mg/kg bw per day or more of haloxyfop-R methyl ester caused similar effects on the livers of male rats to those seen after repeated oral doses of rats with haloxyfop. A NOAEL was not identified.

Combined long-term studies of toxicity and carcinogenicity were conducted with haloxyfop in mice and rats. In mice, hepatocellular hypertrophy and liver tumours were seen. A dose-related positive trend in the number of animals having one or more liver masses, and statistically significant increases in the incidences of liver tumours (adenoma, and adenoma plus carcinoma in males; carcinoma in females) occurred. Only the increased incidence of liver carcinomas was greater than the historical control range. The LOAEL for production of liver tumours in mice was 0.6 mg/kg bw per day and the NOAEL was 0.065 mg/kg bw per day. Hepatocellular hypertrophy was seen in rats in the absence of an increase in tumour incidence, and the NOAEL was therefore identified as the highest dose tested (0.1 mg/kg bw per day in males, and 1 mg/kg bw per day in females). Haloxyfop was not carcinogenic in rats.

Haloxyfop and haloxyfop-R methyl ester gave negative results in a comprehensive range of studies of genotoxicity in vitro and in vivo. The Meeting concluded that haloxyfop, haloxyfop-R and haloxyfop-R methyl ester are unlikely to be genotoxic.

In order to confirm the hypothesis that the mouse liver tumours observed at 0.6 mg/kg bw per day were the result of a mode of action involving peroxisome proliferation, a series of special studies were performed, including studies in vitro and in vivo in mice, rats, guinea pigs, dogs and cynomolgus monkeys treated orally. It was shown by electron microscopy that exposure to haloxyfop caused proliferation of hepatocellular peroxisomes in mice and rats at 10 mg/kg bw per day, but not in dogs or cynomolgus monkeys at up to 20 mg/kg bw per day. In mice, hepatic CAT was induced at 0.5 mg/kg bw per day or more, and other enzymes were induced at higher doses: catalase, GPD, and FAOX. Furthermore, several genes (ACO, CYP4A10 and CTE-I) that are associated with peroxisome proliferation were upregulated at 0.6 mg/kg bw per day. In rats, CAT, catalase, GPD and FAOX were induced. In guinea-pigs, ACO activity was increased modestly at 30 mg/kg bw per day. In dogs, electron microscopy showed no peroxisome proliferation at doses of up to 20 mg/kg bw per day, but there was increased activity of hepatic FAOX at doses of 5 mg/kg bw per day or more. Doses of up to 30 mg/kg bw per day did not cause hepatocellular peroxisome proliferation in cynomolgus monkeys and FAOX activity in hepatic peroxisomes was unaffected. Studies in vitro showed that peroxisome proliferation did not occur in human hepatocytes exposed to haloxyfop acid or to a positive-control peroxisome proliferator (WY14,643) at doses that caused peroxisome proliferation in hepatocytes from mice.

The negative results of tests for mutagenicity indicate that the tumours in mice were not caused by a genotoxic mechanism. The Meeting used the IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans (Boobis et al., 2006) in considering whether the mode of action by which the liver tumours were produced in mice was relevant to humans. It was concluded that the production of liver tumours in mice was by a mode of action that involved hepatocellular peroxisome proliferation and it was noted that this effect was not seen in dogs, cynomolgus monkeys or in cultured human hepatocytes. Hepatocellular peroxisome proliferation has been shown elsewhere to be associated with hepatocellular hyperplasia and liver tumours in rodents, but not in non-rodent species. It has been well established (Klaunig et al., 2003) that chemicals that induce mouse liver tumours via peroxisome proliferator-induced receptor alpha (PPARα) agonism do not pose a risk of hepatocarcinogenesis in humans, because of quantitative dynamic differences in PPARα activation.

The Meeting concluded that haloxyfop is unlikely to pose a carcinogenic risk to humans based on the absence of genotoxicity and the recognition of a mode of action for production of the liver tumours in mice that is not relevant to humans.

Multigeneration studies with haloxyfop in rats showed no effects on reproduction at doses that were not maternally toxic. Low pup body weights (F_{1a}, F_{1b} and F_{2a} pup body weights, 5–10% lower than controls) and low litter weights (6–7% lower in litters of F_{1b} pups on postnatal days 14–21) were reported at 1 mg/kg bw per day in Sprague-Dawley rats in the two-generation study. The NOAEL was 0.065 mg/kg bw per day. In the three-generation study in Fischer 344 rats, the NOAEL was 0.05 mg/kg bw per day on the basis of low pup body weights seen at a dose of 1 mg/kg bw per day in the F_{1b} and F_{2a} pups during weaning and up to termination of the study at age 28 days. The overall NOAEL for the effects on pup body weight was 0.065 mg/kg bw per day.

In a study of developmental toxicity, the fetuses of rats given haloxyfop at a dose of 7.5 mg/kg bw per day showed delayed ossification of thoracic vertebrae. The NOAEL was 1 mg/kg bw per day. Two studies of developmental toxicity were performed in rabbits. In the first, the NOAEL was 7.5 mg/kg bw per day on the basis of an increased incidence of a minor skeletal variation at 20 mg/kg bw per day. Increased maternal mortality, increased number of resorbed implantations and increased number of litters with resorptions were also seen at this dose. In the second study, there was no adverse effect on development, but there was increased maternal mortality at 15 mg/kg bw per day. The NOAEL was 7.5 mg/kg bw per day. The Meeting concluded that haloxyfop is not teratogenic.

No cases of adverse effects were reported in France between 1995 and 2002 among personnel involved in the production and formulation of haloxyfop-based products. A medical review of 50 workers at the plant showed no adverse health findings that could be attributed to exposure to haloxyfop. In China, between January 1999 and January 2002, there were 82 reported incidents of alleged human health effects associated with an herbicidal product that contained haloxyfop-R methyl ester. The majority of the reported incidents concerned small accidental exposures involving minor symptoms. Intentional oral exposures to the commercial preparation were associated with drowsiness, lethargy, seizures, coma and death. These non-specific signs can be attributed either to the petroleum distillate solvent used as coformulant or to haloxyfop, or both, since they are both contained at high concentrations (about 20% and 10%, respectively) in the commercial preparation.

The Meeting noted that there were no qualitative or quantitative differences in the toxicological properties of haloxyfop-R methyl ester and haloxyfop. None of the studies revealed any unexpected effects of haloxyfop-R methyl ester, and the quantitative dose–effect relationships of racemate and *R*-enantiomer were very similar. The data on haloxyfop could therefore be used for the toxicological evaluation of haloxyfop-R methyl ester and haloxyfop-R.

The Meeting concluded that the existing database was adequate to characterize the potential hazard of haloxyfop, haloxyfop-R and their methyl esters to fetuses, infants and children.

Toxicological evaluation

The Meeting concluded that there were sufficient data to bridge the toxicology studies between haloxyfop and haloxyfop-R methyl ester. As the relative molecular mass of haloxyfop methyl ester is very close to that of haloxyfop, there is no need to compensate for differences in relative molecular mass when considering doses.

The Meeting established a group ADI of 0–0.0007 mg/kg bw for racemic haloxyfop, haloxyfop-R and their methyl esters based on a NOAEL of 0.065 mg/kg bw per day for low pup body weight in multigeneration studies in rats, and applying a safety factor of 100.

The Meeting established a group ARfD of 0.08 mg/kg bw for racemic haloxyfop, haloxyfop-R and their methyl esters on the basis of a NOAEL of 7.5 mg/kg bw per day for maternal mortality and increased number of resorptions at higher doses in a study of developmental toxicity in rabbits, and applying a safety factor of 100.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Thirty-six-week study of toxicity[a,e]	Toxicity	0.2 mg/kg bw per day	2 mg/kg bw per day
	Two-year study of carcinogenicity[a,fe]	Toxicity	0.6 mg/kg bw per day[d]	—
		Carcinogenicity	0.065 mg/kg bw per day[d]	0.6 mg/kg bw per day
Rat	Sixteen-week study of toxicity[a,b]	Toxicity	0.2 mg/kg bw per day	2 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a,e]	Toxicity	0.1 mg/kg bw per day	—
		Carcinogenicity	0.1 mg/kg bw per day	—
	Two-generation study of reproductive toxicity[a,e]	Maternal toxicity	1 mg/kg bw per day	—
		Offspring toxicity	0.065 mg/kg bw per day	1 mg/kg bw per day
	Developmental toxicity[b,e]	Maternal toxicity	7.5 mg/kg bw per day	—
		Fetotoxicity	1 mg/kg bw per day	7.5 mg/kg bw per day
Rabbit	Developmental toxicity[b,e]	Maternal toxicity	7.5 mg/kg bw per day	15 mg/kg bw per day
		Embryo- and fetotoxicity	7.5 mg/kg bw per day	15 mg/kg bw per day
Dog	1-year study of toxicity[a,e]	Toxicity	0.5 mg/kg bw per day	5 mg/kg bw per day
Monkey	13-week study of toxicity[c]	Toxicity	2 mg/kg bw per day	10 mg/kg bw per day

[a] Dietary administration with dietary concentrations adjusted weekly to maintain the intended dosages.

[b] Gavage administration.

[c] Nasogastric administration.

[d] Highest dose tested.

[e] Study performed with haloxyfop.

Estimate of acceptable daily intake for humans

0–0.0007 mg/kg bw

Estimate of acute reference dose

0.08 mg/kg bw

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

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

Critical end-points for setting guidance values for exposure to haloxyfop (including haloxyfop-R and haloxyfop-R methyl ester)

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and extensive
Distribution	Widely distributed with highest levels in liver and kidneys.
Potential for accumulation	None
Rate and extent of excretion	$T_{½}$ = 6.3 days for clearance from plasma to urine (human). Mostly excreted in urine. Main route of excretion is faeces in mice, male rats and dogs but urine in female rats, cynomolgus monkeys and men.
Metabolism in mammals	Oral haloxyfop methyl ester rapidly transformed to haloxyfop acid on absorption. Little primary metabolism, but some conjugation.
Toxicologically significant compounds in animals, plants and the environment	Haloxyfop
Acute toxicity	
Rat, LD_{50}, oral	300–337 mg/kg bw in males, 545–623 mg/kg bw in females
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rabbit, dermal irritancy	Not irritating
Rabbit, eye irritancy	Not irritating
Guinea-pig, skin sensitization	Not sensitizing (Buehler, and Magnusson & Kligman)
Short-term studies of toxicity	
Target/critical effect	Liver
Lowest relevant oral NOAEL	0.2 mg/kg bw per day (serum alkaline phosphatase activity in mouse)
Lowest relevant dermal NOAEL	< 40 mg/kg bw per day (erythrocytes)
Genotoxicity	
	Not genotoxic in vitro or in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	None
Lowest relevant NOAEL	0.1 mg/kg bw per day (highest dose tested in male rats)
Carcinogenicity	No carcinogenic potential in the rat. Hepatocellular carcinoma in female mice associated with peroxisome proliferation (mode of action not relevant to humans)
Reproductive toxicity	
Reproductive target/critical effects	No reproductive effects Low pup weight
Lowest relevant reproductive NOAEL	Parental: 1 mg/kg bw per day (highest dose tested) Offspring: 0.065 mg/kg bw per day (pup weight)
Developmental target critical effect	Delayed ossification, resorptions
Lowest relevant developmental NOAEL	Maternal: 7.5 mg/kg bw per day (mortality) Developmental: 1 mg/kg bw per day (delayed ossification)
Neurotoxicity/delayed neurotoxicity	
	No specific study; no findings in other studies
Other toxicological studies	
Mechanism studies	Increased hepatocellular peroxisome proliferation in mice, rats and hepatocytes from guinea-pigs No effect on peroxisome proliferation in dogs, monkeys or hepatocytes from humans
Medical data	
	No adverse effects on health of manufacturing workers

Summary for racemic haloxyfop, haloxyfop-R and their methyl esters

	Value	Study	Safety factor
Group ADI	0–0.0007 mg/kg bw per day	Rat, two-generation study of reproductive toxicity	100
Group ARfD	0.08 mg/kg bw	Rabbit, studies of developmental toxicity	100

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