The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

EMEA/MRL/196/97-FINAL
November 1997

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TRICLABENDAZOLE

SUMMARY REPORT (3)

1. Triclabendazole is a benzimidazole anthelmintic used in food animals, where it is mainly employed in the control of the liver fluke, *Fasciola hepatica*, in sheep and cattle. Typically, an oral dose of 10 or 12 mg/kg bw is administered to sheep and cattle, respectively, at 8-10 week intervals during the fluke season, or at 5-6 week intervals in acute or sub-acute cases. Triclabendazole has been used in clinical trials for the treatment of parasitic infestations (fascioliasis and paragonimiasis) in humans as single or double oral doses of 10 mg/kg bw.

2. The Committee for Veterinary Medicinal Products (CVMP) has previously considered triclabendazole. The outstanding issues that needed to be addressed prior to permitting an Annex I entry for these MRLs was the adoption of an agreed ADI (insufficient data), and to establish the fraction of the total residue content of edible tissues that could be measured by the applicant's HPLC routine analytical method for the marker residue. The CVMP recommended the following provisional MRLs based on data available at the time of their initial assessment as laid down in Council Regulation (EEC) No. 2377/90:

Pharmacologically active substance(s)	Marker residue	Animals species	MRLs	Target tissues	Other provisions
Triclabendazole	Sum of the extractable residues that may be oxidised to ketotriclabendazole	Bovine, ovine	150 µg/kg 150 µg/kg 150 µg/kg 50 µg/kg	Kidney Liver Muscle Fat	Provisional MRLs expire on 1 July 1997

3. Peak plasma levels were reached by 8 hours in rabbits and dogs given single oral radiolabelled doses of up to 26 and 5 mg/kg bw, respectively. In dogs given a dose of 40 mg/kg bw peak plasma levels were reached at 24 hours and maintained for 2-3 days. Studies in the rat, dog, sheep, goat and rabbit demonstrated that the majority of an oral dose of triclabendazole was eliminated in the faeces with minimal urinary excretion. In the rat, approximately 93% of oral doses of 0.5 or 25 mg/kg bw were eliminated within 48 hours and 98% after 144 hours, of which 88-95% was in faeces, 4-10% in urine and only up to 1% remaining in tissues; no sex or dosage-related effects were found. In a bile cannulated rat, 34% of a 5 mg/kg bw oral dose was excreted in bile in 49 hours. Tissue residues were measured in rats after six days and sheep and goat after ten days, and were generally below 1%-2%. The highest concentrations were found in the heart and brain of rats, and in the liver and thyroid of sheep and goats.

4. In a GLP radiometric sheep study, 28 days after receiving a 10 mg ^{14}C-triclabendazole/kg bw dose urine and faeces elimination over 7 days post-treatment accounted for 5% and 77% of the administered dose respectively. Urine, faeces and cage washings equalled a total 7 day elimination of 85.35% of the administered dose. Absorption (contents of urine and unrecovered dose) accounted for 19.34% of the administered dose (the same as in cattle).

5. In a new radiometric study in cattle, urine and faeces elimination accounted for 2.2% and 76% of the 12 mg/kg bw dose respectively, equalling a total 7 day elimination of 81.49% of the administered dose. Absorption (urine content and unrecovered dose) accounted for 21% of the administered dose.

7 Westferry Circus, Canary Wharf, London E14 4HB, UK
Tel: (+44-171) 418 84 00 Fax: (+44-171) 418 84 16

6. Pharmacokinetic studies in rats, rabbits, dogs, sheep, cattle, goats and humans indicated qualitative similarities in metabolism with sulphone, sulphoxide, ketone and the 4-hydroxy-derivatives of triclabendazole identified in plasma and faeces; the only metabolite identified in urine was 2-benzimidazolone. In the rat the predominant identifiable metabolites in faeces were the sulphoxide and 4-hydroxy-derivatives. In sheep and goats, triclabendazole and 4-hydroxy-derivatives were the major components. Plasma kinetics studies of sulphoxide and sulphone derivatives in various species after oral dosing showed the sulphoxide to predominate in rabbits, sheep and humans, and the sulphone in the horse, dog and cattle. The pharmacokinetics in most species appear to be linear, although there is evidence of a deviation from linearity in the rabbit, possibly due to coprophagy. The plasma T_{max} for the sulphoxide was around 6-12 hours in most species, 22 hours in cattle, at oral doses of 10-12 mg triclabendazole/kg bw. Plasma T_{max} for sulphone was around 12-30 hours in most species and 72 hours in cattle.

7. Preliminary studies in humans indicate that triclabendazole is well absorbed from the gut. In fasted patients, peak plasma levels occurred 2 hours after a single oral dose of 10 mg/kg bw. Administration after a meal resulted in plasma levels approximately 3 times higher those in fasted subjects. The sulphoxide and sulphone metabolites were identified in plasma, with the sulphoxide predominating. Parent compound was undetectable after 8 hours, peak levels of the sulphoxide and sulphone were found at about 4 hours and were still present at low levels at 24 hours.

8. Triclabendazole is of low acute toxicity when administered by the oral, intraperitoneal, dermal and inhalation routes in rats and mice, oral LD_{50} values being in excess of 8000 mg/kg and the intraperitoneal LD_{50} in the rat being 1666 mg/kg. However, the oral LD_{50} value in the rabbit was 206 mg/kg. The sulphoxide and sulphone metabolites of triclabendazole also had low acute oral toxicity. triclabendazole produced minimal skin irritation and no eye irritation in the rabbit. An optimisation test in the guinea pig produced sensitisation on intradermal challenge but not on epidermal exposure.

9. Repeated-dose toxicity studies in rats fed diets containing 10, 100 or 1000 mg/kg feed triclabendazole revealed minor transient haematological effects (decreased erythrocyte and lymphocyte counts, and haemoglobin concentration) and some effects on clinical chemistry (increased albumin, alkaline phosphatase and cholesterol) were seen at high doses (equal or greater than 100 mg/kg feed). Decreased food intake and growth retardation were observed at 1000 mg/kg feed. No effects were seen at 10 mg/kg feed (NOEL = 0.7 mg/kg bw). A 13-week feeding study in dogs (10, 100 or 1000 mg/kg feed triclabendazole) revealed slight hepatotoxicity, growth retardation, delayed onset of sexual maturity, reversible electrocardiogram changes and haemolysis at 1000 mg/kg feed. Elevated alkaline phosphatase was also seen at 100 mg/kg feed. The NOEL was 10 mg/kg feed (0.35 mg/kg bw).

10. In sheep, single oral doses of 250, 500 or 1000 mg triclabendazole/kg bw as a 10% suspension caused deaths in 1/20, 6/20 and 5/5 animals, respectively. Congested lungs and renal damage were seen at necropsy and a number of dose-related effects on haematological and biochemical parameters were observed. A dose of 125 mg/kg bw was tolerated with no reported adverse effects. Single oral doses of 50, 100 and 200 mg triclabendazole/kg bw were given to sheep as a 5% suspension. At doses equal or greater than 100 mg/kg, reduced appetite, increased the blood urea nitrogen (BUN) and shifts in serum alpha-2-globulin were observed. Slight increases in absolute liver weight were seen at all doses. A single oral dose of 200 mg/kg caused inappetance, transient weight loss and slight effects on motor activity and serum glucose lactate dehydrogenase (GLDH) in calves.

11. In a two-generation study in rats, animals were exposed to dietary levels of 3, 15 and 75 mg/kg feed triclabendazole. Neonatal survival and bodyweight were decreased at 15 and 75 mg/kg feed in the F_2 generation but not in the F_1 generation. Post-mortem examinations of weanlings revealed decreased lung/brain-weight, testis/brain-weight and adrenal/bodyweight ratios in F_1 males in the top dose group. Histology revealed minimal fatty changes in perilobular hepatocytes in a few top dose F_1 animals of each sex and in two mid-dose F_2 females. There was a dose-related decrease in liver-weight in F_2 females. These effects were considered to be treatment-related, and a NOEL was determined at 3 mg/kg feed (0.15 mg/kg bw/day).

12. There was no evidence of teratogenicity in rats following oral gavage with daily doses of 10, 30 or 100 mg triclabendazole/kg bw on gestation days 6-16. Foetal development was retarded (low foetal bodyweight and delayed ossification) at 100 mg/kg associated with maternal toxicity. In rats exposed to 10, 25, 50 or 100 mg/kg on gestation days 8-15, decreased maternal and foetal bodyweight gains were observed at 100 and 200 mg/kg. The overall NOEL for these studies was 50 mg/kg bw. Chinchilla rabbits were exposed to doses of 3, 10 and 20 mg/kg on gestation days 6-18. Maternal toxicity and retarded foetal development was observed at 10 and 20 mg/kg. The NOEL was 3 mg/kg bw. Oral administration to sheep of single or multiple doses of 10-50 mg/kg bw had no adverse effects on reproductive parameters or offspring. However, oral administration to pregnant ewes in combination with fenbendazole at high doses (single doses of 150 mg/kg bw of a 1:1 mixture on day 12, 17, 21 or 28) caused kidney and skeletal abnormalities in some offspring of ewes exposed on days 12 or 21. In cattle, doses of 15-30 mg/kg bw during the first or 2-7 months of pregnancy caused no adverse effects. Single or four weekly oral doses of 50 mg/kg bw had no effect on testis weights or sperm concentration or quality in male sheep.

13. Triclabendazole was clearly negative in numerous *in vitro* and *in vivo* mutagenicity tests, including the Ames test (0.5-1250 μg/plate with 5 strains of *Salmonella typhimurium*) with and without metabolic activation; V79 Chinese hamster cells (3.5-70 μg/ml with, and 0.025-0.5 μg/ml without metabolic activation); *in vitro* autoradiographic DNA repair test in rat hepatocytes (0.3-40 μg/ml) and human fibroblasts (0.4-60 μg/ml); *in vivo* micronucleus and SCE tests in Chinese hamster bone-marrow (173-692 mg/kg).

14. A chronic toxicity/carcinogenicity study was conducted in mice (3, 15, 60 or 300 mg/kg feed triclabendazole in the diet for 737-752 days. Survival, clinical findings, haematology and urinalysis were unaffected by treatment. Bodyweight gain and food consumption slightly increased in treated animals. Serum glutamate pyruvate transaminase (SGPT), serum glutamic-oxalacetic transaminase (SGOT) and alkaline phosphatase were raised in treated animals. The increases were sporadically statistically significant and occasionally showed significant positive trends, but no obvious dose-relationship. Liver weights were increased in treated animals. In males at 1 year absolute weights were significantly increased at 60 mg/kg feed and relative weights at 300 mg/kg feed and at 2 years absolute and relative weights were increased at 15-300 mg/kg feed. In females at 2 years absolute weights were significantly increased at all doses and relative weights at 60 and 300 mg/kg feed. The only pathological finding was increased incidence of benign hepatomas in females in the top dose group only; there was an indication of similar but lesser effects in the top dose males and at lower doses in females, but the available evidence was insufficient to demonstrate an effect. Minor effects on liver weight and serum enzymes seen at all doses may have been mild indicators of the same pathological process that led to the production of hepatomas in the top dose females, but these effects should not be taken in isolation as toxicologically significant endpoints in themselves. The NOEL for this study was 60 mg/kg feed (5.35 mg/kg bw).

15. A well-conducted chronic toxicity/carcinogenicity study in rats (3, 13, 30 or 100 mg/kg in the diet for 2 years) demonstrated no statistically or biologically significant effects on survival, clinical findings, food or water intake, haematology, biochemistry, urinalysis or tumor incidences at any dose. Bodyweight gain was significantly depressed in the high dose females and kidney weights were lower in the high dose males at 52 weeks. The NOEL was 30 mg/kg feed (equivalent to about 1.5 mg/kg bw/day).

16. Triclabendazole has no significant antibacterial activity.

17. Triclabendazole has been used in clinical trials for the treatment of parasitic infestations in humans. Single and double oral doses of 10 mg/kg bw were well tolerated. Transient epigastric pain was attributed to the death of the parasites.

18. The CVMP is aware of the differences in the interpretation of the toxicological data made by JECFA. The JECFA report concludes that the reported increase in mortality and lower bodyweights of pups in the F2 generation of the 15 mg/kg feed group (0.75 mg/kg bw/day) of the 2-generation rat study were not treatment-related. As stated in paragraph 14, the CVMP considered that these effects were treatment-related. JECFA determined an ADI of 0-0.003 mg/kg bw/day, based on the NOEL of 15 mg/kg feed (equivalent to approximately 0.27 mg/kg bw per day) in the chronic toxicity study in mice. As stated in paragraph 14, the CVMP considered that the higher dose level of 60 mg/kg feed (5.35 mg/kg bw per day) was the NOEL for this study.

19. The CVMP ADI was set on the basis of the increased postpartum mortality of the F2 generation in the two-generation rat reproduction study (NOEL = 0.15 mg/kg bw/day) using a safety factor of 100:

    ADI = 0.0015 mg/kg bw (i.e. 0.09 mg/kg person).

20. A GLP compliant radiometric residue depletion study has previously been considered. In this old study cattle (1 animal/timepoint) were orally dosed 12 mg ^{14}C-triclabendazole/kg bw and tissues residues quantified by liquid scintillation counting (limit of detection = 2-7 µg/kg). Tissue residue concentrations 28 days after dosing (first timepoint) were: 241, 106, 131, and 13 µg/kg in liver, kidney, muscle and fat respectively. Forty-two days after treatment (last timepoint) these concentrations had depleted to 93, 69, 97, and less than 8 µg/kg respectively. Based on these total residues data it was estimated that the routine analytical method was able to detect 33-50 % of the total residues in cattle tissue samples (28 days after dosing).

21. A new GLP-compliant radiometric residue depletion study in cattle (2 animals/timepoint) was presented where cattle were orally dosed 12 mg ^{14}C-triclabendazole/kg bw and tissue residues quantified by liquid scintillation counting (limit of detection = 2-7 µg/kg). Mean tissue residue concentrations 28 days after dosing (only timepoint) were: 462, 195, 339, and 63 µg/kg in liver, kidney, muscle and fat respectively. By comparing the results of the total residues and marker residue depletion data, it was estimated that the routine analytical method was able to detect 28-51 % of the total residues in cattle tissue samples (28 days after dosing).

22. Two non-GLP compliant cattle studies were previously considered in which cattle were orally dosed 12 mg triclabendazole/kg bw and tissues residues quantified by HPLC with UV detection. In the first study (2 animals/time point; limit of detection = 40-60 µg/kg), tissue residue concentrations 2 days after dosing (first timepoint) were: 5870, 4300, 1420, and 2470 µg/kg in liver, kidney, muscle and fat respectively. 42 days after treatment (last time point) these concentrations had depleted to 80, 75, 100, and less than 60 µg/kg respectively. In the second study (2 animals/time point; limit of detection = 27-43 µg/kg), tissue residue concentrations 2 days after dosing (first timepoint) were: 3250, 3050, 875, and 1800 µg/kg in liver, kidney, muscle and fat respectively. 28 days after treatment (last timepoint) these concentrations had depleted to 52, 47, 23, and less than 3 µg/kg respectively.

23. A non-GLP compliant study was carried out using a combination product containing triclabendazole and levamisole hydrochloride. Friesian heifers were given a single oral dose equivalent to 12 mg/kg bw triclabendazole + 7.5 mg/kg bw levamisole hydrochloride. The cattle were slaughtered in groups of 4 per time point, 1, 21 and 28 days after dosing. The extractable residues which were oxidised to ketotriclabendazole and determined using HPLC with UV detection. The mean residues in muscle, liver and kidney declined from 1400, 7050 and 5800 µg/kg, 1 day after dosing, to 142, 142 and 102 µg/kg, 28 days after dosing. The mean residues in fat were 5800 µg/kg, 1 day after dosing, and were below the limit of detection (40 µg/kg) in all samples taken at later time points.

24. A non-GLP compliant tissue in sheep has previously been considered. In this study, sheep were orally dosed 10 or 15 mg triclabendazole/kg bw and tissues residues were quantified by HPLC with UV detection. In this study (2 animals/time point; limit of detection = 29 µg/kg), marker residue concentrations 2 days after dosing (first timepoint) were: 3500, 3100, 1420, and 1350 µg/kg in liver, kidney, muscle and fat respectively. Twenty-eight days after treatment (last timepoint) these concentrations had depleted to 127, 115, 95, and less than 29 µg/kg respectively.

25. A non-GLP compliant study was carried out in South Africa. Sheep (unstated breed and sex) were given a single oral dose of 10 mg/kg bw triclabendazole. The sheep were slaughtered in groups of 3 per time point, 7, 14, 21, 28, 42 and 56 days after dosing. The extractable residues which were oxidised to ketotriclabendazole and determined using HPLC with UV detection. The mean residues in muscle, liver and kidney depleted from 230, 540 and 290 µg/kg, 7 days after dosing, to 100, 76,and 49 µg/kg. Twenty-eight days after dosing. The mean residues in fat were 73 µg/kg, 7 days after dosing, and were below the limit of detection (30 µg/kg), 14 days after dosing.

26. Another non-GLP compliant study was carried out using a combination product containing triclabendazole and levamisole hydrochloride. Black-faced ewes were given a single oral dose equivalent to 10 mg/kg bw triclabendazole + 7.5 mg/kg bw levamisole hydrochloride. The sheep were slaughtered in groups of 4 per time point, 1, 21 and 28 days after dosing. The extractable residues which were oxidised to ketotriclabendazole and determined using HPLC with UV detection. The mean residues in muscle, liver and kidney declined from 2100, 7400 and 6800 µg/kg, 1 day after dosing, to 150, 200 and 65 µg/kg, 28 days after dosing. The mean residues in fat were 10100 µg/kg, 1 day after dosing, and were below the limit of detection (50 µg/kg) in all samples taken at later time points.

27. A new GLP compliant radiometric residue depletion study in sheep (2 animal/timepoint) was presented, where sheep were orally dosed with 10 mg ^{14}C-triclabendazole/kg bw and tissues residues quantified by liquid scintillation counting (limit of detection = 2-7 µg/kg). Tissue residue concentrations 28 days after dosing (only timepoint) were: 238, 198, 321, and 23 µg/kg in liver, kidney, muscle and fat respectively. By comparing the results of the total residues and the marker residue depletion data, it was estimated that the routine analytical method was able to detect 29-53% of the total residues in sheep tissue samples (28 days after dosing).

28. In a special study to determine the ratio of total to marker residue,animal tissues from previous studies were re-analysed. The tissues were taken from one steer and one ram. The total ^{14}C-residues in the samples were determined initially, and then after partition into methylene chloride (to determine extractability), after clean-up, in the peak fraction collected after HPLC and following the method described in paragraph 30. The results of this study indicated that the percentage of total residues detected as marker residue were 46%, 20% and 26% for bovine muscle, liver and kidney respectively and 42% and 26% for ovine muscle and liver. The radioactivity present in ovine kidney was too low for a reliable determination.

29. Bile duct cannulated rats were used to investigate the bioavailability of triclabendazole-related residues. In the first study,described as a feasibility study, lyophilised cattle muscle was fortified with ^{14}C-triclabendazole (approximately 100 µg/kg triclabendazole-equivalents), mixed with a commercial rodent diet and then fed to bile-duct cannulated rats; a total of 52-74% of the dose was recovered from the carcass and 0-48 hour urine and bile samples. In the second study, cattle and sheep were given an oral dose of 12 mg/kg bw or 10 mg/kg bw ^{14}C-triclabendazole, respectively, and killed 28 days later. Tissue samples were lyophilised and mixed with a commercial rat feed and fed or orally administered by stomach tube to groups of bile-duct cannulated male rats. The bioavailability, calculated as the sum of the radioactivity in urine and bile (over 48 hours) and the residues remaining in the tissues and carcass was 8.8%, 13.7% and 3.7% for cattle liver, kidney and muscle, respectively. The corresponding values for sheep were 8.15%, 7.0% and 5.5%. No fat samples were evaluated in the experiment.

30. An analytical method was described in which extractable residues were hydrolysed under alkaline conditions and oxidised to keto-triclabendazole. Determination was by HPLC with UV detection. The method was presented in the ISO format and had been satisfactorily validated for muscle, liver, kidney and fat of sheep and cattle. For all these tissues, the limit of quantification was 100 µg/kg. No validation data were provided for goats.

Conclusions and recommendation

Considering that:

- an ADI of 0.0015 mg/kg bw (0.09 mg/person) had been established,
- the mean concentrations of the marker residue in the muscle, liver and kidney of cattle slaughtered 28 days after treatment were 99, 105 and 71 µg/kg; at this time point the marker residue accounted for 46%, 20% and 26% of the total residues present in these tissues,
- residues in fat were rapidly depleted, fat was not a target tissue and so no MRLs were required,
- a similar distribution of the residues was found in sheep,
- an analytical method, based on HPLC with UV detection, had been validated for edible tissues of sheep and cattle;

the Committee recommends the inclusion of triclabendazole in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animals species	MRLs	Target tissues	Other provisions
Triclabendazole	Sum of the extractable residues that may be oxidised to ketotriclabendazole	Bovine, ovine	100 µg/kg	Muscle, liver, kidney	Not for use in animals producing milk for human consumption

The same MRL values for sheep were elaborated at the meeting of the Joint WHO/FAO Expert Committee on Food Additives (JECFA) held in June 1992. JECFA elaborated higher MRL values for cattle.

Based on these MRLs, it was calculated that consumer intake would amount to a maximum of approximately 134 µg/day. Although this represented 149% of the ADI, it was considered that this would not present a risk to consumers because the bioavailability of the incurred residues was less than 20%, leading to a more realistic estimate of consumer intake of less than 134 µg/day.