



COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

TRICLABENDAZOLE (Modification of Maximum Residue Limits)

SUMMARY REPORT (4)

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 to 10 week intervals during the fluke season, or at 5 to 6 week intervals in acute or sub-acute cases.
2. The Committee for Medicinal Products for Veterinary Use (CVMP) previously considered triclabendazole and agreed a toxicological ADI of 0.0015 mg/kg bw (i.e. 0.09 mg/person) based on the basis of the increased postpartum mortality of the F₂ generation in the two-generation rat reproduction study (NOEL equal to 0.15 mg/kg bw/day) using a safety factor of 100. A validated analytical method based on HPLC with UV detection was available and had been validated for edible tissues of sheep and cattle. The CVMP recommended the following final MRLs based on data available 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	100 µg/kg 100 µg/kg 100 µg/kg	Muscle Kidney Liver	Not for use in animals producing milk for human consumption

The applicant requested scientific advice relating to the modification of the MRLs for triclabendazole.

An application has now been submitted for the modification of the MRLs for triclabendazole.

The applicant provided data to determine bioavailability and thereby revise the MRLs in light of the new information submitted that proved a considerable lower bioavailability of residues and binding to tissue components.

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 to 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 to 95% was in faeces, 4 to 10% in urine and only up to 1% remaining in tissues; no sex or dose-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 to 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 ¹⁴C-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 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.
6. Pharmacokinetic studies in rats, rabbits, dogs, sheep, cattle, goats and humans indicated qualitative similarities in metabolism with sulphone, sulfoxide, 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 sulfoxide and 4-hydroxy-derivatives. In sheep and goats, triclabendazole and 4-hydroxy-derivatives were the major components. Plasma kinetics studies of sulfoxide and sulphone derivatives in various species after oral dosing showed the sulfoxide 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 sulfoxide was around 6 to 12 hours in most species, 22 hours in cattle, at oral doses of 10 to 12 mg triclabendazole/kg bw. Plasma T_{max} for sulphone was around 12 to 30 hours in most species and 72 hours in cattle.
7. A GLP-compliant radiometric residue depletion study in cattle (2 animals/timepoint) was carried out where cattle were orally dosed 12 mg ¹⁴C-triclabendazole/kg bw and tissue residues quantified by liquid scintillation counting (limit of detection equal 2 to 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 to 51 % of the total residues in cattle tissue samples (28 days after dosing).
8. Two non-GLP compliant cattle studies were carried out 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 equal 40 to 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. Forty-two 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 equal to 27 to 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. Twenty-eight days after treatment (last time point) these concentrations had depleted to 52, 47, 23, and less than 3 µg/kg respectively.
9. 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 plus 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.

10. A non-GLP compliant tissue in sheep was carried out where 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 equal to 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.
11. A non-GLP compliant study was carried out where 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.
12. 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 plus 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.
13. A GLP compliant radiometric residue depletion study in sheep (2 animals per timepoint) was carried out, where sheep were orally dosed with 10 mg ¹⁴C-triclabendazole/kg bw and tissues residues quantified by liquid scintillation counting (limit of detection equal to 2 to 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 to 53% of the total residues in sheep tissue samples (28 days after treatment).
14. 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 ¹⁴C-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 analytical method described below. 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.

Digestion of tissues with alkali followed by oxidation with hydrogen peroxide converted triclabendazole and its metabolites to ketotriclabendazole. The conversion to ketotriclabendazole following oxidation of incurred radioactive residues has been determined in two studies in cattle. The recovery of ketotriclabendazole from muscle, liver and kidney was shown to be 42%, 19% and 24% in one study and 32%, 24% and 27% in another study respectively. Marker residue levels were not detected in fat.

In another study in sheep to determine bioavailability of muscle, liver and kidney from animals slaughtered 28 days after dosing, gave values of 5%, 8% and 7% respectively and the marker to total residue ratios for muscle and liver were 38% and 24% respectively. A marker residue ratio was not calculated for kidneys or fat.

15. Bile duct cannulated rats were used to investigate the bioavailability of total triclabendazole-related residues. The approach of using total residues was considered acceptable as concentrations of metabolites would be very low and not reflective of those bioavailable due to further metabolism in the rat.

In the first study, described as a feasibility study, lyophilised cattle muscle was fortified with ¹⁴C-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 to 74% of the dose was recovered from the carcass and 0 to 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 ¹⁴C-triclabendazole, respectively, and killed 28 days later. Tissue samples were lyophilised and mixed with a commercial rat feed (80:20 ratio) 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 9%, 14% and 4% for cattle liver, kidney and muscle, respectively. The corresponding values for sheep were 8%, 7% and 5%. No fat samples were evaluated in the experiment. The recovery of radioactivity varied between 76 to 131%. Most of radioactivity was eliminated in faeces.

In another new radiolabelled study using a similar design, bile fistulated rats were given freeze dried tissues mixed with molasses and syrup to improve palatability. Urine, bile and faeces were collected over a 72 hour period and recovery of total radioactivity was less variable than the above study (90.6 to 116.7%) and the pattern of excretion was similar (mainly via faeces). The bioavailability was 17%, 4% and 20% from liver, kidneys and muscle respectively. Bioavailability calculated using the areas under the curve (AUC) following oral and intravenous dosing to rats gave values of 6% and 10% for muscle and liver respectively. Although no fat samples were evaluated in the experiment, for the calculation of residue ingested the fat bioavailability was estimated 10%.

Bioavailability in rats of triclabendazole in the feed was found to be about 70%.

As metabolism is similar in rats, sheep, goats and cattle it was concluded that the residues of toxicological concern would be similar and accordingly the MRLs could be revised based on their bioavailability.

Bioavailability of incurred residues was very low and these were determined by both the Gallo-Torres method and by the comparison of the areas under the curve following administration of triclabendazole intravenously and incurred residues orally.

A number of extraction solvents (including rigorous methods) were employed but triclabendazole and/or its metabolites remained tightly bound to tissues indicating a strong binding (possibly a covalent bond) and hence low bioavailability.

16. 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 liver, kidney, muscle and fat of sheep and cattle. For all these tissues, the limit of quantification was 50 µg/kg. Limited validation data were provided for goats, the method could measure marker residue levels with acceptable accuracy and precision.
17. Taking into account the Note for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL) and considering that the MRLs in bovine and ovine species would be identical it was considered appropriate to recommend the extension of the MRLs for bovine and ovine species to all ruminants. The MRLs should be extrapolated in such a way that the same MRLs values would apply to all ruminants.

Conclusions and recommendation

Considering that:

- an ADI of 0.0015 mg/kg bw (0.09 mg/person) has previously been established,
- the metabolic profile of triclabendazole in laboratory animals and the target species (rats, sheep, goats and cattle), were similar,
- the data submitted demonstrated that triclabendazole and its metabolites were tightly bound to tissues and therefore showed low bioavailability,
- only 20%, 17% and 4% of the triclabendazole residues were bioavailable from muscle, liver and kidney tissues from sheep and cattle, which would allow for an increase of the previously established MRLs,
- the marker to total residue ratio in cattle is 0.3, 0.32, 0.24 and 0.27 and in sheep 0.3, 0.38, 0.24 and 0.24 in fat, muscle, liver and kidney respectively,
- a similar distribution of the residues was found in sheep and goats,
- fat was added to the list of target tissues,
- an analytical method for the extractable residues that may be oxidised to ketotriclabendazole, based on HPLC with UV detection, had been validated for muscle, liver, kidney and fat tissues of sheep and cattle,
- MRLs recommended in bovine and ovine species are identical,
- validated analytical methods are available for the residue determination of the extractable residues that may be oxidised to ketotriclabendazole in edible tissues of bovine and ovine species which should be applicable to all ruminants;

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	All ruminants	225 µg/kg 100 µg/kg 250 µg/kg 150 µg/kg	Muscle Fat Liver Kidney	Not for use in animals producing milk for human consumption

Based on these MRLs, and taking into account the bioavailability of the residues in the tissues, the daily intake will represent about 70% of the ADI (approximately 63 µg/day).