European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

FENBENDAZOLE
(Extrapolation to all ruminants)

SUMMARY REPORT (4)

1. Fenbendazole is a benzimidazole anthelmintic that is metabolised in mammals to a series of other benzimidazoles including oxfendazole. It is used for the control of gastrointestinal roundworms, lung worms and tape worms. Fenbendazole is currently included in Annex I of Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Fenbendazole	Sum of extractable residues which may be oxidised to oxfendazole sulphone	Bovine, ovine, porcine, *Equidae*	50 µg/kg 50 µg/kg 500 µg/kg 50 µg/kg	Muscle Fat Liver Kidney	
		Bovine, ovine	10 µg/kg	Milk	

2. In reviewing the availability of endo- and ectoparasiticides for sheep and goats, fenbendazole was considered for extrapolation from bovine and ovine species to all ruminants. The considerations and criteria leading to the identification of fenbendazole are described in the Position Paper Regarding Availability of Veterinary Medicines – Extrapolation of MRLs (EMEA/CVMP/457/03-FINAL).

3. The scientific justification for this extrapolation was assessed in accordance with the Notes for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL) and on the Establishment of Maximum Residue Limits for Minor Animal Species (EMEA/CVMP/153a/97-FINAL).

4. In setting the ADI in the original assessment of fenbendazole, the data summarised in the paragraphs below were considered.

5. In ruminants the rumen acts as a reservoir releasing the benzimidazoles slowly into the remainder of the gastro-intestinal tract; in monogastrics there is no reservoir effect and repeat doses over several days are needed for greater efficacy. Absorption of fenbendazole is slow but more rapid in monogastrics, the C_{max} in blood after oral treatment is around 8 hours in rats and rabbits, 24 hours in the dog and 2 to 3 days in sheep. The half-life for plasma elimination for fenbendazole in rats is 6 hours, rabbits 13 hours, dogs 15 hours and sheep 2 to 3 days. Elimination of fenbendazole is predominantly by the faecal route. The liver appears to be the main target tissue in all species tested. The metabolic pathway for fenbendazole, febantel and oxfendazole appears to be similar in most species that have been investigated.

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6. Fenbendazole was shown to be of low acute toxicity. Oral LD_{50} values in laboratory rats and mice were greater than 10000 mg/kg.

7. No treatment related effects were observed in a repeated-dose toxicity study in which groups of Wistar rats (10 per sex) were given daily oral doses of 0, 25, 250 or 2500 mg/kg bw per day of fenbendazole for 30 days. In a 90-day study, groups of Wistar rats (15 per sex) were given daily oral doses of 0, 25, 200 or 1600 mg/kg bw per day of fenbendazole. For 5 per sex the top dose was increased to 2500 mg/kg bw per day from day 61 onwards. Two rats from the 1600 mg/kg bw group and 5 rats from the 2500 mg/kg bw group had tremors; there were no other treatment-related effects.

8. In a series of studies in dogs, fenbendazole was administered in gelatin capsules for periods of 6 days to 6 months. The main toxic effect was lymphoid hyperplasia in the gastric mucosa and mesenteric lymph nodes. The overall NOEL was 4 mg/kg bw per day.

9. In a 3-generation study Charles River CD rats were given fenbendazole in the diet at doses equivalent to 0, 5, 15, 45 or 135 mg/kg bw per day. At doses of 45 mg/kg bw and above, parental animals had diarrhoea, reduced bodyweight gain and pathological changes in the liver. At these doses there were also reductions in fertility, survival and growth of the neonates during lactation. The NOEL was 15 mg/kg bw per day.

10. Fenbendazole had no effect in testicular function tests in sheep and horses.

11. In a teratogenicity study in Wistar rats, groups of 20 mated females were given daily oral doses of 0, 25, 250 or 2500 mg/kg bw per day fenbendazole from days 7 to 16 of gestation. There was no evidence of maternal toxicity, foetotoxicity or teratogenicity at any dose level. Groups of 10 mated yellow silver rabbits were given daily oral doses of 0, 10, 25 or 63 mg/kg bw per day from days 7-19 of gestation. An increase in delayed ossification was observed in the 63 mg/kg bw group. The NOEL was therefore 25 mg/kg bw per day. There were no treatment-related effects in the offspring of dogs, pigs, sheep and cattle, administered fenbendazole at various times during gestation.

12. Fenbendazole gave negative results in the Ames test with *Salmonella typhimurium*, and in an *in vitro* assay for DNA repair in primary rat hepatocytes. Negative results were also obtained in an *in vivo* cytogenetic assay in Chinese hamster bone marrow and in an *in vivo* mouse bone marrow micronucleus test. Fenbendazole and the 2-amino metabolite were positive, in the presence of metabolic activation only, in mouse lymphoma forward mutation assay. Many benzimidazole compounds are known to be mitotic spindle poisons. The microtubules of exposed cells are affected in such a way as to impair normal cell division and cause mis-segregation of chromosomes into the daughter cells resulting in aneuploidy. The mutagenicity data available for febantel, fenbendazole and oxfendazole show no clear evidence of genotoxicity and although no specific tests for aneugenicity have been conducted, the clastogenicity studies that have been conducted are generally reassuring.

13. There was no evidence of carcinogenicity in a study in which groups of 60 per sex per dose Charles River CD-1 mice were given fenbendazole in the diet at concentrations designed to produce 0, 45, 135 or 405 mg/kg bw per day for up to 2 years. Survival was reduced in treated groups compared to controls. In Charles River CD rats, the animals were exposed to dietary doses of fenbendazole of 0, 5, 15, 45 or 135 mg/kg, including an initial *in utero* phase where the dams received the same dosages. Effects on survival were seen at the high dose and bodyweight gain was affected at 45 and 135 mg/kg. Alkaline phosphatase was consistently elevated at 15 to 135 mg/kg and serum glutamic-oxalacetic transaminase (SGOT) at 135 mg/kg only. Histological changes were seen primarily in the liver including hepatocellular hypertrophy, hyperplasia and vacuolation, bile duct proliferation and biliary cyst formation. The overall NOEL was 5 mg/kg.

14. Fenbendazole had no significant antibacterial activity (no effects on human gut flora).

15. An ADI of 7 µg/kg bw per day for oxfendazole has been established by applying a safety factor of 100 to the NOEL of 0.65 mg/kg bw per day for hepatic vacuolation seen in a carcinogenicity study in rats treated with oxfendazole.

16. For the extension to include all ruminant species in Annex I the information summarised in the paragraphs below was taken into account.

17. Four pharmacokinetic studies were presented for fenbendazole in cattle, three in sheep, two in pigs and one in the horse. Most of these studies were GLP compliant but none of them represented a full absorption, distribution, metabolism and excretion (ADME) assessment. The $t_{1/2}$ of fenbendazole following therapeutic dosing (7.5 mg/kg bw cattle and 5 mg/kg bw sheep) with suspension and pellet formulations were 36 and 27 hours respectively in cattle and 33 and 14 hours respectively in sheep. In horses following oral treatment with suspension formulation of fenbendazole at a dose of 10 mg/kg bw the elimination half life of fenbendazole was approximately 9.5 hours. Additionally, the area under curve (AUC) values were 4.7 times higher when fenbendazole was administered to cattle as a pellet as opposed to a suspension formulation but 1.5 times higher in sheep. The pharmacokinetic data and residue depletion studies indicated that the persistence of fenbendazole metabolite residues in treated animals was dose presentation (macro or micronised particles) dependent.

18. GLP compliant tissue depletion studies meeting the requirements of Volume 8 of the Rules Governing Medicinal Products in the European Community were presented for fenbendazole in cattle (two), sheep (two) and pigs (three). In all of these studies tissues residues were quantified (using HPLC) as being the sum of the extractable residues when oxidised (by peracetic acid) to oxfendazole sulphone. Fenbendazole residues were preferentially partitioned into liver tissues after therapeutic dosing resulting in liver concentrations greater than 10 times that of other edible tissues in treated cattle (7.5 mg/kg bw) and sheep (10 mg/kg bw).

19. In the studies provided for cattle, 7 days after oral treatment with fenbendazole (7.5 mg/kg bw), tissue concentrations of oxidised fenbendazole residues were less than 5, 7, 8, and 194 µg/kg respectively for fat, kidney, muscle and liver. At 21 days after treatment, residue concentrations in all cattle tissues were below the analytical limit of quantification (5 µg/kg). No radiometric total residue studies were available in cattle.

20. Fenbendazole residue concentrations in milk depleted from 270.5 µg/l at 10 hours after treatment with a micronised suspension formulation (7.5 mg fenbendazole/kg bw) to below the analytical limit of quantification (5 µg/kg) at all time points greater than 120 hours after treatment. When lactating cattle were dosed (7.5 mg fenbendazole/kg bw) using bolus preparation, the concentration of residues in milk decreased from 639 µg/kg 12 hours after treatment to 20 µg/kg (above the limit of quantification) at the final 137 hour time point measured.

21. In the studies provided for sheep, 5 days after oral treatment with fenbendazole (10 mg/kg bw), tissue concentrations of oxidised fenbendazole residues were: 33.5, 79.0, 29.3 and 3658 5 µg/kg respectively for fat, kidney, muscle and liver. Nine days after treatment, these concentrations had depleted to, less than 5, less than 5.7, 6.2 and 744.5 µg/kg respectively for kidney, fat, muscle and liver. These residue concentrations detected in sheep tissues were consistent with those found in a radiometric study previously assessed by the CVMP.

22. Data on the depletion of fenbendazole residues from sheep's milk samples were uncollated (raw data with samples unidentified).

23. In a study in pigs, 5 days after oral treatment with fenbendazole (5 mg/kg bw), fenbendazole residue concentrations were below the analytical limit of quantification (less than 5 µg/kg) in all edible tissues. Tissue concentrations of fenbendazole residues at earlier time points were not reported. In an old radiometric (5 mg ^{14}C-fenbendazole/kg bw) study in the pig previously reviewed by the CVMP, residue concentrations in the liver were: 260, 70 and less than 20 µg/kg respectively and in kidney 50, 30 and 10 µg/kg respectively on days 5, 14 and 21 after treatment. Muscle tissues contained residue concentrations below the analytical limit of quantification (less than 10 µg/kg) at all time points (concentrations in fat were not reported). Based on the data in these two studies it can be estimated that the routine analytical method was only able to measure a small fraction of the tissue residue content of pig tissues 5 days after treatment (liver: less than 5µg/kg by HPLC or 260 µg/kg radiometric).

24. In a new study in horses 5 days after repeated oral treatment for 5 days with fenbendazole (10 mg/kg bw) concentrations of the combined residues of fenbendazole, oxfendazole and oxfendazole sulphone were below the analytical limit of quantification (10 µg/kg) in muscle, fat, liver and kidney. Tissue concentrations of fenbendazole at earlier time points were not reported.

25. The Joint FAO/WHO expert Committee on Food Additives (JECFA) proposed temporary MRLs for febantel, oxfendazole and fenbendazole of 500 µg/kg in liver and 100 µg/kg in muscle, kidney and fat. However, these MRLs do not reflect the tissue distribution according to the new data submitted for febantel in cattle, sheep and pig tissues.

26. Ruminant species such as bovine, ovine and caprine share a similar gastro-intestinal physiology. The available pharmacokinetic and residues depletion data do not indicate any significant variability between cattle and sheep, therefore, it was considered that other ruminants were unlikely to show any significant differences in these parameters. The existing MRLs for bovine and ovine species are identical and so it was considered appropriate to recommend the extension of the MRLs so that that the same MRL values would apply to all ruminants, including milk.

27. A routine analytical method was presented for quantifying fenbendazole residues in tissues from cattle, sheep, pigs, and horses. With slight changes to the solvent extraction process this method was also proposed for the routine analysis of milk samples. In the method, residues were extracted from sample matrices in acetonitrile, then oxidised (peracetic acid) to oxfendazole sulphone and quantified by HPLC with fluorescence detection. Sample extracts were quantified by comparison to calibration standards made by extracting blank matrix samples spiked with fenbendazole, oxfendazole and oxfendazole sulphone (1:1:1 w/w/w; 5 to 1000 µg/kg or litre). The method and its validation data were well presented and both met the requirements of Volume VI[1] of the Rules Governing Medicinal Products in the European Community. The validation data demonstrated no interference from blank sample matrices but no other compounds were tested. The limit of quantification of the method was shown (in terms of accuracy and precision) to be 5 µg/kg or litre for all samples matrices. When investigated the limit of detection of the analytical method was shown to be 2 to 3 µg/kg or litre (equivalent to a signal of 3.5 to 5 times the background noise). This method should be applicable to other ruminants and therefore from this aspect extrapolation to the tissues and milk of all ruminants is possible.

Conclusions and recommendation

Having considered that:

- *in vivo*, fenbendazole mainly exists in its oxidised oxfendazole form,
- an ADI of 7 µg/kg bw (i.e. 420 µg/person) for oxfendazole was previously established,
- MRLs were previously established in bovine and ovine species; these MRLs are identical,
- an analytical method for the monitoring of residues in tissues and milk of all ruminant species was available;

the Committee for Medicinal Products for Veterinary Use recommends the modification of the current entry for fenbendazole for bovine and ovine species in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Fenbendazole	Sum of extractable residues which may be oxidised to oxfendazole sulphone	All ruminants	50 µg/kg 50 µg/kg 500 µg/kg 50 µg/kg 10 µg/kg	Muscle Fat Liver Kidney Milk	

Based on these MRL values, the daily intake will represent about 17% the ADI; this margin allows for total residue correction.

[1] Now Volume 8 following revision in September 2001 and June 2003