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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

FENBENDAZOLE

SUMMARY REPORT (3)

1. Fenbendazole is a benzimidazole anthelmintic compound that is metabolised in mammals to a series of other benzimidazoles including oxfendazole; it may be used for the control of gastrointestinal roundworms, lung worms and tape worms. It is already administered to cattle, sheep, horses, pigs and goats. This compound is not used in human medicine.

2. The Committee for Veterinary Medicinal Products (CVMP) agreed to adopt the oxfendazole ADI for fenbendazole and its prodrug febantel as all three compounds share a common metabolism and oxfendazole (the common metabolite formed *in vivo*) was the most toxic. Using the sum of fenbendazole, oxfendazole and oxfendazole sulphone oxidised to the common oxide (oxfendazole sulphone) as the marker residue, provisional MRLs were adopted as stated in Council Regulation (EEC) No 2377/90:

Pharmacologically active substance (s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Febantel	Combined residues of oxfendazole, oxfendazole sulphone and fenbendazole	All food producing species	1000 µg/kg 10 µg/kg 10 µg/kg	Liver Muscle, kidney, fat Milk	provisional MRLs expire on 1 July 1997. The MRLs cover all residues of febantel, fenbendazole and oxfendazole
Fenbendazole	Combined residues of oxfendazole, oxfendazole sulphone and fenbendazole	All food producing species	1000 µg/kg 10 µg/kg 10 µg/kg	Liver Muscle, kidney, fat Milk	provisional MRLs expire on 1 July 1997. The MRLs cover all residues of febantel, fenbendazole and oxfendazole
Oxfendazole	Combined residues of oxfendazole, oxfendazole sulphone and fenbendazole	All food producing species	1000 µg/kg 10 µg/kg 10 µg/kg	Liver Muscle, kidney, fat Milk	provisional MRLs expire on 1 July 1997. The MRLs cover all residues of febantel, fenbendazole and oxfendazole

3. When fenbendazole, oxfendazole and febantel were previously considered by the CVMP, the applicants were asked to provide further information on the following: (1) additional residues data in milk, (2) additional residues data in edible tissues and (3) additional information on the nature of extractable residues. The applicants' responded jointly the form of a supplementary residue file containing all the residue studies undertaken using the modified analytical methods proposed for routine surveillance for all three compounds.

4. 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 dosing is around 8 hours in rats and rabbits, 24 hours in the dog and 2-3 days in sheep. The half-life for plasma elimination for fenbendazole in rats is 6 hours, rabbits 13 hours, dogs 15 hours and sheep 2-3 days. Elimination

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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.

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

6. 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.

7. 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.

8. 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.

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

10. 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 7-16 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.

11. 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.

12. 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 15-135 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.

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

14. An ADI of 7 µg/kg bw per day for oxfendazole has been estimated 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.

15. 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 T½ 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.

16. New GLP compliant tissue depletion studies with animals per timepoint meeting the requirements of Volume VI 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).

17. No tissue residue depletion studies were conducted in the horse for fenbendazole.

18. In new studies provided for cattle, 7 days after oral dosing 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 dosing, 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.

19. Fenbendazole residue concentrations in milk depleted form 270.5 µg/l at 10 hours after dosing 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 dosing. 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 dosing to 20 µg/kg (above the limit of quantification) at the final 137 hour time point measured.

20. In new studies provided for sheep, 5 days after oral dosing 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.

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

22. In a new study in pigs, 5 days after oral dosing 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 timepoints 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 dosing. 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 may 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 dosing (liver: less than 5µg/kg by HPLC or 260 µg/kg radiometric).

23. In a new study in horses 5 days after repeated oral dosing 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.

24. 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.

25. 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; 5-1000 µg/kg or litre). The method and its validation data were well presented and both met the requirements of Volume VI 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 2-3 µg/kg or litre (equivalent to a signal of 3.5-5 times the background noise).

Conclusions and recommendation:

Having considered that :

- *in vivo*, fenbendazole mainly exists in its oxidised oxfendazole form,
- an ADI of 7 µg/kg bw per day for oxfendazole has been established,
- a validated analytical method for residues monitoring purposes is available;

the Committee recommends the inclusion of fenbendazole 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	

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