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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

OXFENDAZOLE

SUMMARY REPORT (3)

1. Oxfendazole is a benzimidazole anthelmintic that is administered orally to cattle and sheep for treatment and control of gastro-intestinal roundworms, lung worms and tapeworms.

2. The Committee for Veterinary Medicinal Products (CVMP) agreed a common ADI for the benzimidazoles oxfendazole, fenbendazole and its prodrug febantel as all three compounds share a common metabolism with oxfendazole (the common metabolite formed *in vivo*) being the most toxic. None of the three compounds is used in human medicine. 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

2. When oxfendazole, fenbendazole 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.

3. In ruminants the rumen acts as a reservoir releasing the drugs 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. Studies in rats and feeder calves using oxfendazole show similar systemic exposure, but occurring within 24 hours in the rat and 168 hours in the calf. For oxfendazole, faecal elimination was reported to be greater in the calf than the rat. The liver appears to be the main target tissue in all species tested.

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4. Oxfendazole was of low acute toxicity. Oral LD_{50} values in laboratory rats and mice were greater than 6400 mg/kg for oxfendazole.

5. In Beagle dogs given oral (gavage) doses of 0, 11, 33 or 100 mg/kg bw per day of oxfendazole for 2 weeks, reduced myeloid maturation was observed in the bone marrow of all treated dogs. Reduction of splenic lymphoid tissue and thymic atrophy were observed in males but not females. In a second study using oral doses of 0, 3, 6 or 11 mg/kg bw per day oxfendazole in a different suspension formulation, no substance-related effects were bserved. No effects were reported in a 3-month study in which dogs were given oral doses of 0, 1.5, 3.0 or 6.0 mg/kg bw per day oxfendazole in gelatin capsules.

6. Oxfendazole caused haematological effects in Sprague-Dawley rats given doses of 33 mg/kg or more for 14 days. In a 3-month study in Long-Evans rats, hepatic hypertrophy, vacuolation and hepatic necrosis were observed at a dietary concentration of 600 mg/kg feed. Other pathological changes included testicular atrophy, splenic necrosis or atrophy and bone marrow hyperplasia. At 200 ppm, only mild hepatic hypertrophy was observed. The NOEL was 100 mg/kg feed (equivalent to 7.3 and 7.7 mg/kg bw per day in males and females respectively).

7. Oxfendazole caused increased liver weights, elevated clinical chemistry values and/or increased fatty vacuolation in mice exposed to dietary doses of 3000 mg/kg or more for one month or 75 mg/kg or more for 3 months, NOELs were 750 or 37.5 mg/kg bw per day, respectively.

8. Reduced testicular weights and hypospermatogenesis were observed in mice exposed to 3000 or 7500 mg/kg oxfendazole in the diet for one month; the NOEL was 750 mg/kg. Hypospermatogenesis was also reported in mice exposed to 300 mg/kg oxfendazole for 3 months; the NOEL was 150 mg/kg. Decreased testicular weights and inhibition of spermatogenesis was seen in rats exposed to 100 mg/kg oxfendazole for 14 days; the NOEL was 33 mg/kg. Testicular atrophy was observed in rats given a dietary dose of 54 mg/kg oxfendazole for 3 months; the NOEL was 17 mg/kg.

9. In a 2-generation reproduction study in rats, oxfendazole at dietary doses of 0.9, 2.3 and 7.7 mg/kg caused maternal toxicity at the highest dose and effects on the offspring of the mid and high doses. These included increased mortality, lower weight gain, hepatocellular vacuolation and decreased fertility in the F_1 generation. F_2 pup weights were also lower at these doses. The NOEL was 0.9 mg/kg.

10. In mice foetotoxicity was reported at 360 mg/kg bw oxfendazole with a NOEL of 108 mg/kg bw (dietary administration, gestation days 6-15). No teratogenic effects were seen at 34 or 108 mg/kg. In the rat, two gavage studies using doses of 10-60 or 5-20 mg/kg on gestation days 6-15 or 7-16 respectively, indicated foetotoxic effects (increased resorptions, decreased foetal viability, reduced foetal weight and/or delayed development) at doses greater than 10 mg/kg. No teratogenic effects were reported. Unspecified teratogenic effects were reported in rats exposed to 21 mg/kg oxfendazole for an unspecified period during pregnancy. Increased foetotoxicity, external and skeletal abnormalities were reported in rats after oral exposure to 15.75 mg/kg oxfendazole through gestation days 8-15 or 31.5 mg/kg on gestation day 12 and 13.

11. In an old and poor quality study, pregnant sheep received single oral doses of 7.5 or 22.5 mg/kg oxfendazole on gestation days 12, 17 or 23. No effects were seen at either dose at gestation days 12 or 23. In animals exposed to 22.5 mg/kg on gestation day 17 lower birth rates and malformations of the axial skeleton, face and organs were reported. No effects were seen at 7.5 mg/kg. In two other reported studies in sheep, no adverse effects on the foetus were seen following single doses of oxfendazole at 10 mg/kg bw on gestation day 17, or 10 or 15 mg/kg bw on gestation day 14, 17 or 20.

12. No effects on the foetus were reported following maternal exposure to oxfendazole during pregnancy in pigs (4 doses of 4.5 or 13.5 mg/kg at 7-day intervals between gestation days 12 and 37), cattle (8 doses of 13.6 mg/kg at 4-day intervals between gestation days 11 and 39) or horses (3 doses of 20 mg/kg on gestation days 26, 180 and 280).

13. The recent developmental toxicology study of oxfendazole in the rabbit indicated no evidence of teratogenicity or foetotoxicity following maternal exposure to oral gavage doses of 10-45 mg/kg bw through gestation days 7-19.

14. No evidence of genotoxicity was observed in an *in vitro* assay for gene mutation in *Salmonella typhimurium*. 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 oxfendazole, febantel and fenbendazole 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.

15. A 78-week study was conducted in which groups of CD-1 mice (50 per sex) were fed diets doses containing the equivalent of 15, 45 or 150 mg/kg bw per day of oxfendazole. A control group of 100 mice per sex were fed untreated diet. No effects on tumour incidence or survival were noted. Increased incidences of hepatocellular hypertrophy, lipid vacuolation and focal necrosis were seen in the high dose; the NOEL was 45 mg/kg. In a one-year study, groups of Sprague-Dawley rats (25 per sex) were fed diets containing the equivalent of 0, 0.65/0.76, 2.0/2.4 or 6.6/7.8 mg/kg bw per day of oxfendazole in males and females respectively. Increased liver weights and decreased male acessory sex organ weights were seen at the high dose, and increased incidences of hepatic discolouration and hepatocellular vacuolation in the mid and high doses. The NOEL was 0.65/0.76 mg/kg bw per day in males and females respectively. In a 2-year carcinogenicity study, groups of Sprague-Dawley rats (50 per sex) were fed diets containing the equivalent of 0.7/0.9, 2/2.5 or 7/8.8 mg/kg bw per day in males and females respectively. Groups control rats (100 per sex) received untreated diet. No significant effects on bodyweight, food consumption, clinical condition, haematology or survival were noted. There was no evidence of carcinogenicity. A dose-related increase in hepatocellular lipid vacuolation was seen in the mid and high dose animals; the NOEL was 0.7 mg/kg bw per day for males and 0.9 mg/kg bw per day for females.

16. Oxfendazole had no significant antibacterial activity (no effects on human gut flora).

17. 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.

18. No pharmacokinetic studies were presented for oxfendazole in any of the target species.

19. GLP compliant tissue depletion studies (4 animals per timepoint) were presented for oxfendazole in cattle, cow's milk and sheep. In cattle dosed with 4.5 oxfendazole mg/kg bw, liver residue concentrations fell steadily from 55.5 µg/kg 10 days after treatment to the analytical limit of quantification (5 µg/kg) by day 18 after treatment. Fat contained residues above the analytical limit of quantification at day 10 after treatment (12 µg/kg). In muscle and kidney the residues were below the analytical limit of quantification at all timepoints studied. There were no data on tissue residue concentrations at timepoints earlier than 10 days after dosing. However, marker residue concentrations were also confirmed (HPLC with co-chromatography) in four blank cattle tissue samples (30, 33, 142 and 18 µg/kg). Due to a number of discrepancies in the residues data in this study it was not considered in the proposal for tissue MRLs.

20. After oral dosing of lactating cattle (4.5 mg oxfendazole/kg bw), the average milk residue concentrations were: less than 5, 87, 221, 186, 106, 55, 19, 10, less than 6, less than 5 and less than 5 µg/kg at 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, and 132 hour timepoints after treatment. Differences in the average oxfendazole residue concentrations detected in milk from high (30.9-26.5 litres/day) and low (15.2-23.3 litres/day) milk yielding cattle were not statistically significant.

21. In sheep (5 mg oxfendazole/kg bw), liver residue concentrations fell steadily from 476 µg/kg 10 days after treatment to 12 µg/kg by day 24 after treatment. In all other tissues residues concentrations were below the analytical limit of quantification (10 µg/kg) at all timpoints

assessed (10-24 days after dosing). No residue depletion studies were presented for oxfendazole in sheep's milk.

22. In horses no specific studies were conducted with oxfendazole. However, in a study with fenbendazole, 5 days after repeated oral dosing for 5 days (10 mg/kg bw) concentrations of the combined fenbendazole, oxfendazole and oxfendazole sulphone residues were below the analytical limit of quantification (10 µg/kg) in muscle, fat liver and kidney. Tissue concentrations at earlier points were not reported.

23. 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.

24. A routine analytical method was presented for quantifying oxfendazole 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 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 :

- an ADI of 7 µg/kg bw per day for oxfendazole has been determined,
- due to the inadequacies of the new tissue deplection study in cattle additional data for fenbendazole and febantel (compounds metabolised to oxfendazole *in vivo*) were taken into account for the establishment of MRLs,
- a validated analytical method for residues monitoring purposes is available;

the Committee recommends the inclusion of oxfendazole 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
Oxfendazole	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 17% ADI; this margin allows for total residue correction.