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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

OXIBENDAZOLE

SUMMARY REPORT (2)

1. Oxibendazole is a broad-spectrum anthelmintic belonging to the benzimidazole family and is used in the treatment of adult and larval forms of intestinal nematodes in piglets at weaning and at batch forming (i.e. at approximately 30 kg bw). It is given in single oral administration (15 mg/kg bw) or in feed (40 mg/kg feed per day for 10 days, i.e. 2 mg/kg bw/day). Provisional MRLs were previously elaborated and published in Commission Regulation EC No 2703/94 of Council Regulation (EC) No 895/93 of the 16 April 1993 and extended for another period of two years in Council (EC) No 1798/96 of the 17 September 1996 :

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Oxibendazole	Oxibendazole	Bovine, ovine, porcine, equidae	100 µg/kg	Muscle, liver, kidney, fat	Provisional MRLs expire on 1.1.1998
		Bovine, ovine	50 µg/kg	Milk	

2. The use of oxibendazole is now restricted to porcine species. The recommended dosages are either single oral administration of 15 mg/kg bw or repeated administrations of 40 mg/kg feed per day for 10 days (2 mg/kg bw/day).

3. Administered orally, this compound has low acute toxicity. In rodents, the LD_{50} generally exceeds 10 g/kg bw. In livestock species, tolerance exceeds 300 mg/kg. Subacute toxicity tests carried out over periods of one to three weeks confirm this low toxicity in livestock.

4. Of the 98-day subchronic toxicity tests carried out on rats (0, 3, 10 and 30 mg/kg bw/day) and dogs (0, 3, 10 and 30 mg/kg bw/day), only the rodent study showed any effects on the blood. Although significant differences for hematocrit values were observed at one month, these values were in the range of normal values (36-55 %). Furthermore as there were no clinical signs associated (pallor of the mucous membranes, weakness, lethargy, reduced tolerance to exercise, loss of appetite, increased heart and respiratory rates etc.), these decreases of hematocrit values should not be considered oxibendazole-related. The mean cell volume and the mean cell haemoglobin concentration, more accurate haematological indices, did not present significant differences vs. control. Therefore, 30 mg/kg bw/day was retained as the NOEL.

5. Many tests for the possible effects of oxibendazole on reproduction were carried out on various animal species.

   Three teratogenicity studies were carried out : two in rats, one in mice.

   In rats, the oral administration of oxibendazole (0, 1, 3, 10, 30 mg/kg bw/day from 6 to 15 day of gestation or 0, 25, 150 mg/kg bw/day from day 6 to 15 of gestation) did not induce teratogen effects. Only an embryotoxic effect was reported at 150 mg/kg bw.

   In mice, no toxic effects on dams or on new-born were reported after oral administration of 0, 1, 3, 10 or 30 mg/kg bw/day of oxibendazole from day 6 to 15 of gestation.

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6. The post natal effect of oxibendazole was studied by oral administrations of 0, 30, 60, 120 mg/kg bw/day from day 16 of gestation to 21 days of the lactating period in rats. The highest dose induced a delay in opening eyes and in teething.

7. Effects on fertility and reproduction were studied in sheep, cattle and horses. None of these studies showed the product to have any teratogenic or embryotoxic properties.

8. Mutagenic potential of oxibendazole was tested in three *in vitro* tests (Ames test, L5178Y TK+/- mouse lymphoma mutation assay and chromosomal aberrations in Chinese hamster ovary (CHO) cells and in one *in vivo* micronucleus test (mice). Oxibendazole was negative in these tests.

   Polyploidy was observed in cultured Chinese hamster ovary cells with and without S9 metabolic activation for concentrations equal or higher than 10 µg/ml.

9. No carcinogenicity study was carried out.

10. An ADI of 0.06 mg/kg bw, i.e. 3.6 mg per person, can be established from the NOEL obtained in the chronic toxicity studies (in rats and dogs), 30 mg/kg bw/day using a first safety factor of 100 and, exceptionally, an additional factor of 5 as oxibendazole induced polyploidy

11. The available metabolic studies, though incomplete, indicate that oxibendazole was readily metabolized in the liver and that a significant proportion of the radioactivity measurable in the liver and kidneys during isotopic studies no longer bears any structural relation to oxibendazole.

12. Kinetic studies on residues in pig after a single oral administration of 15 mg/kg bw of ^{14}C-labelled oxibendazole or repeated administrations of ^{14}C labelled oxibendazole at a level of 40 mg/kg feed per day for 10 days (2 mg/kg/day), showed a rapid decrease in residue levels in tissues.

    After the single oral administration, the total residue levels were higher than those assayed after repeated administrations, the mean values being : in the liver, 3160 vs. 3000 µg equivalents oxibendazole/kg; in kidneys, 398 vs. 68 µg equivalent oxibendazole/kg; skin, 395 vs. 85 µg equivalent oxibendazole/kg; lower than 100 µg equivalent oxibendazole/kg in muscle and fat at 48 hours after treatment.

13. In order to determine the amounts of ^{14}C-oxibendazole extractable residues in liver, liver samples were submitted to repeated solvent extractions [methanol (6 times), acetonitrile (once), ethyl acetate (twice)]. 34.5 % of the radioactivity was extractable from the 24-hour withdrawal liver and only 10.5 % of the radioactivity from the 7-day withdrawal liver.

14. Contaminated liver powders, prepared from liver samples collected from pigs treated with ^{14}C-oxibendazole at a level dose of 15 mg/kg bw and slaughtered at 24 hours and at 7 days post treatment, were incorporated into rat feed. It was shown that a maximum of 40% of the total radioactive residues of swine liver is available for rats instead of 90% after oral ingestion of ^{14}C-oxibendazole in medicated lyophilised liver.

15. The majority of the total radioactive material remained associated with the liver homogenates. In liver, the ratio of oxibendazole towards total radioactivity has been evaluated as 1% at 2 days post-dosing. In kidney, this ratio was in the same magnitude as that in liver; however in muscle, oxibendazole represented the majority of the residues.

    These maximum residue limits can be monitored using a fully validated HPLC method with fluorescence detection, which has limits of detection of 10 µg/kg (fat+skin, muscle), 25 µg/kg (kidney), and 50 µg/kg (liver). The limits of quantification are 50 µg/kg for kidney and muscle, 100 µg/kg for liver and 250 µg/kg for fat+skin. These require final MRL values higher than the provisional ones for fat and liver in order to respect the concept than the MRL values should be established at least at twice the value of the limit of quantification of the monitoring analytical method.

Conclusions and recommendation

Having considered:

- the toxicological ADI of 60 µg/kg bw allowing a permitted daily intake of 3600 µg for a 60 kg person,
- the bioavailability of liver residues representing only 40% of the total residue,
- the tissue distribution at 48 hour-slaughtering time, after single oral administration of 15 mg/kg bw of oxibendazole,
- the marker residue, the parental compound and its ratio to total residue : 1% in liver and kidney, 100 % in muscle and fat,

the Committee recommends the inclusion of oxibendazole into Annex I of Council Regulation (EEC) N° 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Oxibendazole	Oxibendazole	Porcine	100 µg/kg 500 µg/kg 200 µg/kg 100 µg/kg	Muscle Skin + fat Liver Kidney	

Based on these MRLs expressed as oxibendazole, the daily intake will reach 1355 µg, which corresponds to 38% of the ADI 60 µg/kg i.e. 3600 µg/person.

The total residues measured in tissues collected from animals slaughtered 48 hours after a single oral administration of 15 mg/kg bw of ^{14}C-labelled oxibendazole amounts to 156 µg, i.e. about 4.35% of acceptable daily intake.

Seven days after a single oral administration of 15 mg/kg bw of ^{14}C labelled oxibendazole, the oxibendazole residue intake is 77 µg, i.e. less than 2% of the ADI.