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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

RIFAXIMIN

SUMMARY REPORT (2)

1. Rifaximin is an antibiotic belonging to the family of naphthalene-ringed ansamycins (Rifampicin, Rifamycin). Rifaximin possesses a broad spectrum of action against gram-positive bacteria (*Staphylococci* and *Streptococci*, *Corynebacterium* sp.) and against gram-negative bacteria (*E. coli*, *Pasteurella* sp., *Pseudomonas* sp., *Proteus* sp.). In veterinary medicine, it is intended, in cattle, for the treatment and prevention of mastitis during the dry period by intramammary route and for the treatment of post-partum metritis by intrauterine route. The recommended dose is 100 mg of rifaximin per quarter at dry-off and 50-200 mg per animal for intrauterine treatments, respectively. In accordance with the requirements of the Council Regulation (EEC) No. 2377/90, Annex III and Annex II entries for this species were published in Council Regulation (EEC) No 2034/96 of 24 October 1996 as follows :

Annex III					
Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Rifaximin	Rifaximin	Bovine	60 µg/kg	Milk	Provisional MRL expires on 1 June 1998

Annex II		
Pharmacologically active substance(s)	Animal species	Other provisions
Rifaximin	Bovine	For intramammary use - except if the udder may be used as food for human consumption - and intrauterine use only

2. Analytical data on the precision of the analytical method for a level corresponding to half the value of the provisional MRL (30 µg/kg) were provided for inclusion of the current entry for milk into Annex I.

3. The pharmacokinetic studies were carried out by oral route in rats and man, and by intramammary route in dairy cows. An oral or topical administration of rifaximin leads to a negligible systemic absorption of the active ingredient.

   After oral administration of rifaximin to rats, plasma concentrations remained to a low level, represented less than 0.1 % of the administered dose (10 mg/kg or 100 mg/kg). After 168 hours, the faecal excretion amounts represented more than 95 % of the administered dose.

   In lactating dairy cows or in cows at drying-off, no trace of rifaximin could be detected in plasma after administration by intramammary route of 100 mg of rifaximin per quarter (2 or 4 quarter treated). The HPLC quantification and detection limits were 0.02 µg/ml and 0.01 µg/ml.

4. Single oral toxicity was only tested for rats, and the LD_{50} was higher than 2000 mg/kg.

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5. Three and six month-toxicity studies were performed in rats and in dogs (0, 25, 50, 100 mg/kg bw/day). The most sensitive species was rats. In rats, liver steatosis was described for dosages higher than 25 mg/kg bw/day. The blood biochemistry revealed a significant increase in the cholesterolemia and a diminution of the ratio of esterified cholesterol to total cholesterol in animal groups at the doses of 50 and 100 mg/kg bw/day. A NOEL of 25 mg/kg bw/day could be retained.

6. No embryotoxic and teratogenic effects of rifaximin could be seen in rats and rabbits after administration of 0, 50 and 100 mg of rifaximin/kg bw/day during the organogenesis period.

7. From the toxicological data set, a toxicological ADI of 0.25 mg/kg bw/day could be derived from the three-month toxicity studied carried out in rats.

8. Rifaximin gave negative results in five *in vitro* tests (Ames's test, chromosomal aberration and gene conversion tests in yeasts, chromosomal aberration test in human lymphocytes and CHO/HGPRT test) and in one *in vivo* test Micronucleus test in rats after oral administration. It could be concluded that rifaximin is unlikely devoid of mutagenicity.

9. In the dairy technology, especially in the fabrication of yoghurts, the concentration of rifaximin without effect on Streptococcus thermophilus growth was established at 0.098 µg/ml.

10. For the assessment of the microbiological risk, use was made of the formula that was recommended by the CVMP:

$$\text{ADI (µg/kg bw)} = \frac{\dfrac{\text{Geometric mean } MIC_{50} \times CF2}{CF1} \text{ (µg/ml) x daily faecal bolus (150 ml)}}{\text{fraction of an oral dose available for microorganisms} \times \text{weight of human (60 kg)}}$$

Based on the above formula and on the *in vitro* results obtained on sensitive strains of human gut flora, the microbiological ADI can be calculated as follows:

$$\text{ADI} = \frac{\dfrac{0.20 \times 4}{1} \times 150}{1 \times 60} = 2 \text{ µg/kg bw i.e. } 120 \text{ µg/person}$$

The following assumptions were made:

- CF1 = 1 as the MIC variability being evaluated by the calculation of the one-tailed 10 % lower confidence limit of sensitive strains
- CF2 = 4 as the influence of the inoculum size been tested on intermediate or resistant strains.
- 150 g was the weight of the daily faecal bolus;

This microbiological ADI (0.002 mg/kg bw per day) is about 100 times lower than the toxicological ADI of 0.250 mg/kg bw retained from the three-month toxicity in rats.

11. Two studies of depletion of rifaximin in milk were performed to quantify the rifaximin residues after treatment of lactating cows (100 mg of rifaximin per quarter by intramammary route - two quarters treated in a first study, four quarters in the second study). Milk residues were below the limit of detection from the 18th milking. The HPLC and microbiological limits of detection were 0.01 and 0.025 µg/ml respectively.

12. In a third study, it is shown that no residues of rifaximin could be detected in milk after calving when animals had been treated at drying - off (100 mg of rifaximin per quarter). The limits of detection are the same as previously.

13. After intrauterine administration to cows immediately after calving, with pessaries (1200 mg/animal) or foam (200 mg/animal), plasma and milk concentrations up to 96 hours are below the quantification limit of the HPLC method used (0.01 µg/ml).

14. There is no study of residues depletion in tissues either after intramammary or intrauterine administrations. As plasma concentrations of rifaximim were always below the limit of quantification (0.010 µg/ml) after intramammary or intrauterine administrations, the CVMP concludes that depletion studies are not required for this compound.

15. A validated routine HPLC method for the assaying of rifaximim residues in milk is available. The limit of quantification of this method is 30 µg/kg and the limit of detection 22 µg/kg.

Conclusion and recommendation:

Having considered that :

- after intramammary use, residues of rifaximin can be detected in milk;
- after intramammary use, plasma concentrations of rifaximim are always below the limit quantification of the HPLC method used (0.01 µg/ml);
- after intrauterine administration, plasma concentrations of rifaximin are below the quantification limit of the HPLC method used (0.01 µg/ml),
- the analytical method for monitoring residues of rifaximin in milk is fully validated according to the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community,

The Committee recommends the inclusion of rifaximin for bovine milk in Annex I to 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
Rifaximin	Rifaximin	Bovine	60 µg/kg	Milk	