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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

NATAMYCIN

SUMMARY REPORT

1. Natamycin is an antifungal agent active against yeast and yeast-like fungi.

   In veterinary medicine, natamycin is applied topically to skin or mucous membranes for the treatment of mycotic infection (ringworm) in cattle and horses at a dose of 100 mg per animal (dose per kg/bw not stated) twice with an interval of 4 to 5 days between treatments.

   Natamycin has been used as a topical therapeutic in animals and humans for over 30 years, and it is also used as an antifungal agent in food processing. It is authorised as a food additive for surface treatment of certain cheese and sausages; it is listed in Part C of Annex III to Council Directive No 95/2/EC.

2. Natamycin is thought to work by reaction with the sterol molecules in such a way that the permeability of the membrane for potassium ions and other molecules is greatly enhanced; this enhancement leads ultimately to the death of the fungal cell. However, some workers have shown that natamycin appears not to affect the permeability of cell membranes in this way. Instead its fungicidal activity may be explained by the formation of molecular complexes with the sterol compounds, resulting in a weakening of the cell membrane. The membranes are thus rendered fragile and rupture very easily.

   No data on the development of resistance to natamycin in certain strains of yeasts and fungi are presented.

3. Natamycin is recommended for use by direct external application to the body surface of the animal (cattle and horses). As the product is not intended for systemic use, the pharmacokinetic studies undertaken with natamycin have been entirely focused on investigating the possibility of systemic absorption of the product following external or oral application.

   In a GLP-compliant study in 4 ruminating calves treated with 150 mg natamycin/animal, natamycin was not detectable in plasma at any time point after treatment (sampling times 0.25 to 72 hours). The HPLC-method used in this study had a limit of detection of 10 µg/l. It is concluded that absorption through the skin does not lead to detectable plasma levels.

   The conclusion that penetration of natamycin through skin is negligible is supported by three *in vitro* experiments, one using ^{14}C-labelled natamycin detected by liquid scintillation counting and two performed with unlabelled natamycin detected by HPLC (limits of detection of 20 µg/l and 10 µg/l, respectively).

   The three *in vitro* studies involved the use of prepared cattle and horse skin (previously frozen, hair removed) being fitted between the donor and acceptor sides of a skin penetration chamber. Conditions were designed to maximise the possibility of absorption. Penetration of the radiolabelled natamycin in 24 hours was calculated as percentage of the mean amount of natamycin applied to the skin samples (66947.4 decays per minute) and

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amounted to 0.02% for cattle skin and 0.63% for horse skin. In the non-radiolabelled studies penetration though the skin was not detectable; 0.5 µg were applied per sample.

Two studies were carried out to investigate absorption through the gastrointestinal tract, one in rats and one in a cow.

The study in rats involved the detection of gut passage and absorption of the ^{14}C-labelled natamycin orally administered at a single dose of 50 mg/kg bw, using both autoradiography and bioautography. At all sampling points (1 to 24 hours after treatment) radioactivity and biological activity against *Saccharomyces cerevisiae* were only detectable in the lumen of the gastro-intestinal tract. At 24 hours after dosing the autoradiographic results showed radioactivity only in the caecum and the colon, while no biological activity was detectable at this time point. No absorption from the gastrointestinal tract was observed.

The study in the cow involved administration of natamycin via a rumen fistula for 21 consecutive days at a dose of 5000 mg/animal and assaying natamycin levels in blood, urine and milk with a microbiological method using *Saccharomyces cerevisiae*. The limit of detection was 200 µg/l for blood and urine and 500 µg/l for milk. Samples were taken daily in the morning after treatment. The results were negative.

The results of the studies conducted indicate that if percutaneous absorption occurs at all, it does so at a very low level whereas gastrointestinal absorption has not been observed.

4. Natamycin has low acute toxicity after oral administration. The reported LD_{50} values were 1420 mg/kg bw for oral administration in the rabbit, 120 mg/kg bw for intramuscular administration in the rabbit, and 18 mg/kg bw for intravenous administration in the dog.

   In addition to the studies discussed above, an acute intraperitoneal toxicity study was performed in mice with natamycin and three potential metabolites which could be formed either by the treated animal or by decomposition during storage. It was found that the three potential metabolites were less toxic, as measured by the intraperitoneal route, than the parent compound.

5. Pre-GLP studies on the effect of long term oral administration of natamycin were performed in rats, dogs and cats. In all species tested, the only adverse effects seen at the high doses tested were inappetence and diarrhoea. Doses of up to 500 mg/kg feed in rats and 250 mg/kg feed in dogs were tolerated without adverse effects over a 2 year period. For dogs a NOEL of 6.25 mg/kg bw can be calculated based on reduced bodyweight gain.

6. During treatment, natamycin comes into contact with sensitive areas of the body such as the mucous membranes of the eye and nose. Two direct contact tolerance studies, one in cattle and one in horses, were undertaken. No adverse effects attributable to natamycin were observed in either study.

   Following application, the animals may ingest amounts of natamycin by licking their own or other treated animals' coats. No statistically significant effects on the biological activity of the bovine rumen liquor were observed when 5000 mg natamycin/day was given via a rumen fistula to a cow for 21 days. This quantity is 50 times of the therapeutic dose (200 mg; 100 mg/animal twice) administered topically.

7. Investigation of the effect of natamycin on the reproductive performance in rats was conducted as part of the pre-GLP 2-year long term toxicity study, and in young rats (aged 77 days at mating). The only dose level studied was 1000 mg natamycin/kg feed (approximately 133 mg/kg bw for male and 107 mg/kg bw for female rats). At this dose levels the reproductive performance of treated and control rats did not differ.

   Further studies on reproduction toxicity and teratogenicity in rats and rabbits are cited from the 1976 evaluation of natamycin by the Joint FAO/WHO Expert Committee on Food Additives. However neither this report, nor the full study reports, were made available and therefore no conclusion could be drawn.

8. The mutagenicity of natamycin was tested in a set of GLP-compliant studies. A *Salmonella*/mammalian microsome mutation assay in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100, with and without metabolic activation (S 9 mix from arochlor 1254 induced rat liver preparations), a mouse lymphoma mutation assay at the TK locus with and without metabolic activation and a chromosomal aberration assay with Chinese hamster ovary (CHO) cells *in vitro* have been carried out. In none of the experiments undertaken was there any observed evidence that natamycin might have any mutagenic potential.

9. Carcinogenicity studies have not been provided but were not considered necessary in view of the absence of mutagenic effects of natamycin.

10. No specific tests have been carried out to investigate the immunotoxic potential of natamycin.

11. No specific tests have been carried out to investigate the potential effects on the human gut flora. In studies conducted in cattle, the effects of very high doses of natamycin (5000 mg/animal/day for 21 days) had no measurable effect on the digestive capacity of the rumen gut flora.

12. The very low level of residues that might be observed in the food of treated animals will be of no practical significance for industrial processing of food of animal origin.

13. No data on effects of natamycin in humans were submitted. Reported side effects associated with oral use of natamycin in human medicine include nausea, vomiting and diarrhoea. These symptoms have occasionally been caused by oral doses of 300 to 400 mg natamycin daily (5 to 8 mg/kg bw); no changes in peripheral blood have been observed. In a group of 10 patients with systemic mycoses, who received oral doses of 50 to 1000 mg/day of natamycin for 13 to 180 days, nausea, vomiting and diarrhoea occurred in those receiving 600 to 1000 mg/day.

    In 8 reported studies in humans (including one in new born babies), natamycin was applied to the skin, the eyes, the mouth and the lungs (via an aerosol); the compound was well tolerated and no adverse reactions were observed.

14. In 1976 the FAO/WHO Expert Committee on Food Additives noted that the NOEL for dogs, which appeared to be more sensitive than rats, was about 6 mg/kg bw. The Committee further stated that the NOEL for humans was about 3 mg/kg bw. Using a safety factor of 10, an ADI of 0.3 mg/kg bw was derived. As the report and the studies cited therein were not submitted, this ADI cannot be accepted. However, from the two-year study in dogs a NOEL of 6.25 mg/kg bw can be established. Using a safety factor of 100, an ADI of 0.06 mg/kg bw (3.6 mg for a 60 kg person) can be derived.

15. No radiometric or alternative residue depletion studies in tissues and milk of the target species have been carried out. However, in absence of percutaneous absorption, these data were not considered necessary.

16. No routine analytical method has been provided but was not consideerd necessary for this substance.

Conclusions and recommendation

Having considered that:

- percutaneous and gastrointestinal absorption of natamycin either does not occur, or occurs at a very low level,
- natamycin is intended for topical application on individual animals only (cattle and horses),
- the treated animal is unlikely to be sent for slaughter immediately after treatment,
- natamycin is of low toxicity after oral administration;

the Committee considers that there is no need to establish an MRL for natamycin and recommends its inclusion in Annex II to Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Natamycin	Bovine, equidae	For topical use only