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

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ERYTHROMYCIN - ERYTHROMYCIN THIOCYANTE - ERYTHROMYCIN STEARATE

SUMMARY REPORT (1)

1. Erythromycin, a macrolide antibiotic, is effective *in vitro* against Mycoplasma, Gram positive Cocci (Straphylococcus, Streptococcus), Neisseria, some strains of Haemophilus, Corynebacterium, Listeria, *Pasteurella multocida*, Brucella, Rickettsiae, and Treponemes. Proteus, Pseudomonas, *E. Coli* are relatively resistant to the drug. For most of the sensitive organisms, the MIC ranged from 0.01 to 0.5 µg/ml. In veterinary medicine, it is used for the treatment of clinical and subclinical mastitis in lactating cows, for the treatment of infectious diseases due to erythromycin-sensitive bacteria (cattle, sheep, swine, poultry) and for the treatment of chronic respiratory diseases due to mycoplasma in poultry.

2. The site of action of macrolides is thought to be the ribosomal subunits located in cytoplasm. Erythromycin resistance genes may be carried on plasmids or transposons. The mechanism of resistance to macrolides is related to an enzymatic modification (methylation) of the 23S ribosomal RNA which prevents the macrolides from binding to the ribosome.

3. Erythromycin is rather slowly absorbed after oral administration. Peak serum concentrations ranged from 0.1 to 4.8 µg/ml according to the form and the coating of erythromycin administered. The oral absorption is less than 50% and erythromycin is degraded by gastric acid. It is absorbed in the small intestine (mainly in duodenum for humans) as erythromycin base.

4. Twenty hours after an oral administration of 10 mg erythromycin (N-methyl-^{14}C-erythromycin-8 µC$_i$) to rats, about 37-43% of the administered radioactivity was recovered in the intestinal tract plus faeces, 27.2 to 36.1% in the urine, 21-29% in the expired air. It was rapidly metabolised in the liver, mainly through demethylation process, and excreted in the bile as des-N-methyl-erythromycin, the major metabolite present only in the bile and in the intestinal contents of rats. The isotopic methyl group was eliminated in the expired air as CO_2.

5. Erythromycin is highly (approximately 70-90%) bound to human plasma proteins, mainly to alpha-lacid glycoprotein and to a minor extent to albumin. Erythromycin undergoes a relatively low extent of binding (38-45%) to bovine serum proteins.

6. Several tolerance studies, carried out in cattle, sheep and swine showed that the clinical signs observed at the injection sites disappeared 6 days after the treatment. No side effect was observed after intramammary administration.

7. The acute toxicity of erythromycin base has been determined using different routes of administration in various laboratory animals (mice, rats, hamsters, guinea pigs, rabbits and dogs). The LD_{50} was higher than 300 mg/kg bw after oral administration, and close to 400 mg/kg bw after intraperitoneal administration (374 mg/kg bw for rat and 413 mg/kg bw for guinea pig).

8. Several short and long-term studies in rats, mice, dogs and cats, monkeys were performed. No toxic effects were reported for animals treated up to 370 mg/kg bw for rats (13 weeks erythromycin base) and 100 mg/kg bw for dogs (one year - erythromycin heptonate).

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9. In a reproduction study, two groups of 21 rats received 21 ppm of erythromycin thiocyanate in feed during 100 days before mating. Females of parent generation (FO) were mated three times, and female offspring of these three matings were followed through three subsequent generations (F1, F2, F3). No statistically detectable difference between the control and erythromycin thiocyanate mean values for fertility, foetotoxicity could be detected. No malformation were observed in rats.

10. Erythromycin stearate appears to be non mutagen in Ames test (TA 96, TA 100, TA 1535 or TA 1537), in sister chromatid exchange test or chromosomal aberrations test (CHO cells) in either presence or absence of S9. Erythromycin stearate demonstrated equivocal mutagenicity in the Mouse L5178Y lymphoma cell assay in the absence of S9; it was not mutagen in presence of S9. Finally, it was concluded that erythromycin has no mutagen properties.

11. In carcinogenicity studies, no carcinogenic effect could be seen in animals after oral administrations of either erythromycin ethylsuccinate (rats) or erythromycin stearate given diet containing 0, 2500, 5000 or 10000 ppm (rats and mice).

12. Erythromycin may possess an immunostimulating activity. Epidemiological data in humans indicate that the hypersensitivity reaction to erythromycin is rare and generally mild (hepatic lesions). Orally administered erythromycin in volunteers at therapeutic doses (1 to 3 g/day) or higher doses for 5 days to 3 weeks has been shown to induce a transient decrease or suppression of sensitive bacteria on the intestinal microflora. Administered up to one month, erythromycin-resistant strains were induced. In gnotobiotic mice inoculated with human faecal flora, erythromycin induced suppression of the sensitive strains but does not disturb microbiological antagonism to a great extent. Anaerobes are relatively sensitive to erythromycin.

13. Based on the *in vitro* results, a microbiological ADI of 5 µg/kg bw/day was calculated according to the following formula :

$$\text{ADI (µg/kg bw)} = \frac{\dfrac{\text{lowest MIC (0.1 µg/ml)} \times CF_2\ (10)}{\text{---------------------------------------}} \times \text{daily faecal bolus (150 ml)} \times CF_2\ (1)}{\text{fraction available for microorganism (0.5)} \times \text{human weight (60 kg)}}$$

10, was retained as CF_2 as it had been demonstrated that the MIC of erythromycin for *E. Coli* decreased by a factor 5 to 10 as pH increased from pH 7.4 to 8.

14. Residue depletion studies with unlabelled drug were performed in cattle, pigs, chickens, sheep and swine either after oral routes of administration including administration via drinking water or intramuscular injections. Most of the residues studies have been performed with a formulation of erythromycin base. The amounts of residues were measured by a microbiological method which detection limit was 0.2 µg/g for tissues, 0.06 µg/g for whole egg and 0.02 µg/ml for milk. The dosage for the oral formulation in chicken or for the intramammary paste was similar to those indicated for the marketing authorisations. However, for the injectable formulations, only 6.6 mg/kg bw was the dosage tested. There is no information for depletion studies carried out with higher dosages (10 or 20 mg/kg bw).

15. No residues could be detected in tissues, two or three days after the end of the treatment and at the injection sites 7 days post injection.

16. Residues studies in milking cows and laying hens indicated that erythromycin passes into milk and egg. After intramuscular injections of 5 mg/kg/day/5 days erythromycin (in a propylenglycol diester vehicle), the residues in milk reached the limit of quantifications (0.025 µg/ml) at 130 hours after the last injection. Six days after the end of the medication via drinking water (257000 IU/l drinking water), no residues could be detected in egg.

17. As erythromycin was assayed by microbiology, the compounds measured are the microbiologically active ones.

18. As there is no available (physicochemical) analytical method validated according to the recommendations of Volume VI of the Rules Govering Medicinal Products in the European Community; the following MRLs based on all active antimicrobial residues should be proposed as provisional :

    Cattle, swine, sheep, poultry

liver	0.40 mg/kg
kidney	0.40 mg/kg
fat	0.40 mg/kg
muscle	0.40 mg/kg
milk	0.01 mg/kg
egg	0.20 mg/kg

    The residue marker should be expressed as equivalent erythromycin. The WHO reference of the microbiological activity of anhydrous erythromycin is 920 IU per milligram.

19. These MRL values expire on 01.06.2000.

LIST OF QUESTIONS

Before the 01.06.1999, the following information is requested.

1. The applicant should provide information about the name and the structures of the impurities and complete the criteria of solubility of erythromycin salts in solvents.
2. The applicant should propose a residue marker for all edible tissues (muscle, liver, kidney, milk and eggs) and evaluate the ratio of the marker residue towards the microbiological active residues.
3. The applicant should provide depletion studies for maximal dosages (10 or 20 mg/kg bw of erythromycin thiocyanate or erythromycin base) in target species according the recommended therapeutic regimen.
4. The applicant should provide suitable analytical physicochemical methods for the determination of residues in edible tissues of target species. These methods should be validated according the recommendations of Volume VI and described according a standard layout (Norm ISO 78/2).