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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ERYTHROMYCIN

SUMMARY REPORT (2)

1. Erythromycin (CAS-no 114-07-8), a macrolide antibiotic, is used in veterinary medicine 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, and poultry) and for the treatment of chronic respiratory diseases due to mycoplasma in poultry, mainly as the base, thiocyanate ester and stearate salt. The most often recommended doses (as erythromycin base) range from 5 to 20 mg/kg bw/day for bovines including lactating cows, pigs and sheep, for 3 to 5 days by intramuscular route and 20 mg/kg bw/day via drinking water for broiler chickens and laying hens.

   Currently, erythromycin is included in Annex III of Council Regulation No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissue	Other provisions
Erythromycin	MRLs apply to all microbiological active residues expressed as erythromycin equivalent	Bovine, ovine	40 µg/kg	Milk	Provisional MRLs expire on 1.6.2000
		Bovine, ovine, porcine, poultry	400 µg/kg 400 µg/kg 400 µg/kg 400 µg/kg	Muscle Fat Liver Kidney	
		Poultry	200 µg/kg	Eggs	

   Further data were provided to support the establishment of final MRLs for erythromycin.

2. Erthromycin and other macrolides bind reversibly to the 50S subunit of ribosomes, resulting in the blockage of transpeptidation or translocation reactions, inhibition of protein synthesis and hence inhibition of cell growth. Its action is mainly bacteriostatic, but high concentrations are slowly bactericidal against the more sensitive strains. Its actions are increased at moderately alkaline pH (up to about 8.5), particularly in Gram-negative species, probably because of the improved cellular penetration of the non-ionised form of the substance.

3. In humans, erythromycin is slowly absorbed after oral administration. Peak serum concentrations occurred 1 to 6.3 hours after dosing and 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 as erythromycin base.

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4. Twenty hours after an oral administration of 10 mg erythromycin (N-methyl-^{14}C-erythromycin-µCi) to rats, about 37 to 43% of the administered radioactivity was recovered in the intestinal tract plus faeces, 27.2 to 36.1% in the urine, 21 to 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.

   In calves, 2 hours after a single intramuscular administration of 5 mg erythromycin/kg bw the mean highest concentration in plasma (0.652 µg/ml) was reached. Twelve hours after the administration, the concentration of erythromycin in serum was close to 0.22 µg/ml. After repeated intramuscular administrations of 5 mg erythromycin/kg bw/day for 5 days, no accumulation phenomenon was observed.

   In chickens, 30 minutes after the beginning of a repeated administration of erythromycin via drinking water at a dose of 25 000 IU/kg bw/day of for 3 days (approximately 27 mg/kg bw), the average serum levels ranged from 0.108 to 0.22 µg/ml. After the last administration, they declined to approximately 0.040 µg/ml.

5. Erythromycin is highly (approximately 70 to 90%) bound to human plasma proteins, mainly to alpha-acid glycoprotein and to a minor extent to albumin. Erythromycin undergoes a relatively low extent of binding (38 to 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 (oedema, haemorrhage, irritating) 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 repeated dose toxicity studies in rats, dogs and monkeys were performed.

   After repeated oral administrations of erythromycin base at a dose of 800 mg/kg bw/day for 6 weeks mortalities were reported. No compound related adverse effects were reported in rats treated orally with 90 to 370 mg/kg bw/day for 13 weeks or with 2 to 100 mg/kg feed (dose in mg/kg bw not stated) of erythromycin for 68 weeks.

   No compound related adverse effects were noted in rabbits after repeated oral administration of erythromycin (salt not stated) at doses of 100 and 200 mg/kg bw per day for 31 days.

   No compound related adverse effects were reported in dogs after repeated oral administrations of erythromycin at doses of 50 to 100 mg/kg bw/day for 3 months or of 50 mg/kg bw/day as its glucoheptonate salt for 12 months. Repeated intravenously administrations of 170 mg/kg bw of erythromycin as its glucoheptonate salt twice daily for 3 months did not induce adverse effects in dogs.

   In monkeys after repeated oral administrations of 75 mg/kg bw of erythromycin (salt not stated) per day for 64 days, no compound related adverse effects were reported.

9. In a reproduction study, 2 groups of 21 rats received 0 and 21 mg of erythromycin thiocyanate/kg feed (dose in mg/kg bw non stated) during 100 days before mating. Females of parent generation (F0) were mated 3 times, and female offspring of these 3 matings were followed through 3 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 was observed in rats.

10. In a teratogenicity study, oral doses of 2000 mg/kg bw/day of erythromycin (in gum arabic) did not induce teratogen effects in mice. No further details were available.

11. Erythromycin stearate was not mutagenic in the *Salmonella*-microsomal assay (strains TA 96, TA 100, TA 1535 or TA 1537), in the sister chromatid exchange test or the chromosomal aberrations test (CHO cells) in either presence or absence of metabolic activation. Erythromycin stearate demonstrated equivocal mutagenicity in the mouse L5178Y lymphoma cell assay in the absence of metabolic activation (the increase of mutant fraction was not related to the dose), but it was not mutagenic in presence of metabolic activation. It was concluded that erythromycin has no mutagenic properties.

12. In carcinogenicity studies, no carcinogenic effect could be seen in animals after oral administrations of either erythromycin ethylsuccinate (rats) or erythromycin stearate given for 2 years in diet containing 0, 2500, 5000 or 10000 mg/kg of feed (corresponding to 125, 250 and 500 mg/kg in rats and to 350, 700 and 1400 mg/kg bw in mice).

13. Erythromycin may possess an immunostimulating activity. Epidemiological data in humans indicate that the hypersensitivity reaction to erythromycin is rare and generally mild (hepatic lesions).

14. 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. For the microbiological evaluation also MIC_{50} values were determined for the most relevant micro-organisms of the human gut (*Bifidobacterium, Clostridium, Streptococcus, Bacteroides* spp and *Bacteroides fragilis, Fusobacterium, Eubacterium*). The MIC_{50} of the most sensitive micro-organism, *Bifidobacterium*, was 0.1 µg/ml.

15. The maximum concentrations of erythromycin without effect on lactic ferments were 40 µg/l for *Streptococcus thermophilus* and 6 µg/l for *Lactobacillus bulgaricus*. However, in the industrial fabrication of yoghurt, *Streptococcus thermophilus* grows the first and it has to be considered as the sensitive reference. Therefore, it was concluded that 40 µg/l is the maximal concentration of erythromycin without effect on the industrial fabrication of yoghurts.

16. In humans, doses of 2 g/day or more can induce signs of gastrointestinal disturbance (nausea, vomiting, diarrhoea) in 5 to 30% of the patients. A transient hearing loss could be observed, especially in elder patients with use of large doses or with renal failure. Hepatotoxicity was reported in patients after 10 to 16 days of treatment at dose of 1 g/day of erythromycin estolate, in children treated with 40 mg/kg bw/day and in pregnant women who have received 3 treatments; hepatic injury was rare. Local irritation can be observed after intramuscular administration of 100 mg, and an intravenous infusion of 1 g is regularly followed by phlebitis.

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

$$\text{ADI } (\mu g/kg\ bw) = \frac{\dfrac{MIC_{50} \text{ of the most sensitive species} \times CF2}{CF1} (\mu g/ml) \times \text{daily faecal bolus (150 ml)}}{\text{fraction of an oral dose available for microorganisms} \times \text{weight of human (60 kg)}}$$

Based on the above formula, the microbiological ADI can be calculated as follows:

$$\text{ADI} = \frac{\dfrac{0.1 \times 10}{1} \times 150}{0.5 \times 60} = 5\ \mu g/kg\ bw \text{ i.e. } = 300\ \mu g/person$$

The following assumptions were made :

- MIC_{50} of the most sensitive micro-organism, *Bifidobacterium*, was retained: 0.1 µg/ml
- CF1 = 1 because MIC_{50} of the most sensitive organism (0.1 µg/ml, *Bifidobacterium*) was used,
- CF2 = 10 to take into account the differences between *in vitro* and *in vivo* findings; the *in vivo* data show that a CF2 = 1 would be too conservative.
- 150 g was the weight of the daily faecal bolus,
- Fraction of an oral dose available for micro-organism = 0.5 as determined from human data.

18. Residue depletion studies with unlabelled drug were performed in cattle, pigs, chickens, sheep and swine after oral routes of administration including administration via drinking water or intramuscular injections. Most of the residue studies were performed with formulations of erythromycin base. The residues were quantified by a microbiological method with a detection limit of 200 µg/kg for tissues, 60 µg/kg for whole egg and 20 µg/kg for milk. The oral dose in chicken was similar to that indicated in the marketing authorisations. However, for the injectable formulations, only a dose of 6.6 mg/kg bw was tested. There is no information for depletion studies carried out with higher doses (10 or 20 mg/kg bw). No residues could be detected in tissues, 2 or 3 days after the end of the treatment and at the injection sites 7 days post injection.

19. In a non-radiometric depletion study, groups of 4 non ruminating calves received daily intramuscular doses of erythromycin base on 5 consecutive days at a target dose of 5 mg/kg bw/day. Animals were slaughtered at 1, 3, 7, 14 and 21 days after the last dose. The total antimicrobial activity of residues was measured by a microbiological plate assay, using agar medium and *Micrococcus luteus ATCC9341* as test organism, with a limit of quantification of 100 µg/kg for muscle and of 200 µg/kg for liver, kidney and fat. The concentrations of erythromycin A and its metabolite, N-demethyl-erythromycin A, were simultaneously assayed using a liquid chromatography method with mass detection (LC-MS/MS) proposed for monitoring purposes, the limit of quantification being 100 µg/kg for all edible tissues. Erythromycin B and erythromycin C could also be detected by this analytical method.

    One day after the last treatment, the microbiologically active residues could only measured in muscle and in fat in 1 out of 4 animals (366 and 1067 µg equivalents erythromycin/kg, respectively), and in liver and kidney in 2 animals (550 and 1000 µg equivalents erythromycin/kg for liver and 643 and 1561 µg equivalents erythromycin/kg for kidney respectively). At day 3, no antimicrobial activity could be measured in muscle, fat, liver, and kidney except in one kidney sample (296 µg equivalents erythromycin/kg). However, the injection site presented a significant amount of antimicrobiologically active residues: 18889, 3653, 1194, 713 and 599 µg equivalents erythromycin/kg at 1, 3, 7, 14 and 21 days after the last treatment.

    One day after the last administration, the mean concentration of erythromycin A in kidney was 447 µg/kg (n=4). In the other edible tissues, erythromycin A could be measured in 1 animal for muscle (223 µg/kg) and for fat (924 µg/kg) and in 2 animals for liver (634 and 278 µg/kg). At later samples erythromycin A concentrations were below the limit of quantification in the muscle, liver, kidney and fat. However, the injection site contained a significant amount of erythromycin A: 45908 (n=4), 3058 (n=4), 185 (n=4) µg/kg at 1, 3, 7 after the last treatment. Thereafter erythromycin A concentrations at the injection site were below the limit of quantification.

    One day after the end of treatment, N-demethyl-erythromycin A could only be quantified in a very limited number of tissue samples e.g. in 1 sample of fat (211 µg/kg) and of kidney (320 µg/kg) and in 2 liver samples (332 and 121 µg/kg). At later sampling times, concentrations were below the limit of quantification in muscle, kidney, fat and liver, respectively). However, the injection site contained a significant amount of N-demethyl-erythromycin A: 521 µg/kg (n=4) and 111 (n=1) at 1 and 3 after the last treatment, respectively. Thereafter N-demethyl-erythromycin A concentrations at injection site were below the limit of quantification.

    Erythromycin B and C could only be detected as traces in 1 kidney sample collected 1 day after the last dose and in injection site samples.

Whenever the concentrations were sufficient to be assayed by both analytical methods, erythromycin A was the main antimicrobial active residue and the ratio of erythromycin A with regard to the total antimicrobiologically active residues were 0.64, 0.57, 0.65 and 0.86 in muscle, liver, kidney and fat, respectively. Erythromycin A was retained as marker residue.

20. In a non-radiometric depletion study, 9 lactating cows received daily intramuscular doses of erythromycin base on 5 consecutive days at a target dose of 5 mg/kg bw/day. Milk was collected during 9 days (2 milkings per day) after the end of treatment. The total antimicrobial activity of residues was measured by a microbiological plate assay, using agar medium and *Micrococcus luteus ATCC9341* as test organism, with a limit of quantification of 20 µg/kg. The concentrations of erythromycin A and its metabolite were simultaneously assayed using the LC-MS/MS method proposed for monitoring purposes, the limit of quantification being 10 µg/kg. Erythromycin B and erythromycin C could also be detected by this analytical method.

    At the first milking after the end of the treatment the mean concentrations of residues with a antimicrobial activity was 803 µg equivalents erythromycin/kg, which then declined to 348, 114 and 87 µg equivalents erythromycin/kg at the 2^{nd}, 4^{th} and 5^{th} milking respectively. At later time points such activity could be only measured on a limited number of samples. At the 8^{th} milking, a mean value of 50 µg/kg was determined from 6 samples. No antimicrobial activity could be detected in milk of the 16^{th} milking.

    At the first milking after the end of the treatment the mean concentrations of erythromycin A were 1223 µg/kg, then declined to 421, 110 and 88 µg/kg at the 2^{nd}, 4^{th} and 5^{th} milking respectively. Thereafter erythromycin A could only measured in a limited number of samples. At the 8^{th} milking, a mean value of 51 µg/kg was determined from 6 samples. The concentrations of erythromycin A in all the samples collected were below 40 µg/kg at 16^{th} milking.

    N-demethyl-erythromycin A concentrations were very low: 141, 69 and 22 µg/kg at the 1^{st}, 2^{nd} and 4^{th} milking. At further milkings, this compound could be quantified in a limited number of samples and the concentrations were in the magnitude of 10 µg/kg or below. In all samples, no erythromycin B was detected. Only traces of erythromycin C were seen (less than 0.1 µg/kg).

    Whenever the concentrations were sufficient to be assayed by both analytical methods, Erythromycin A accounted for about 100% of the antimicrobial active residue. Therefore, the ratio of erythromycin A to the total antimicrobially active residues was 1 for bovine milk.

21. In a non-radiometric depletion study, groups of 4 pigs received daily intramuscular doses of erythromycin base on 5 consecutive days at a target dose of 5 mg/kg bw/day. Animals were slaughtered at 1, 2, 3, 4, 5 and 7 days after the last dose. The total antimicrobial activity of residues was measured by a microbiological plate assay, using agar medium and *Micrococcus luteus ATCC9341* as test organism, with a limit of quantification of 100 µg/kg for all edible tissues. The concentrations of erythromycin A and its metabolite were simultaneously assayed using the LC-MS/MS method proposed for monitoring purposes, the limits of quantification being 100 µg/kg for all edible tissues.

    In all edible tissues except the injection site, neither antimicrobially active residues nor erythromycin A or N-demethyl-erythromycin A could be detected in pig edible tissues at day 1 after the last dose and later on. At the injection site, the concentrations of antimicrobially active residues were 677 µg/kg and 327 µg equivalents erythromycin/kg at days 1 and 2 after the last dose, respectively. At later sampling, residue concentrations in the magnitude of 160 µg equivalents erythromycin/kg could be measured in one sample collected at days 3 and 4. Then, the concentrations were below 100 µg/kg.

    As antimicrobially active residues and erythromycin A could be quantified at the injection site of pigs collected 1 and 2 days after the end of the treatment, erythromycin A was retained as the marker residue, but no ratios were determined.

22. In a non-radiometric depletion study, groups of 6 broiler chickens received erythromycin at a dose of 20 mg/kg bw/day on 3 days via drinking water. The total antimicrobial activity of residues was measured by a microbiological plate assay, using agar medium and *Micrococcus luteus ATCC9341* as test organism, with a limit of quantification of 100 µg/kg for all edible tissues. The concentrations of erythromycin A and its metabolite were simultaneously assayed using the LC-MS/MS method proposed for monitoring purposes, the limit of quantification being 100 µg/kg for all edible tissues.

    Whatever the analytical method used (LC-MS/MS and microbiological assay), the concentrations of residues in edible tissues were below the limits of detection at all time points. The limits of detection for erythromycin A were 3, 25, 5 and 30 µg/kg for muscle, kidney, skin + fat and liver, respectively and those for N-demethyl-erythromycin A were 5, 25, 24 and 48 µg/kg for muscle, kidney, skin + fat and liver, respectively.

23. In a non-radiometric depletion study, 25 laying hens received erythromycin at a dose of 20 mg/kg bw/day for 3 days via drinking water. Eggs were collected daily during the treatment and until 10 days after the last treatment. The total antimicrobial activity of residues was measured by a microbiological plate assay, using agar medium and *Micrococcus luteus ATCC9341* as test organism, with a limit of quantification of 100 µg/kg for eggs. The concentrations of erythromycin A and its metabolite were simultaneously determined using the LC-MS/MS method proposed for monitoring purposes, the limits of quantification of both compounds being 50 µg/kg.

    During treatment, the mean antimicrobial activities measured ranged from 158 (n=4) to 198 µg equivalents erythromycin/kg (n=14). After the end of the treatment, they declined from 221 µg/kg (n=15), at day 1 to 118 µg/kg (n=3) at day 3. At later sampling time, antimicrobially active residues could not be quantified (below 100 µg/kg).

    Erythromycin A could only be quantified in 25% and 12.5% of the eggs collected at day 1 and 2 respectively after the end of the treatment. The concentrations measured ranged from 50 to 78 µg/kg. At later sampling time, the concentrations were below 50 µg/kg. N-demethyl erythromycin A was measured in a single egg.

    Whenever the concentrations were sufficient to be assayed by both analytical methods, erythromycin A accounted for about 25% the antimicrobially active residue in eggs.

24. In a non-radiometric study, sheep received daily intramuscular doses of erythromycin base on 5 consecutive days at a target dose of 10 mg/kg bw. Animals were slaughtered at 1, 3, 6, 12 and 15 days after the end of the treatment. The total antimicrobial activity of residues was measured by a microbiological plate assay, using agar medium and *Micrococcus luteus ATCC9341* as test organism, with limits of quantification of 200 µg/kg for kidney, muscle and fat and of 250 µg/kg for liver. The concentrations of erythromycin A and its metabolite were simultaneously assayed using the LC-MS/MS method proposed for monitoring purposes, the limits of quantification of both compounds being 100 µg/kg.

    One day after the last treatment, the microbiologically active residues could only be measured in the muscle, liver and kidney of 2 or 3 of the 4 animals slaughtered. The mean concentrations were 420 (n=2), 1218 (n=3), 767 (n=3) µg equivalents erythromycin/kg in muscle, liver and kidney, respectively. At later sampling time, no antimicrobiologically active residues could be measured. However, the injection site presented a significant amount of antimicrobiologically active residues: 17 396, 1 996, 707, 759, 470 and 368 µg equivalents erythromycin/kg at 1, 3, 6, 9, 12 and 15 days after the last treatment.

    One day after the last administration, the mean concentrations of erythromycin A in muscle, liver and kidney were 272 (n=3), 405 (n=4) and 589 (n=3) µg/kg. At later sampling, erythromycin A concentrations were below the limit of quantification in muscle, liver and kidney. However, the injection site contained a significant amount of erythromycin A: 12 364 (n = 4), 2 567 (n = 4), 460 (n = 1) µg/kg at 1, 3, 6 days after the last treatment. Thereafter erythromycin A concentrations were below the limit of quantification in the injection site.

N-demethyl-erythromycin A concentrations were below the limit of detection in all edible tissues except the injection site. At the injection site, this metabolite could only be measured in 3 samples of injection site collected at days 1 and 6 after the end of the administration and the concentrations were below 200 µg/kg.

Whenever the concentrations were sufficient to be assayed by both analytical methods, Erythromycin A was the main antimicrobially active residue and accounted for 88, 50 and 76% of the total antimicrobiologically active residues in muscle, liver, kidney, respectively. No figures are available for fat as neither antimicrobiologically active residues nor erythromycin A could be quantified. Erythromycin A was retained as marker residue.

25. Analytical methods, using liquid chromatography with mass detection (LC-MS/MS), have been validated according to the recommendations of Volume VI of the Rules Governing Medicinal Products in the European Community to measure residues of erythromycin A. The limit of quantification for erythromycin A was 100 µg/kg for all edible tissues of bovine, porcine, ovine and poultry. The limits of detection ranged from 0.5 to 39 µg/kg according to the edible tissue and the target species. For milk, the limits of quantification and detection were 10 and 0.43 µg/kg and for eggs, they were 10 and 0.91 µg/kg, respectively.

Conclusions and recommendation

Having considered that:

- a microbiological ADI of 5 µg/kg bw (i.e. 300 µg/person) was established,
- erythromycin A was identified as the marker residue for all edible tissues including milk and eggs,
- the marker residue represents approximately 70, 87, 55 and 65% of the total residues with an antimicrobial activity in muscle, fat or fat + skin, liver and kidney of bovine and ovine, and approximately 100% and 25% of the total residues with an antimicrobial activity in milk and eggs, respectively,
- no residue data are available for ovine milk,
- a validated analytical method for monitoring residues is available;

the Committee for Veterinary Medicinal Products recommends the inclusion of erythromycin 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
Erythromycin	Erythromycin A	Bovine	200 µg/kg 200 µg/kg 200 µg/kg 200 µg/kg 40 µg/kg	Muscle Fat Liver Kidney Milk	
		Ovine	200 µg/kg 200 µg/kg 200 µg/kg 200 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption
		Porcine	200 µg/kg 200 µg/kg 200 µg/kg 200 µg/kg	Muscle Skin + fat Liver Kidney	
		Chickens	200 µg/kg 200 µg/kg 200 µg/kg 200 µg/kg 150 µg/kg	Muscle Skin + fat Liver Kidney Eggs	

Based on these MRLs values, the daily intake will represent about 90% of the ADI.