



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ENROFLOXACIN (extension to sheep, rabbits and lactating cows)

SUMMARY REPORT (3)

1. Enrofloxacin (CAS: 93106-60-6) is a synthetic fluoroquinolone antimicrobial agent. In veterinary medicine it is administered by subcutaneous injection to cattle and intramuscular injection to pigs and orally to cattle, pigs, turkeys and chickens, for the treatment of infections of the respiratory and alimentary tract. The recommended doses are 2.5 to 5 mg enrofloxacin/kg bw/day for 3 to 5 days (cattle and pigs) or 10 mg enrofloxacin/kg bw/day for 3 to 10 days (chickens and turkeys). In some Member States, enrofloxacin is authorised for use in sheep, goats and rabbits. The dosage regime for sheep is 2.5 mg/kg bw per day for 5 days, administered parenterally. For treatment of watery mouth disease in neonatal lambs, a single oral dose of 5 to 7 mg/kg bw is administered. Rabbits may be dosed at 2.5 to 5 mg/kg bw per day for 3 to 5 days, by oral administration (drinking water or gavage) or by intramuscular injection. Ciprofloxacin, a major metabolite of enrofloxacin, is widely used in human medicine.

Currently, enrofloxacin is included in Annex I of Council Regulation (EEC) No. 2377/90 as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Enrofloxacin	Sum of enrofloxacin and ciprofloxacin	Bovine, porcine, poultry	30 µg/kg	Muscle, liver, kidney	

The microbiological ADI currently agreed by the CVMP is 6.2 µg/kg bw per day. This is lower than the toxicological ADI of 30 µg/kg bw per day which was calculated by applying a safety factor of 100 to the NOEL of 3 mg/kg bw per day in a 90-day repeated-dose (dietary) study in 3-month old Beagle dogs, based on arthropathy.

The Applicant has now applied for an extension of the MRLs to tissues of sheep and rabbits, and cows' milk.

2. Target species tolerance data were previously provided for calves, pigs and chickens. No evidence of leg weakness was observed in birds treated at the recommended rate. No evidence of arthropathy was observed in calves and pigs treated according to the recommended dosage regime. However oral administration of 30 mg/kg bw per day for 14 days to calves and 50 mg/kg bw per day to pigs produced histopathological evidence of arthropathy. Evidence of arthropathy was found in calves given oral doses of 90 mg/kg bw per day and pigs given 50 mg/kg bw per day.

3. No adverse effects were observed in neonatal lambs treated orally with 5 to 7 mg/kg bw enrofloxacin and the lambs grew normally over the first 3 months of life. Treatment of rabbits according to the recommended dosage regime had no adverse effects on general health, reproductive performance or development of the offspring. Treatment of rabbits resulted in a sharp decrease in coliform bacteria but no effects were observed on the numbers of total intestinal bacteria, total anaerobic bacteria, *Lactobacilli* or *Bacteroides* spp.
4. In vitro MIC values were determined for both enrofloxacin and ciprofloxacin against 20 dairy starter cultures using both proprietary culture media and sterilised skimmed milk. For both substances, the presence of milk caused a general increase in MIC values. Against enrofloxacin, all strains of *Lactobacillus* spp. and *Bifidobacterium* spp. had MIC values larger than 32 µg/ml when grown in sterilised skimmed milk. Against ciprofloxacin, all of the strains of *Lactobacillus* spp. had MIC values larger than 32 µg/ml when grown in sterilised skimmed milk. Twenty bacterial starter cultures were screened for inhibition by ciprofloxacin and ampicillin. The inhibitory effect of ciprofloxacin was considerably less than that of ampicillin. At the highest concentration of ciprofloxacin (0.1 µg/ml), acid production was inhibited by less than 5% in 70% of the cultures tested and inhibition was greater than 15% for only one culture.
5. No radiometric studies were provided for rabbits. Published pharmacokinetic studies in rabbits showed that enrofloxacin was well absorbed after oral, intramuscular and subcutaneous administration of 5 mg/kg bw. C_{max} values of 0.45, 3.04 and 2.07 µg/ml respectively were attained 1 to 2 hours after administration. The respective bioavailabilities were estimated to be 61%, 92% and 72%. In an *in vitro* study using fractions of rabbit liver, the metabolism of enrofloxacin was shown to be similar to that of the rat, with ciprofloxacin as the main metabolite (12 to 15% of radioactivity). Rabbits were dosed orally (in the drinking water) with 13 mg enrofloxacin/kg bw/day for 7 consecutive days. Three males and 3 females were killed at each time point. Residues of ciprofloxacin were detectable only in liver samples taken 1 day after the end of treatment (less than 10 to 27 µg/kg). Residues of enrofloxacin in liver and muscle were detectable only in samples taken one day after treatment (less than 10 to 20 µg/kg and less than 10 to 22 µg/kg respectively). No residues were detected in fat. In kidney, residues of enrofloxacin declined from 13 to 100 µg/kg, one day after treatment, to less than 10 to 30 µg/kg, 4 days after the end of treatment. All tissues contained marker residue levels below the analytical limit of quantification (10 µg/kg) by day 7 after treatment, with the exception of two kidney samples, which contained 21 and 43 µg/kg enrofloxacin. Taking into account the agreed policy on minor species, it was agreed that the same marker residue which had been adopted for the major species was also applicable to rabbits and radiometric studies were not required.
6. No radiometric studies were provided for sheep. In cattle, the sum of the residues of enrofloxacin plus ciprofloxacin accounted for around 65% of the residues in liver, 80% of the residues in kidney, 88% of the residues in muscle and 50% of the residues in fat. Radiometric studies in pigs and chickens had confirmed that the sum of enrofloxacin and ciprofloxacin was the appropriate marker residue for these species. It was considered reasonable to assume that the same marker residue would be appropriate for sheep, therefore radiometric studies were not needed for sheep. In neo-natal sheep given a single oral dose of 7.5 mg enrofloxacin/kg bw, residues of enrofloxacin and ciprofloxacin (determined by HPLC) in liver, kidney, muscle and fat were each less than or equal to 10 µg/kg in all tissues 16 days after dosing. Two days after dosing, mean residues of enrofloxacin in liver, kidney, muscle and fat were 520, 1370, 993 and 1370 µg/kg and declined to 48, 13, 13 and 13 µg/kg respectively, 4 days after dosing. Over the same time period, mean residues of ciprofloxacin in these tissues declined from 483, 175, 265 and 175 µg/kg in liver, kidney, muscle and fat, to 53, 13, 13, 13 and 13 µg/kg.

7. When a lactating cow was subcutaneously injected with 7.4 mg ¹⁴C-enrofloxacin/kg bw, the total residue concentrations in milk samples declined from 2930 µg equivalents/kg at the first milking (6 hour time point) to the analytical limit of quantification of approximately 2 µg/l by the eighth milking (4 day time point). When 3 lactating cattle were dosed intravenously with 5 mg ¹⁴C-enrofloxacin/kg bw/day for 5 consecutive days (non-GLP study), the total residue concentrations in milk samples fell from approximately 3267 µg/l (3900 µg/l was highest) at 6 hours after dosing to below the analytical limit of detection (17 µg/kg) in one cow 57 hours after dosing; at this time point residues in milk from the other cows were 68 and 140 µg/kg. In this study, the residues in milk samples taken 6 to 24 hours after treatment were characterised; around 80% of the residues in milk were ciprofloxacin and 8% were enrofloxacin; the percentage did not change over the limited time period studied. When lactating cattle were dosed with 2.5 mg enrofloxacin/kg bw/day for 5 days (12 animals, GLP study), the mean residues of enrofloxacin and ciprofloxacin in milk samples fell from 8.3 and 21.9 µg/l at one day after the end of treatment to below the analytical limit of quantification (5 µg/l) in milk from 10 out of the 12 cows, 4 days after the last dose. No data were provided concerning the residues in milk samples during treatment.
8. A routine analytical method was presented in the ISO 78/2 format for determining the combined residues of enrofloxacin and ciprofloxacin (the marker residue) in tissues and milk. In the method, enrofloxacin and ciprofloxacin (the marker residue) are simultaneously extracted into a mixture of ethanol and glacial acetic acid. The extracts of all tissues (except liver and kidney) are evaporated to dryness and re-suspended in HPLC mobile phase. Extracts of liver and kidney are evaporated to a reduced volume then solid phase extracted on a column of Amberlite XAD-4 resin. Residues are eluted from the column in methanol, then evaporated to dryness and dissolved in HPLC mobile phase. Residue extracts were quantified against external standards by HPLC with fluorescence detection. The limits of quantification were 10 µg/kg for enrofloxacin and for ciprofloxacin for rabbit liver, kidney and muscle and 5 µg/l for cows' milk. A version of the method which used different extraction solvents and HPLC mobile phase was provided for sheep tissues; the limit of quantification was 10 µg/kg for enrofloxacin and ciprofloxacin in all tissues; however further data were required concerning specificity, susceptibility and the derivation of the limit of detection for sheep.

Conclusion and recommendations

Having considered that:

- a microbiological ADI of 6.2 µg/kg bw, i.e. 372 µg/person, was established for enrofloxacin,
- the sum of enrofloxacin plus ciprofloxacin accounted for approximately 88% of the residues in cows' milk 6 to 24 hours after treatment,
- in cattle, the sum of the residues of enrofloxacin plus ciprofloxacin (the marker residue) accounted for around 65% of the residues in liver, 80% of the residues in kidney, 88% of the residues in muscle and 50% of the residues in fat, within a few hours of dosing,
- the sum of enrofloxacin plus ciprofloxacin was also a suitable marker residue for tissues of sheep and rabbits; in sheep, residues were most persistent in liver and the distribution of residues resembled that in the bovine whereas in rabbits, residues were most persistent in kidney,
- a routine analytical method was available for the determination of the marker residue in cows' milk and in the tissues of rabbits,
- a routine analytical method was available for the determination of the marker residue in sheep but was not fully validated for sheep tissues;

the Committee recommends the inclusion of enrofloxacin for rabbits and lactating cows in Annex I of Council Regulation (EEC) 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Enrofloxacin	Sum of enrofloxacin and ciprofloxacin	Rabbit	100 µg/kg 100 µg/kg 200 µg/kg 300 µg/kg	Muscle Fat Liver Kidney	
		Bovine	100 µg/kg	Milk	

and recommends the inclusion of enrofloxacin for sheep in Annex III of Council Regulation (EEC) 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Enrofloxacin	Sum of enrofloxacin and ciprofloxacin	Ovine	100 µg/kg 100 µg/kg 300 µg/kg 200 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.7.1999

Based on these MRLs, it was calculated that consumer intake of total residues from the consumption of milk and meat will represent approximately 74% of the ADI.

LIST OF QUESTIONS

1. The Applicant should fully validate the analytical method for edible tissues of sheep, in accordance with Volume VI of The Rules Governing Medicinal Products in the European Community, and re-present it in an internationally recognised format (e.g. ISO 78/2), accompanied by full raw data.