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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ENROFLOXACIN
(modification for bovine, porcine and poultry)

SUMMARY REPORT (2)

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 was not provided for these species. 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 previously agreed by the Committee for Veterinary Medicinal Products was 0.3125 µg/kg bw. This is lower than the toxicological ADI of 12 µg/kg bw which was calculated by applying a safety factor of 100 to the NOEL of 1.2 mg/kg bw per day in a 90-day repeated-dose (dietary) study in 3-month old Beagle dogs, based on testicular toxicity.

   The Applicant has now applied for a modified ADI and modified MRLs for bovine, porcine and poultry.

2. Enrofloxacin was well absorbed after oral administration to rats and the target species. In rats, the bioavailability of enrofloxacin was estimated to be 75% after a single oral dose of 5 mg/kg bw of radiolabelled enrofloxacin. The substance was widely distributed to all tissues with highest concentrations in the liver and kidney. Elimination was rapid via both urine and faeces. Most of the administered radioactivity was excreted during the first 24 hours after administration. Rat urine after dosing (5 times 5 mg/kg bw/day) with ^{14}C-enrofloxacin contained the following substances: enrofloxacin (17%), ciprofloxacin (31%), oxociprofloxacin (5%), enrofloxacin amide (23%), dioxociprofloxacin (9%), desethylene ciprofloxacin (3%), desethylene enrofloxacin (2%), N-formyl ciprofloxacin (less than 2%), oxoenrofloxacin (less than 2%) and hydroxy oxoenrofloxacin (3%).

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3. In an adsorption/desorption study, enrofloxacin was tested on cattle manure and chicken and turkey excreta and ciprofloxacin was tested on cattle manure. Both substances were strongly bound to cattle manure (around 76 to 77%) but enrofloxacin was only poorly bound to poultry excreta. Similar data on the binding to human faeces were not provided. In a separate study, there was a 4-fold increase in the minimum inhibitory concentrations (MICs) of both enrofloxacin and ciprofloxacin in the presence of muscle or liver and a 2 to 3-fold increase in the presence of kidney, fat or milk; these data indicated that the substances were bound to these substrates.

4. The main metabolite ciprofloxacin was also reported to be well absorbed. In humans, the bioavailability of orally administered ciprofloxacin was 63 to 69% and was not significantly affected by co-administration with food. The main sites of absorption of ciprofloxacin in humans were shown to be the duodenum and jejunum. In humans, ciprofloxacin was eliminated by both renal and non-renal routes. The concentrations of ciprofloxacin in human faeces varied widely between individuals. It was calculated that a mean percentage of approximately 14% of an orally-administered dose of ciprofloxacin was excreted in faeces, assuming a daily faecal bolus of 150 g. Competitive binding studies with human faeces suggested that most of the fluoroquinolone residues present in faeces were bound; however no data were provided concerning the extent of ciprofloxacin binding to faeces. In humans, oxociprofloxacin was the main metabolite of ciprofloxacin found in urine.

5. *In vitro* MIC values were determined for enrofloxacin, at 3 different inoculum densities, against 100 bacterial strains of human gut origin comprising 10 isolates from 10 genera. These included *Escherichia coli, Enterococcus* spp., *Lactobacillus* spp., *Proteus* spp., *Bacteroides* spp., *Bifidobacterium* spp., *Fusobacterium* spp., *Eubacterium* spp., *Peptostreptococcus* spp., and *Clostridium* spp. *Escherichia coli* was the most sensitive species to enrofloxacin with a geometric mean MIC value of 0.041 µg/ml (corresponding to an MIC_{50} value of 0.031 µg/ml) at an inoculum density of 10^7 cfu/ml. For most genera, the MIC_{50} value doubled when the inoculum density was increased to 10^9 cfu/ml. For *Escherichia coli*, the MIC_{50} at 10^9 cfu/ml was 0.062 µg/ml.

6. *In vitro* MIC values were determined for ciprofloxacin, against the same 100 bacterial strains of human gut origin. The intended inoculum density was 10^7 cfu/ml. The same or higher mean MIC values against ciprofloxacin, compared with enrofloxacin, were obtained for most genera. Only *Escherichia coli, Proteus* spp., *Bifidobacterium* spp. and *Peptostreptococcus* spp. gave lower MIC_{50} values with ciprofloxacin. For *Escherichia coli*, the MIC_{50} was 0.016 µg/ml.

7. A study was carried out to determine the microbiological activity of enrofloxacin and 9 enrofloxacin metabolites against 164 aerobic bacterial strains of human origin at a single inoculum density of 10^5 cfu/ml. Enrofloxacin and ciprofloxacin were the most active substances. *Escherichia coli* was the most sensitive species tested giving MIC_{50} values of 0.03 and 0.015 µg/ml against enrofloxacin and ciprofloxacin respectively.

8. The effect of pH on MIC_{50} values was investigated against 36 bacterial strains of human gut origin. The pH values tested were 5.2, 6.2 and 7.2. There was a clear trend towards increased MIC values with decreasing pH. *Escherichia coli* was the most sensitive with geometric mean MIC values of 0.031, 0.049 and 0.27 µg/ml at pH values of 7.2, 6.2 and 5.2 respectively.

9. Special studies were carried out to investigate the possible effects of co-culture and anaerobiosis. *In vitro* MIC values were determined under different levels of anaerobiosis for 10 strains of human gut origin (2 strains from each of 5 genera). No growth was observed with the obligate anaerobes except under conditions approaching complete anaerobiosis. For the facultative anaerobes, there was no difference in sensitivity to enrofloxacin under different levels of anaerobiosis. In another study, *in vitro* MIC and MBC (minimum bactericidal concentration) values were determined for enrofloxacin against selected co-culture combinations of human intestinal flora. Of the 10 co-culture pairs, only 2 bacterial strains showed an increased enrofloxacin MBC in co-culture, compared with the result in pure culture. No firm conclusions can be drawn from this limited result.

10. The effect of differences between the *in vitro* and *in vivo* situation on the microbiological activity of enrofloxacin was investigated against 10 bacterial strains of human origin using an *in vitro* model to simulate the human gut. Enrofloxacin was added to the test system at a concentration similar to the geometric mean MIC values and at 0.56 µg/ml, the concentration estimated to occur in the human intestine after consumption of a diet containing residues of enrofloxacin. Viable counts were performed at the beginning and at the end of an 18-hour incubation period. The study was carried out using only 2 strains from each of 5 bacterial species. The results indicated that gut conditions would raise the MIC_{50} values but it was not possible to draw any definite conclusions regarding the size of this effect.

11. In humans, ciprofloxacin is used for selective elimination of potential aerobic and facultative anaerobic pathogens from the gastrointestinal tract, whilst preserving the predominant anaerobic bacterial flora. Because of these properties, ciprofloxacin is clinically useful for selective decontamination prior to colorectal surgery, and in the treatment of immuno-compromised patients. Several studies were provided on the effects of oral doses of ciprofloxacin on the intestinal flora of humans. The doses ranged from 50 mg twice daily to 750 mg three times daily. In all cases the facultative anaerobic bacteria were strongly suppressed. The strict anaerobic microflora were either not affected or were only mildly suppressed by therapeutic doses of ciprofloxacin. There was no evidence to suggest that therapeutic doses of ciprofloxacin weakened the barrier effect.

12. Following a re-evaluation of the results of the 3-month repeated dose toxicity studies in rats and dogs, it was concluded that the effects on the testes in dogs were inconsistent, not dose-related and were of a different type from those observed in rats. It was therefore agreed that the toxicological ADI should be based on the NOEL 3 mg/kg bw per day for arthropathy in juvenile dogs. A revised toxicological ADI of 30 µg/kg bw per day was calculated by applying a safety factor of 100.

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

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

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

$$\text{ADI} = \frac{\frac{0.062 \times 2}{1} \times 150}{0.25 \times 0.2 \times 60} = 6.2 \text{ µg/kg bw, i.e. } 372 \text{ µg/person}$$

The following assumptions were made:

- 0.062 µg/ml is the MIC_{50} value for enrofloxacin against the most sensitive micro-organism, *E.coli*, at an inoculum density of 10^9 cfu/ml.
- CF1 = 1, based on a factor of 1 because of the chromosomal nature of fluoroquinolone resistance and a factor of 1 because the MIC_{50} for the most sensitive, species was used.
- CF2 = 2, to correct for the differences in growth conditions between the *in vitro* and *in vivo* situation including the increase in MIC values following changes in pH, the limited data from simulated gut model did not permit a higher value to be used.

- the bioavailability of enrofloxacin in rats was 75% following oral administration of 5 mg/kg bw; therefore 0.25 was the fraction of the oral dose available to the micro-organisms at the distal part of the gastrointestinal tract; this factor was multiplied by 0.2 to account for the results of an absorption study with ^{14}C-enrofloxacin in human faeces which indicated that binding to the ileocaecal contents was approximately 80%.

The microbiological ADI calculated above 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 for arthropathy in dogs.

14. When poultry were orally dosed with 10 mg ^{14}C-enrofloxacin/kg bw for 7 days, the total residue concentrations in liver samples at time points from 6 to 24 hours after the last dose declined from 4890 µg equivalents/kg to 89 µg equivalents/kg in chickens and 8240 µg equivalents/kg to 2662 µg equivalents/kg in turkeys. In both studies, the residues in tissues taken at the 6-hour time point were characterised. In chickens, 61 to 66%, 51%, 53 to 62% and 85% of the total residues present in kidney, liver, fat and muscle were present as enrofloxacin. The comparative values for turkeys were 58 to 60%, 94 to 97% and 99% for liver, kidney and muscle respectively. Enrofloxacin also accounted for 85% and 98% of the total residues in muscle samples from chickens killed 10 and 15 hours after the end of treatment. In turkeys, enrofloxacin accounted for 81 to 99% of the total residues in birds killed 10, 15 and 24 hours after the last dose. When birds were dosed in a similar manner with unlabelled enrofloxacin, the concentrations of marker residues in chicken liver samples 3 to 15 days after the last dose declined from 42 µg/kg to 11 µg/kg. In turkeys, the marker residue concentrations in liver 1 to 7 days after the last dose fell from 1250 µg/kg to less than 10 µg/kg. In all the turkey studies and in the radiometric chicken study, liver was the tissue containing the highest residue concentrations. However in a non-radiometric study in chickens, samples of skin with its associated fat contained the highest marker residue concentrations. A true comparison of the total residue levels found in the radiometric studies with those determined using the routine analytical method was not possible as there was no overlap in the time points chosen.

15. When pigs were subcutaneously injected with 5 mg ^{14}C-enrofloxacin/kg bw/day for 5 consecutive days, mean total residue concentrations (3 animals) in kidney, liver, muscle and fat fell from 2080, 1790, 862 and 148 µg equivalents/kg respectively at 1 days after the last dose, to 141, 277, 28 and 10 µg equivalents/kg respectively at 5 days (the last time point). The highest residue concentrations were found in injection site tissues (3 animals, 5 sites/animal) which fell from average concentrations of 76600 µg equivalents/kg (highest was 244000 µg equivalents/kg) to 7520 µg equivalents/kg (highest was 98300 µg equivalents/kg) over 0.5 to 5 day time points respectively after the last dose. The nature of the residues in tissues from one gilt and one barrow killed 12 hours after dosing were characterised; approximately 80%, 80%, 90% and 99% of the residues in liver, muscle, kidney and fat were present as enrofloxacin; significant amounts of ciprofloxacin were also present.

    When pigs were dosed by deep intramuscular injection with 2.5 mg enrofloxacin/kg bw/day for 3 consecutive days, marker residue concentrations in kidney, liver, muscle, fat and skin fell rapidly from 721, 671, 356, 96 and 141 µg/kg 1 days after the last dose (first time point) to less than 10 µg/kg in all tissues at 9 days. Comparison of the radiometric and non-radiometric pig studies was not possible as the dosages, routes of administration and time points used were all different. Consequently there was no information of the ratio of marker to total residues at time points later than 12 hours.

16. When cattle were subcutaneously injected with 5 mg ^{14}C-enrofloxacin/kg bw/day for 5 consecutive days, total residue concentrations in liver, kidney, muscle and fat fell from 10050, 7470, 1540 and 625 µg/kg respectively at 8 hours after the last dose (first time point) to 639, 66, 4 and 6 µg/kg respectively at 14 days (last time point). The highest residue concentrations were found in injection site tissues (3 animals, 5 sites/animal) which fell from average concentrations of 380 000 µg/kg (1 940 000 µg/kg was highest) to 160 µg/kg (3432 µg/kg was highest) over the same time points. Residues in the tissues from one steer and one heifer slaughtered 8 hours after the last dose were characterised; enrofloxacin accounted for 21 to 26%, 27 to 31%, 55 to 56% and 35 to 59% of the residues in liver, kidney, muscle and fat respectively. Ciprofloxacin accounted for 38 to 44%, 50 to 55%, 33 to 34% and 5 to 7% of the residues in these same tissues. Oxoenrofloxacin was the major component of the residues in fat. Up to 40% of the residues at the injection site were ciprofloxacin. Investigation of liver samples 7 days after the last dose showed that very little enrofloxacin or ciprofloxacin were present and that the main component of the residue was desethylene ciprofloxacin, accounting for up to 16% of the total residues.

    When cattle were subcutaneously injected with a single dose of 7.5 mg enrofloxacin/kg bw, marker residue concentrations in liver, kidney, muscle and fat fell rapidly from approximately 30, 20, less than 10, and less than 10 µg/kg respectively, 3 days after dosing (first time point) to less than 10 µg/kg in all tissues at 7 days (except for injection site residues 959 µg/kg enrofloxacin highest concentration detected). Comparison of the radiometric and non-radiometric cattle studies was not possible as the number of doses and time points used were different.

17. 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 liver, muscle and fat samples from chickens, turkeys, cattle and pigs, the kidneys from cattle and pigs and the skin from chickens and pigs. 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 limit of quantification for this method was 10 µg/kg for enrofloxacin and for ciprofloxacin for all tissues.

Conclusions and recommendation

Having considered that:

- a microbiological ADI of 6.2 µg/kg bw, i.e. 372 µg/person was established for enrofloxacin,
- 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, within a few hours of dosing,
- the ratios of marker to total residues were similar for the tissues of pigs 12 hours after dosing and poultry for up to 24 hours after dosing,
- in cattle, concentrations of the marker residue in muscle, fat, liver and kidney were in the ratio 1:1:3:2, three days after the end of dosing; the pattern of residues distribution was different in pigs and chickens where concentrations of the marker residue were higher in kidney than in liver,
- a routine analytical method was available;

the Committee recommends that the entry in Annex I of Council Regulation (EEC) 2377/90 should be amended 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	Bovine	100 µg/kg 100 µg/kg 300 µg/kg 200 µg/kg	Muscle Fat Liver Kidney	
		Porcine	100 µg/kg 100 µg/kg 200 µg/kg 300 µg/kg	Muscle Skin + fat Liver Kidney	
		Poultry	100 µg/kg 100 µg/kg 200 µg/kg 300 µg/kg	Muscle Skin + fat Liver Kidney	Not for use in animals from which eggs are produced for human consumption

Based on these MRLs, it was calculated that the consumer intake of total residues from the consumption of meat will represent approximately 27% of the ADI.