COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

SARAFLOXACIN

SUMMARY REPORT

1. Sarafloxacin is a fluoroquinolone antibiotic which works by inhibition of bacterial DNA-topoisomerase II. It has been proposed for use in the drinking water of poultry to treat bacterial disease, and in fish feed to treat diseases such as furunculosis, vibriosis and enteric redmouth.

2. The extent of sarafloxacin absorption varied between species and decreased with increasing dose level. The drug was excreted in both urine and faeces and was not extensively metabolised.

3. Most of the toxicity studies with sarafloxacin were carried out using preparations of relatively low bioavailability.

   Sarafloxacin was of low acute toxicity (oral LDSO >5 g/kg bw in rats and mice)and was well-tolerated by the target species.

   Reversible caecal distension was the most notable finding in repeat dose studies in rats; this effect is a common finding in rats given large doses of antibiotics. The NOEL in a 13-week repeat dose study, based on caecal distension, was 20 mg/kg bw per day.

4. In repeat-dose studies in immature dogs, sarafloxacin caused the arthropathy which is typical of quinolone antibiotics in juvenile animals of this species. The NOEL in a 13 week repeat-dose study, based on arthropathy, was 10 mg/kg bw per day. However, transient reductions in serum globulin levels were observed in female dogs at this dose level. The significance of the latter finding for safety assessment is questionable and the dose of 10 mg/kg bw/day is therefore taken as the no adverse effect level.

5. There was no evidence of matemal toxicity, teratogenicity or foetotoxicity in a teratology study in which dose levels of up to 1000 mg/kg bw per day were administered to rats; the absence of adverse effects in this study was associated with the poor absorption of the test material. Administration of sarafloxacin to pregnant rabbits resulted in increased post-implantation loss, reduced foetal weights and an increase in the incidence of foetuses with malformations. These effects were secondary to the matemal toxicity which is frequently seen in pregnant rabbits given antibiotics and is due to a, disturbance of the intestinal flora NOELs of 15 and 35 mg/kg bw per day were established for foetotoxicity and teratogenicity respectively.

6. Sarafloxacin induced a significant increase in nuclear labelling in an *in vitro* UDS assay. Equivocal results were obtained in two further *in vitro* mutagenicity assays. However *an in vivo* exposure - *in vitro* assay UDS assay and an *in vivo* mouse bone marrow micronucleus test gave negative results. High doses (up to 8 g/kg bw) were used in the latter study, but there was no evidence that sarafloxacin reached the target bone marrow cells. Fluoroquinolones present problems for *in vitro* mutagenicity testing because of their effects on bacterial and mammalian topoisomerase II. In the carcinogenicity studies there was no increase in tumour incidence, although poor survival was observed in both species and the mouse study was of short duration. Although there are difficulties in interpretation of the studies on mutagenicity and carcinogenicity, the balance of evidence indicates that the proposed use of sarafloxacin in poultry does not present a mutagenic or carcinogenic risk to consumers.

7. Sarafloxacin was not a sensitiser when tested in the guinea pig Maximisation test.

8. Sarafloxacin was tested in double-blind placebo controlled trials in human volunteers. No significant effects of the compound were observed. The oral biavailability was low (11-34%).

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9. The *in vitro* antibacterial potency of sarafloxacin was investigated in approximately 900 bacterial strains of human origin. A special study was also carried out to investigate the effects of sarafloxacin on human enteric bacteria under gut-like conditions. The range of species studied were too limited to allow use of the MIC values obtained, but they did indicate that sensitivity of micro-organisms to sarafloxacin would be reduced in the human gut compared to standard *in vitro* assay conditions.

10. The microbiological ADI is calculated as follows from the equation below :

$$ADI = \frac{\dfrac{\text{geometric mean } MIC_{50} \times CF2}{CF1} \text{ (µg/ml) } \times \text{ daily faecal bolus (0.15 l)}}{\text{fraction of an oral dose available for microorganisms} \times \text{weight of human (60 kg)}} \text{ (µg/kg bw)}$$

MIC_{50} The geometric mean value for the most sensitive species is not available, so the value of 0.031 is selected as the lower MIC_{50} value obtained in a number of organisms, including *E.coli.*

CF1 There was no evidence of resistance problems and the range of organisms studied was large, so a value of 1 is used for CF1.

CF2 Although the studies in the gut simulation model did not study the sensitivity of organisms in the model and standard conditions in parallel, the indication was that a factor of at least 5-fold could be used to allow for the difference between the in vitro assays and the situation in the human gut. A factor 5 is therefore taken for CF2

As the bioavailability of sarafloxacin in humans is low, the fraction of the dose available is taken as 0.9.

$$ADI = \frac{\dfrac{0.031 \times 5}{1} \times 150}{0.9 \times 60} \text{ (µg/kg bw)}$$

ADI = 0.4 µg/kg/d
= 24 µg/person/day

The antimicrobial activity of 4 potential metabolites was shown to be less than that of the parent sarafloxacin.

11. The microbiological ADI is well below the toxicological ADI of 100 µg/kg bw/day, based on the overall NOEL of 10 mg/kg bw/day obtained in the 13-week study in immature dogs, and applying a safety factor of 100.

12. Radiolabel studies indicated that, 6 hours after withdrawal of the drug, 83% and 86% of the total residues in the liver of turkeys and chickens respectively were in the form of sarafloxacin plus conjugates hydrolysable to sarafloxacin. The identity of residues in other tissues and at later times could not be determined because the tissue concentrations were too low to permit analysis.

13. A validated HPLC analytical method for parent sarafloxacin has been proposed for chicken tissues. The limit of quantification of the method is 10 µg/kg tisssue for liver, skin and fat and muscle and 50 µg/kg tissue for kidney.

14. In chicken liver, 6 hours after drug withdrawal, free sarafloxacin accounted for about 80% of the

sarafloxacin plus residues hydrolysable to sarafloxacin. Residues in other tissues and at other times were too low to allow identification of the residues. MRLs can be determined for chicken tissues based on the analysis of free sarafloxacin and correcting for the potential presence of conjugates. In the absence of data on tissues other than liver, the same relative proportions have been assumed. This is likely to overestimate the total biologically active residue concentration, since in general, conjugated forms are more abundant in the liver than other tissues. The potential for error in making this extrapolation is small, since the total residue levels in tissues other than liver are relatively low. Thus unconjugated sarafloxacin is acceptable as the marker residue for chicken tissues.

15. MRLs may be calculated for chicken tissues from the relative tissue levels of the total residues at 18 hours after drug withdrawal and correcting for the predicted proportion of free sarafloxacin as described above. The expected concentrations of free sarafloxacin in kidney and muscle are below the limit of quantification of the analytical method and thus no MRLs can be set for these tissues. The predicted total intake of biologically significant residues resulting from consumption of the complete meat package from chickens in which the liver contains free sarafloxacin at the MRL is about 15 µg/person/day, which is well below the ADI of 24 µg/person/day. The MRLs recommended for chicken are :

Species	Marker residue	MRLs	Tissues
Chicken	parent sarafloxacin	100 µg/kg 10 µg/kg	Liver Skin + Fat