



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

MARBOFLOXACIN

SUMMARY REPORT (1)

1. Marbofloxacin is a new fluoroquinolone antibiotic. It acts by inhibition of bacterial DNA-gyrase. It is proposed for oral or parenteral administration to cattle, including lactating dairy cattle, for treatment of bovine respiratory disease and parenteral administration to pigs for the treatment of Mastitis Metritis Agalactiae syndrome. The proposed dosage corresponds to a daily dose of 2 mg/kg bw/day marbofloxacin for up to 5 days.
2. Marbofloxacin was well absorbed after oral and parenteral administration. The values of C_{max} and AUC were proportional to the dose administered. Oral bioavailability approached 100% in several species. It was widely distributed to the tissues. In rats and dogs, concentrations of the drug in tissues such as lung, liver and kidney were higher than the concentrations in plasma. In humans, laboratory animals and in pigs marbofloxacin was weakly bound to plasma proteins (<10%); binding was higher in cattle (around 30%). It was excreted mostly in the urine. In all species, unmetabolised marbofloxacin was the main component of the residues in tissues and excreta. Some residues of marbofloxacin conjugates were found together with small amounts of the desmethyl- and the N-oxide metabolites. The extent of biotransformation was very limited and there were no significant species differences in metabolism.
3. Marbofloxacin was of low acute oral toxicity; acute oral LD₅₀ values ranged from 1781 mg/kg bw (male ICR mice) to 3772 mg/kg bw (male Sprague-Dawley rats). The acute subcutaneous LD₅₀ ranged from 972 mg/kg bw (female ICR mice) to 2094 mg/kg bw (male Wistar rats). The signs of toxicity included decreased activity, tremors and convulsions. The substance was only a mild skin and eye irritant.
4. In a 13-week repeat-dose study in rats, marbofloxacin was administered in the diet at concentrations intended to provide 0, 4, 50 and 600 mg/kg bw per day. Increased mortality observed at the top dose level of 600 mg/kg bw per day was associated with obstructive uropathy. Hyalin droplets were found in the kidneys of 2 rats receiving this dose level. At 50 and 600 mg/kg bw marbofloxacin had a toxic effect on the male reproductive organs causing reduced testes and epididymides weights, testicular tubular atrophy, oligospermia and spermatic granuloma. Dose levels of 50 and 600 mg/kg bw also induced arthropathy. The effects on the testes and the arthropathy were also observed in some animals from the 600 mg/kg bw group which were given untreated diet for 6 weeks at the end of the experiment. The NOEL was 4 mg/kg bw/day.
5. In a 13-week repeat-dose study, adult dogs were given daily oral doses of 1, 4 or 40 mg/kg bw marbofloxacin in gelatin capsules. Typical quinolone-induced changes in the articular cartilage were observed at 40 mg/kg bw/day. Testicular tubular atrophy was observed in one (out of 4) animals given 40 mg/kg bw and spermatic granuloma was also observed in one (out of 4) dogs at this dose level. The NOEL was 4 mg/kg bw.
6. No substance-related effects were observed in a repeat-dose study in immature dogs given doses of up to 6 mg/kg bw/day for 13 weeks.

7. In a teratology study, pregnant rabbits were given oral doses of 10, 30 or 80 mg/kg bw/day of marbofloxacin from days 6-18 of gestation. There was no evidence of teratogenicity at any dose level. 80 mg/kg bw/day was foetotoxic, causing an increased incidence of delayed ossification; the NOEL for foetotoxicity was therefore 30 mg/kg bw. Maternal toxicity was observed at 30 and 80 mg/kg bw but not at 10 mg/kg bw.
8. In a rat teratology study, the dams were given oral doses of 10, 85 or 700 mg/kg bw/day from days 6-15 of gestation. There was no evidence of teratogenicity at any dose level. 700 mg/kg bw was foetotoxic causing an increased incidence of resorptions with a corresponding reduction in the numbers of live foetuses per litter and reduced foetal weights; the NOEL for foetotoxicity was 85 mg/kg bw. Maternal toxicity was observed at 85 and 700 mg/kg bw but not at 10 mg/kg bw.
9. In a 2-generation study, rats were fed diets containing 10, 70 or 500 mg/kg bw/day. At 500 mg/kg bw/day, overt signs of toxicity were observed and male fertility was impaired. A 10-week recovery period indicated that the effect was reversible. The reproductive tissues were not examined microscopically so the aetiology of the effect was unknown. Reductions in implantation rate, litter size and pup weight together with increased pup mortality and a delay in the onset of sexual maturity were also observed at 500 mg/kg bw. Similar though less severe changes were observed at 70 mg/kg bw. The NOEL was 10 mg/kg bw/day.
10. Marbofloxacin was mutagenic in the excision repair deficient strain TA102 of *S. typhimurium*, in both the presence and absence of metabolic activation. Negative results were obtained with 6 further strains. It was also mutagenic in the yeast *Saccharomyces cerevisiae* with respect to gene conversion, gene mutation and mitotic crossing-over. Marbofloxacin induced point mutations in cultured Chinese hamster V79 cells, only in the absence of metabolic activation, at dose levels which were moderately cytotoxic. Negative results were obtained in an *in vitro* UDS assay and in an *in vitro* assay for chromosome aberrations in human lymphocytes. The substance was not mutagenic in an *in vivo* micronucleus test in mouse bone marrow and an *in vivo* unscheduled DNA synthesis assay in rat liver.
11. There were no data on carcinogenicity. The Applicant justified this omission by comparing the results of the mutagenicity assays with marbofloxacin with those obtained for other fluoroquinolones. Similar results had been obtained. Data were provided for several quinolones which showed that their inhibitory action against eukaryotic topoisomerase II was at least 100-fold less than their action against the prokaryotic enzyme. Other fluoroquinolones were not carcinogenic. It was concluded that there was no reason to suspect that marbofloxacin was carcinogenic.
12. The potential effects of marbofloxacin on the microorganisms used for industrial food processing were investigated in the yoghurt inhibitory test. Tolerance thresholds (NOELs) of 1.6 µg/ml and > 50 µg/ml for *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, respectively, were determined.
13. A toxicological ADI of 0 - 0.04 mg/kg bw was calculated by applying a safety factor of 100 to the NOEL of 4 mg/kg bw/day which was established in the 13-week repeated dose study in dogs.
14. Since the ADI should be low enough to eliminate the possibility of any pharmacological, microbiological or other biological effect, in the case of marbofloxacin the microbiological risk of residues for the human gut flora was considered.

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

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

In vitro MIC values were determined for a range of bacteria which had been isolated from the human gut MIC values were also determined under simulated gastrointestinal tract conditions in the presence of meat or milk. In the presence of milk, the geometric mean MIC for *E. coli* was 0.536 µg/ml.

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

$$\text{ADI} = \frac{0.536 \times 1}{0.3 \times 60} \times 150 = 4.5 \text{ } \mu\text{g/kg bw i.e.} = 268 \text{ } \mu\text{g/person}$$

The following assumptions were made:

- CF1 = 1 because the MIC for the most sensitive, predominant organism was used;
 - CF2 = 1 because the MICs were determined under simulated intestinal conditions;
 - 150 g was the weight of the daily faecal bolus;
 - 0.3 was the fraction of the oral dose “available” to the intestinal flora.
15. In a cross-over design study, the pharmacokinetics following oral and intravenous dosing of 2 mg/kg bw marbofloxacin was compared in sows in early pregnancy, late pregnancy and during lactation. In lactating sows, the clearance was higher, the half-life of elimination was shorter and plasma concentrations were lower. Mean values for $T_{1/2}$ were 8.22, 8.27 and 5.92 hours after intravenous administration to sows in early pregnancy, late pregnancy and lactation, respectively. The volume of distribution was large in all the physiological states indicating good tissue penetration (mean values for volume of distribution at steady state were 1.30, 1.13 and 1.27 l/kg) after intravenous dosing to sows in early pregnancy, late pregnancy and lactation, respectively. The oral bioavailability was estimated to be around 80% in both pregnant and lactating sows. Clinical trials indicated a similar degree of efficacy using the same dosage regime for both lactating and non-lactating pigs.
 16. In pigs, almost all of the residues in muscle and fat consisted of marbofloxacin but residues in liver and kidney contained significant amounts of metabolites. In liver, 77% of the residues were present as marbofloxacin, 4 hours after the last dose. 192 hours after the last dose, it was estimated that around 70% of the residues in liver were marbofloxacin though residues in some samples at this time point were below the limit of quantification. In pig kidney, 90% of the residues consisted of marbofloxacin 4 hours after the end of treatment. 48 hours after the last dose, approximately 70% of the residues in pig kidneys consisted of marbofloxacin.
 17. A residue depletion study was carried out in which young pigs of bodyweight approximately 20 kg were given 5 daily subcutaneous injections of 2 mg/kg bw per day radiolabelled marbofloxacin. The residues were rapidly depleted. 4 days after the last injection, residues of marbofloxacin were detectable only in 1 out of 4 samples of liver (49 µg/kg) and 1 out of 4 samples of kidney (82 µg/kg). Residues in fat were detectable only in samples taken 4 hours after the last dose (range 369-1826 µg/kg). 4 hours after the last dose, residues at the injection site were in the range 907-1118 µg/kg and depleted to <21 - 88 µg/kg, 2 days after dosing.
 18. The pharmacokinetics of marbofloxacin were compared in pre-ruminant and ruminant calves. After subcutaneous administration, both absorption and elimination were slower in pre-ruminant animals. This was reflected in higher tissue residues concentrations in pre-ruminant animals. In pre-ruminant calves, absorption and elimination were slower after oral administration compared with subcutaneous administration. Bioavailability approached 100% in pre-ruminant calves after intramuscular and subcutaneous administration. Marbofloxacin was rapidly absorbed after intramuscular and subcutaneous administration to lactating cows and bioavailability approaching 100%.

19. A residues depletion study using radiolabelled marbofloxacin was carried out in which pre-ruminant calves were given daily subcutaneous injections of 2 mg/kg bw for 5 days. The calves were slaughtered in groups of 4 at different time points. The total residues in tissues were determined by scintillation counting and residues of marbofloxacin were determined by HPLC. Depletion was rapid from all tissues including the injection site. The depletion of marbofloxacin residues in muscle and fat closely paralleled the depletion of total residues. In liver, around 90% of the total residues consisted of marbofloxacin, 4 hours after the last dose. Ninety six hours after the last dose, this percentage had declined to approximately 40%. The corresponding values for bovine kidney were 95% and 60% respectively. Residues of marbofloxacin in liver, determined by HPLC, declined from 1327-3105 µg/kg, 4 hours after treatment, to 19-36 µg/kg, 96 hours after the last treatment. Over the same time period, residues in kidney declined from 3602-4782 µg/kg to 39-59 µg/kg; in muscle from 1776-2207 µg/kg to 16-23 µg/kg, in injection site tissue from 2985-4410 µg/kg to 15-25 µg/kg and in fat samples from 504-2023 µg/kg to <8.5-30 µg/kg. 192 hours after treatment, residues of marbofloxacin in most tissue samples were below the LOQ (8.5 µg/kg).
20. Pre-ruminant calves were given daily intramuscular injections of a proposed commercial formulation of marbofloxacin at a dose of 2 mg/kg bw per day. Four calves were slaughtered at each time point. Residues in liver depleted from 30 - 198 µg/kg, 48 hours after dosing to 37 - 61 µg/kg, 96 hours after dosing. Over the same time period, residues in kidney depleted from 53 - 164 µg/kg to 47 - 111 µg/kg; in muscle from <25 - 65 µg/kg to 27 - 44 µg/kg, and, in injection site tissue from <25 - 61 µg/kg to <25 - 43 µg/kg. Residues in all fat samples were below 25 µg/kg. 192 hours after the last dose, residues were detected only in one sample of kidney (36 µg/kg). The Limit of Quantification was 25 µg/kg.
21. Three lactating dairy cows were given 5 daily subcutaneous doses of 2 mg/kg bw/day of radiolabelled marbofloxacin. Samples of milk were collected, the cows were slaughtered at different time points and the residues in milk and tissues were determined using scintillation counting to determine total residues and HPLC to determine residues of marbofloxacin. In the milk, 73 - 89% of the total residues were marbofloxacin. However, there was no information concerning the lactation status and milk yield of the individual cows and the study was not adequate to draw any conclusions regarding the depletion of residues in milk.
22. In another study, 8 lactating cows, 4 high-yielding animals at an early stage of lactation, and 4 low-yielding animals at a late stage of lactation, were given daily subcutaneous doses of a proposed commercial formulation of marbofloxacin at a dose rate of 2 mg/kg bw/day. Residues in milk at the first milking after the end of treatment were in the range 180-679 µg/l. At the 3rd milking, residues had declined to <10 - 34 µg/l. Residues in all samples taken at the 5th milking were below the LOQ (10 µg/kg) of the assay used in this study.
23. The proposed routine analytical method for the determination of residues in tissues and in milk was based on HPLC with UV detection. The method used ofloxacin as an internal standard. Under the HPLC conditions employed, residues of ciprofloxacin, danofloxacin and enrofloxacin did not interfere. The method was of acceptable accuracy and precision at the Limits of Quantification of 5 µg/kg for bovine and porcine liver, kidney and muscle and 1 µg/kg for bovine milk. However the method was not satisfactorily validated for fat samples at the claimed LOQ of 5 µg/kg and information concerning stability in standard solutions, extracts, stored milk and porcine tissues was still required.

Conclusions and recommendation:

- having set a microbiological ADI of 4.5 µg/kg bw (268µg/person);
- having considered the depletion profiles of residues of marbofloxacin in bovine and porcine;
- considering the lack of a validated analytical method for residues monitoring purposes;
- having identified marbofloxacin as the marker residue;

The Committee for Veterinary Medicinal Products recommends that marbofloxacin be entered into Annex III of Council Regulation (EEC) No 2377/90 and that provisional MRLs for marbofloxacin be set as indicated in the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Marbofloxacin	Marbofloxacin	Bovine	150 µg/kg 50 µg/kg 150 µg/kg 150 µg/kg 75 µg/kg	Muscle Fat Liver Kidney Milk	Provisional MRLs expire on 1.7.1998
		Porcine	150 µg/kg 50 µg/kg 150 µg/kg 150 µg/kg	Muscle Skin and Fat Liver Kidney	

Based on these MRLs, consumer intake of residues from meat and milk would represent approximately 85% of the microbiological ADI calculated in paragraph 14.

LIST OF QUESTIONS

Further information should be provided concerning the analytical method, including:

1. Presentation using a standard internationally-recognised format (e.g. ISO 78/2);
2. Validation data in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community to verify the claimed Limit of Quantification for fat samples;
3. Data on the stability of marbofloxacin in standard solutions, extracts, stored milk and porcine tissues;
4. The final report of the stability study in bovine tissues to include clarification of the finding that the concentrations appeared to be significantly higher following storage for 6 months at -20°C.