



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

DIFLOXACIN

SUMMARY REPORT

1. Difloxacin HCl is an aryl fluoroquinolone derivate. Like other quinolones, it acts by inhibiting the structure and function of DNA gyrase, a bacterial topo-isomerase II which is an essential enzyme for DNA replication and transcription.

In vitro difloxacin is very potent against a broad spectrum of bacteria including aerobic and anaerobic gram-positive and gram-negative organisms. This in vitro potency has been confirmed *in vivo* in several experimental infections in mice, including systemic infections in normal and in immunosuppressed mice, pyelonephritis and pneumonia.

For chickens and turkeys, the proposed dosage corresponds to a daily dose of 10 mg/kg bw per day of difloxacin for up to 5 days.

2. Pharmacokinetic studies were performed in rats, rabbits, dogs, monkeys and man. No substantial differences between species were observed. After oral administration, difloxacin was well absorbed from the gastro-intestinal tract and bioavailability was nearly complete. Peak plasma levels occurred at 1-6 hours after oral administration, and plasma concentrations were dose-proportional in most cases. The decline in plasma levels was biphasic in dog and monkey, but monoexponential in rat and human. Depending on dose, elimination half-lives in plasma were 7-8 hours in dogs, 3.5-8.7 hours in rats, 14 hours in monkey and 21-33 hours in humans. In several studies, distribution of radioactivity was measured after oral administration of labelled difloxacin to dogs and rats. Highest radioactivity was found in bile and intestine, followed by liver and to some lower extent bone and kidney. Lowest levels were found in brain. Difloxacin was mainly excreted in faeces: about 80-88% in dogs and rats and about 60% in rabbit. In man and mouse, difloxacin excretion in urine was 33-43% and 27% respectively (faeces not examined). In plasma, over 90% is unchanged difloxacin. In urine, faeces and bile, metabolites were more prevalent. In urine sarafloxacin and difloxacin were most prevalent, followed by the glucuronide and N-oxide. In faeces the predominant compound consisted of parent drug. Smaller amounts of sarafloxacin and glucuronides were also present, but no N-oxide. In bile, glucuronides were the most predominant metabolites which consisted of only small amounts of parent drug. These findings suggest that glucuronide in the bile is subsequently hydrolysed and then free difloxacin is either reabsorbed or excreted via faeces. Hence, difloxacin is subjected to enterohepatic cycling.
3. Acute oral toxicity comprised decreased activity, ataxia, squinting, dyspnea, tremors and a decreased bodyweight. The oral LD₅₀ (males-females) for mice and rats were 1.38-1.60 and 5.51-6.27 g/kg bw, respectively, so difloxacin was considered slightly to moderately toxic. Difloxacin was non-corrosive and non-irritant in ocular and dermal irritation studies in rabbits.
4. Several oral repeated dose toxicity studies were performed with rats and dogs. A decreased food consumption and bodyweight, convulsions, dehydration and effects on haematology and clinical chemistry were noted in both species. In dogs, the photoreceptor cells of the retina were also affected, as was the articular cartilage of leg joints. Pathological target organs included liver and/or biliary system and kidney. An overall NOEL of 1 mg/kg bw/day can be established, based on effects on articular cartilage in immature dogs, which was observed in a 3-month repeated dose study and was the most sensitive parameter.

5. Tolerance studies were performed in chickens and turkeys at dosages representing up to 10x and 7x the recommended dose for chickens and turkeys, respectively. Transient decreases in water and food consumption in the highest dose groups was the only significant finding for both species, so it can be concluded that difloxacin is well tolerated in the target species.
6. Reproductive toxicity of difloxacin was studied in rats after oral administration. In a fertility and general reproductive performance study and a peri- and postnatal study no significant effects were found on reproductive performance, on physical development, reflex responses and behaviour of pups. Effects were seen on maternal bodyweight, food and water consumption, and foetal and pup bodyweight at 45 and/or 150 mg/kg. In a 3-generation reproduction study, a number of effects on parents and pups were seen and fertility was strongly affected at 100 mg/kg (the highest dose). The NOEL for reproductive and neonatal toxicity in this study was 50 mg/kg bw/day and for parental toxicity 25 mg/kg bw/day. Embryo- and foetotoxicity, including teratogenicity was studied in the rat and the rabbit. In the rat, litter resorption occurred and foetal survival was decreased at the highest dose (275 mg/kg). An ossification delay, apparent from unossified and unfused sternebrae, was noted at 65 and 275 mg/kg, but this was most probably due to inhibition of pup growth. In the rabbit study, no teratogenic effects were observed at 15 and 35 mg/kg. As a result of increased embryotoxicity at the highest dose level (75 mg/kg), the number of foetuses was too low for a teratogenic examination.
7. Genotoxicity of difloxacin was only investigated *in vitro*. Tests in bacterial systems were not conducted in view of the compound's microbiological effects. Difloxacin was negative in all 3 mutagenicity studies conducted in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community. Difloxacin gave positive results in some other tests (UDS, SCE, DNA-repair, alkaline elution). However, due to the limited solubility of difloxacin, precipitates were formed at relatively low doses, which in UDS and DNA tests may interfere with tritiated thymidine as a result of intracellular trapping of thymidine by microaggregates of the drug. When the precipitate was removed, the UDS test was negative. Therefore, these results were judged to be false positives. Furthermore, an indirect effect on DNA replication is not uncommon for fluoroquinolones, since they act as a gyrase-inhibitor. Sarafloxacin (N-desmethyl-difloxacin), a metabolite of difloxacin, showed also some positive results in the *in vitro* UDS tests. However, an *in vivo* UDS test with sarafloxacin gave negative results, and sarafloxacin was not considered to be a genotoxic agent.

In view of the explanations given above and as other fluoroquinolones are not genotoxic, despite certain mutagenicity tests being positive, difloxacin contains no structural alert and is considered a non-genotoxic compound.

8. Carcinogenicity studies with difloxacin were carried out in the rat and in the mouse. The tumour incidences in the rat and in the female mouse were not affected. In the male CD-1 mouse a shift from alveolar/bronchiolar adenomas to carcinomas was observed which was only statistically significant in the highest dose group (150 mg/kg bw/day). At lower doses also a slight increase in the incidence of carcinomas was found, but this effect was not considered toxicologically relevant since the mice strain used in this study is known for relative high incidences of spontaneous lung tumours. Given the results of the rat and the mouse carcinogenicity studies, in spite of the low survival in male rats, difloxacin is considered not carcinogenic. Bearing in mind the results of the mutagenicity studies and the above data, difloxacin is not considered carcinogenic.
9. The microbiological properties of difloxacin were studied *in vitro*. MIC₅₀ values were determined in different species of bacteria originating from the human gut, and at different conditions, including a pH and inoculum size which are representative for the *in vivo* human gut situation. In most cases MIC₅₀ values were not different at inoculum sizes of 10⁷ or 10⁹ cfu/ml. In some cases, an increased pH (from 6.5 to 8.0) resulted in a higher or lower MIC₅₀ value, but differences were not more than 1 or 2 dilution steps. The MIC₅₀ for the most sensitive species tested was 0.5 µg/ml at pH 8 and a density of 10⁹ cfu/ml.

10. Oral administration of difloxacin to humans at (single) doses ranging from 100 to 600 mg resulted in a low incidence of adverse reactions including headache, dizziness, disturbed sleep, nausea, vomiting and upset stomach. Some subjects reported visual disturbance. Also the prothrombin time was slightly increased, in some cases accompanied by a decrease in Factor VII. However, the prothrombin time delay was only slightly above the normal range, and was not observed in extensive pharmacodynamic investigations in animals.
11. The toxicological ADI for difloxacin is calculated to be 10 µg/kg bw, based on the overall NOEL of 1 mg/kg bw/day, derived from a 3-month oral toxicity study in dogs, and a safety factor of 100.

Since the ADI should be low enough to eliminate the possibility of any pharmacological, microbiological or other biological effect, in the case of difloxacin 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:

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

Due to the low number of isolates used for the determination of MIC₅₀ values, it seems most appropriate to use the MIC₅₀ of the most sensitive species tested instead of the geometric mean MIC₅₀. In this case, CF1=1. Since MIC₅₀ values are also determined at inoculum size and pH representative for the human gut, it is, however, preferable to use the lowest determined MIC₅₀ value at these conditions, which is 0.5 µg/ml (for various species), and in this case no CF2 is needed. However, the biliary excretion of difloxacin glucuronides and the subsequent hydrolysis in the gut should be taken into account. Since difloxacin was the most predominant faecal residue, the faecal excretion data can be used to estimate the fraction available for microorganisms. Faecal excretion in humans was not determined, but urinary excretion was about one third of the dose, so a faecal excretion of about 70% can be estimated. Therefore, a fraction of 0.7 will be used in the formula.

Based on the above, the microbiological ADI can be established as:

$$\text{ADI} = \frac{0.5 \times 150}{0.7 \times 60} = 1.8 \mu\text{g/kg bw} = 108 \mu\text{g/day for a 60 kg person}$$

This microbiological ADI is much lower than the toxicologically derived ADI of 10 µg/kg bw.

12. From pharmacokinetic studies in broiler chickens and turkeys it appeared that maximum plasma levels of difloxacin are reached within 1-2 hours after oral administration. Elimination half-lives were approximately 7 hours in these species. At the same dose level, difloxacin plasma levels in turkey are about 3-4 times lower than those in broilers. This might be explained by the 2-fold

larger distribution volume and the lower bioavailability in turkeys (56%) compared with broilers (92%). Radiolabel studies revealed that extractable residues were the predominant fraction of the total radioactive residues in tissues (>90%) and excreta (>99%), and difloxacin was the predominant radioactive compound. In tissues, the major residue was difloxacin or its conjugates (60-90%) with a smaller proportion of its demethylated form, sarafloxacin (5-15%). In excreta, difloxacin was demethylated to sarafloxacin or N-oxidized to N-oxide-difloxacin, all of which were conjugated to base and/or acid-labile conjugates.

13. Residues of difloxacin after oral treatment for 5 days at 1-2x the recommended dose showed that highest residues in broilers and turkeys were found in liver and kidney, but that residues in skin were most persistent. Residues in skin were still above 10 µg/kg at 7 and 10 days after treatment at the recommended dose for chickens and turkeys, respectively. Only in the first few days after treatment small amounts of sarafloxacin (broiler/turkey), N-oxide difloxacin (turkey) and conjugates (broiler/turkey) could be detected in the tissues (mainly liver).
14. Taking into account the microbiological ADI of 1.8 µg/kg bw and the tissue distribution of difloxacin and the concentration of residues at 1-6 hours and 24 hours; the following MRLs can be proposed for chickens and turkeys: 200 µg/kg for liver, 150 µg/kg for kidney, 50 µg/kg for muscle and 200 µg/kg for skin+ fat.
15. The proposed routine analytical method for the determination of difloxacin in edible tissues of turkey and broiler (HPLC with fluorescence detection) is validated and suitable for residues surveillance. The limit of quantification (LOQ) is 10 µg/kg in all tissues, and the limits of detection were all well below 2.3 µg/kg, even after deglucuronidation. The method also provides for measuring glucuronides in liver samples, and has the capability of measuring sarafloxacin.

Conclusion and recommendation:

- Having set a microbiological ADI of 1.8 µg/kg bw (108µg/person);
- Having considered the depletion profiles of residues of difloxacin in chickens and turkeys;
- Having checked the availability of a validated analytical method for residues monitoring purposes;

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

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Difloxacin	Difloxacin	Chicken Turkey	200 µg/kg	Liver	
			150 µg/kg	Kidney	
			50 µg/kg	Muscle	
			200 µg/kg	Skin +fat	

Based on these MRLs, the predicted total intake of biologically significant residues resulting from consumption of the complete meat package of chickens/turkeys is about 54.5 µg/person, which is well below the ADI of 108 µg/person.