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

EMEA/MRL/154/96-FINAL
January 1997

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

DIFLOXACIN
(Modification)

SUMMARY REPORT (2)

1. Difloxacin hydrochloride 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.

2. *In vitro* difloxacin has a broad antibacterial spectrum with high bacterial activity against common avian pathogens. It is indicated for the treatment and control of difloxacin-sensitive infections in chickens and turkeys at a dosage of 10 mg/kg bw daily for 5 days via the drinking water.

3. Upon initial evaluation, the CVMP established for difloxacin hydrochloride a toxicological ADI of 10 µg/kg bw (based on a NOEL of 1 mg/kg bw/day in a 3-month dog study and a safety factor of 100) and a microbiological ADI of 1.8 µg/kg bw. The latter was based on the following formula:

$$ADI = \frac{0.5 \mu g/ml \text{ x } 150 \text{ ml}}{0.7 \text{ x } 60 \text{ kg bw}} = 1.8 \ \mu g/kg \text{ bw}$$ (equivalent to 108 µg/person)

in which:

- 0.5 µg/ml is the lowest MIC_{50} value for difloxacin determined at inoculum size and pH representative for the human gut. In this case CF1 and CF2 are 1;
- 150 ml is the daily faecal bolus;
- 0.7 is the fraction of an oral difloxacin dose available for microorganisms, based on an estimated human faecal excretion of 70%. As no data were submitted on the potential binding of difloxacin to human faeces, and thereby reduced availability to intestinal microorganisms, the value of 0.7 is a conservative estimate;
- 60 kg is the human bodyweight.

4. As the microbiological ADI is lower than the toxicological ADI, the former served as basis for the establishment of MRLs for difloxacin hydrochloride for the tissues of chickens and turkeys. The following MRLs for the marker residue difloxacin were included in Annex I of Council Regulation (EEC) Nº 2377/90 :

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target Tissues	Other provisions
Difloxacin	Difloxacin	Chicken, turkey	200 µg/kg 150 µg/kg 50 µg/kg 200 µg/kg	Liver Kidney Muscle Skin/fat	

7 Westferry Circus, Canary Wharf, London E14 4HB, UK
Tel: (+44-171) 418 84 00 Fax: (+44-171) 418 84 16

5. Data provided were in line with the CVMP guideline on the Safety evaluation of antimicrobial substances regarding the effects on human gut flora, where it is stated with respect to the fraction of an oral dose available for microorganisms that: "if the applicant provides data from studies which show that after exposure to acidic conditions a certain percentage of the active component is no longer microbiologically active or that binding to macromolecules occurs, this information might be used to alter the value of this fraction in an appropriate way".

   The following *in vitro* studies were conducted :

   · aerobic biodegradation potential of ^{14}C-difloxacin hydrochloride in manure of domestic livestock (chicken, cow, swine, turkey; moisture content 62.6-78.1%) determined by means of the carbon dioxide evolution method;

   · aerobic biodegradation potential of ^{14}C-difloxacin hydrochloride in different soils determined by means of the carbon dioxide evolution method;

   · sorption/desorption performed according to the FDA Environment Assessment Technical Assistance using the batch equilibrium method to evaluate the soil leaching potential of difloxacin hydrochloride;

   · microbial growth inhibition to examine the impact of difloxacin hydrochloride on the microbial density in soil;

   · adsorption/desorption of difloxacin on human faeces.

6. From the studies provided in the present dossier it can be concluded that difloxacin binds strongly to organic matter, including human faeces, and so precludes for the most part its availability to enteric bacteria.

7. In order to establish the amount of difloxacin assumed to be available to the microorganisms, the following points were considered:

   · sorption coefficients determined at equilibrium;
   · sorption is pH-dependent, depending on the pK_a of the substance;
   · distribution in the gastrointestinal-tract depending on the K_{oc}, the solid/water ratio, and the percentage organic matter;
   · sorption coefficient (if according to Freundlich isotherm) per se not dependent on the soil/water ratio in the system, nor on the concentration of the substance. However, the solid/water ratio tested was chosen in such a way that the relative decrease in the water phase was greater than 10%, and preferably ca. 50%, to reduce analytical problems.

8. In a worst case situation the data presented substantiate that only 4.4% difloxacin is present in the liquid phase in the gastrointestinal tract. Recalculation (using the formula as stated in paragraph 3, and adjusting the available fraction from 0.7 to 0.7 x 0.044) results in a microbiological ADI that is higher than the toxicological ADI (40.6 and 10 µg/kg, respectively), the latter therefore becoming most relevant for the derivation of MRLs.

9. The ratio of marker (i.e. parent compound) towards total residues has been well established for the liver (at 12 and 24 h after administration): 0.78 and 0.76 for chickens and turkeys respectively. In the other edible tissues the ratio marker/total residues has not been established directly, but from the metabolite characterisation profile it appears that, in chickens, the ratio at 12 and 24 h after administration was 0.94, 0.98, 0.9 and 0.94 for kidney, muscle (mean of leg/thigh and breast), fat and skin/fat, respectively. In turkeys, the ratio was a little bit lower in these tissues: 0.73, 0.81, 0.78 and 1 respectively.

10. It was noted that the proposal for new MRLs should also take into account the tissue distribution of total radioactive residues (in µg/kg) in chickens and turkeys after the recommended oral treatment of 10 mg difloxacin/kg bw/day for 5 consecutive days.

Conclusions and recommendation

Having considered that :

- the distribution in chickens and turkeys is roughly comparable;
- the residues in muscle and skin/fat are of the same magnitude;
- the residues in kidney are slightly higher than in muscle and skin/fat (approximately a factor of 2);
- the residues in liver are the highest (approximately 6 times higher than in muscle and skin/fat);

the Committee recommends the inclusion of difloxacin into Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table :

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target Tissues	Other provisions
Difloxacin	Difloxacin	Chicken, turkey	300 µg/kg 400 µg/kg 1900 µg/kg 600 µg/kg	Muscle Skin/fat Liver Kidney	

These MRLs will lead to a maximum daily intake of 399 µg/person (66% of the toxicological ADI of 600 µg/person), and provide a safety margin for other uses.