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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

DIFLOXACIN (extension to swine and cattle)

SUMMARY REPORT

1. Difloxacin is an arylfluoroquinolone derivative, which 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. The compound has a broad antibacterial spectrum and is currently used in chickens and turkeys for the treatment and control of difloxacin-sensitive infection.

   Difloxacin was evaluated by the CVMP in 1995 and 1996, and a toxicological ADI of 10 µg/kg bw (i.e. 600 µg/person) was established, which was calculated from the overall NOEL of 1 mg/kg bw/day (based on effects on articular cartilage in immature dogs) and a safety factor of 100. The toxicological ADI was lower than the microbiological ADI based on effects on the human gut flora, which was set at 40.6 µg/kg bw.

   Currently, difloxacin is included in Annex I of Council Regulation (EEC) No 2377/90 for chicken and turkey 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	

3. These MRLs may result in a maximum intake of 399 µg/person, which represents 66% of the toxicological ADI.

4. An application has now been submitted for the extension of the MRLs to cattle and pigs. For both target species, difloxacin is indicated for the treatment of respiratory diseases. For pigs it is also indicated for the treatment of Salmonellosis, piglet scours, and enteritis/toxaemia. The recommended dose for both cattle and pigs is 2.5 to 5 mg/kg bw, once daily for 5 consecutive days by the subcutaneous (cattle only) or intramuscular (cattle and pigs) route of administration.

5. In general, pharmacokinetic data showed that difloxacin is well absorbed from the intramuscular and subcutaneous injection site, and is effectively eliminated, mainly as the parent compound, in faeces and urine. Only a minor part was metabolised; of the metabolites identified, N-desmethyldifloxacin (synonym: sarafloxacin) and the N-oxide of difloxacin were most important.

6. In two cross-over studies, the bioavailability of difloxacin was assessed in cattle and pigs using the recommended routes of administration at a single dose of 5 mg/kg bw. In cattle, the bioavailability as calculated in comparison to the intravenous route, was 88% following subcutaneous administration and 95% after intramuscular administration. In pigs, the bioavailability was 92% after intramuscular administration.

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7. The plasma kinetics of difloxacin following (the recommended) repeated administration of 5 mg/kg bw/day for 5 consecutive days were studied in cattle (intramuscular and subcutaneous) and pigs (intramuscular). In cattle, comparison of the pharmacokinetic parameters at day 1 and day 5 gave some indication of accumulation over the dosing period. Following subcutaneous treatment a mean maximum plasma level of 1397 µg/l was found at day 1 and 1951 µg/l at day 5. Intramuscular treatment revealed mean maximum plasma levels of 1838 µg/l at day 1 and 2125 µg/l at day 5. Also the elimination half-lives were somewhat longer after 5 days (7.7 to 8.2 hours at day 5 and 6.3 to 6.6 hours at day 1). The results obtained in pigs showed no evidence for accumulation. Mean maximum plasma levels of 1266 and 1359 µg/l were reached at 2.7 and 2.3 hours after the first (day 1) and last dose (day 5), respectively.

8. The dose proportionality of difloxacin was studied in cattle and pigs in two cross-over studies, using the intravenous route of administration at single doses of 2.5, 5 and 10 mg/kg bw. Comparison of the area under the blood-concentration-time curves ($AUC_{0-\infty}$) showed that the plasma kinetics were proportional with the dose in both cattle and pigs.

9. The total drug related residues, excretion and metabolic profiles after subcutaneous and intramuscular administration were investigated in a radiolabelled study in which cattle were dosed with [^{14}C]-difloxacin formulated as the commercial solution at the recommended dose of 5 mg/kg bw/day at 5 consecutive days. More than 80% of the radioactivity was excreted in the faeces and less than 10% was excreted in the urine within 14 days after both routes of administration.

   Highest residues were found in the injection sites (in subcutaneous sites somewhat higher than in intramuscular sites) and were still 5 to 6 mg/kg at 28 days after treatment. The total radioactive residues in the other tissues were comparable after intramuscular and subcutaneous administration, being highest in the liver, followed by kidney. Lowest levels were found in muscle and fat. At day 3 after treatment the levels of total radioactivity in liver, kidney, muscle and fat were (intramuscular/subcutaneous) 1.19/1.06, 0.53/0.44, 0.27/0.130, and 0.10/0.09 mg/kg, respectively, whereas for the parent compound values of 0.84/0.73, 0.42/0.35, 0.25/0.13 and 0.06/0.12 mg/kg, respectively, were found. Determinations of the parent compound revealed that the approximate ratio parent:total residues in liver was declining from 90% at 12 hours to 50% at 14 days. In kidney, the ratio also declined from 90% at 12 hours to 70% at 14 days. In fat the ratio was around 60% (although one exceptional value of 138% was found) without an apparent decline. Three days after treatment marker residue represents 70, 80, 100 and 60% of the total residues in liver, kidney, muscle and fat, respectively.

   In muscle (including injection sites) all radioactivity was attributed to the parent compound. The metabolite sarafloxacin was identified in some liver and kidney samples, as well as in urine. The N-oxide of difloxacin was identified in faeces and exceptionally in fat. Relatively low levels of unknown metabolites were found in urine, liver, kidney, muscle, injection sites and fat.

10. The total drug related residues, excretion and metabolic profiles in pigs after intramuscular administration were investigated in a radiolabelled study in which pigs were dosed with [^{14}C]-difloxacin formulated as the commercial solution at the recommended dose of 5 mg/kg bw/day at 5 consecutive days. Approximately 70% of the radioactivity was excreted in the faeces and less than 20% was excreted in the urine, the majority excreted within 4 days after administration.

    Highest residues were found in the injection sites and were still between 20 to 21 mg/kg at 14 days after treatment. The total radioactive residues in the other tissues were highest in the liver, followed by kidney. Lowest levels were found in muscle and skin plus fat. At day 2 after treatment the levels of total radioactivity in liver, kidney, muscle and skin plus fat were 0.50, 0.38, 0.27, and 0.18 mg/kg, respectively, whereas for the parent compound values of 0.26, 0.27, 0.16 and 0.04 mg/kg, respectively, were found.

Determinations of the parent compound revealed that the approximate ratio parent:total residues in liver was declining from 80% at 12 hours to 22% at 9 days. In kidney, the ratio also declined from 85% at 12 hours to 53% at 9 days. In skin plus fat the ratio was around 50% without an apparent decline. Two days after treatment the marker residue represents 50, 70, 95 and 50% of the total residues in liver, kidney, muscle and skin plus fat, respectively, at 2 days after treatment,

In muscle (including injection sites) the results indicated that all radioactivity was attributed to the parent compound. The metabolite sarafloxacin was identified in some liver and kidney samples, as well as in urine. The N-oxide of difloxacin was identified in urine and in fat and skin plus fat. Relatively low levels of unknown metabolites were found in urine, liver, kidney, fat and skin plus fat.

11. The residue depletion of the parent compound difloxacin was studied in cattle and pigs in non-radiolablled residue studies using both proposed routes of administration. The commercial formulation was administered at the recommended dose of 5 mg difloxacin/kg bw/day for 5 consecutive days. In the residue depletion study in cattle, animals were slaughtered at 7, 21, 28, 35 and 42 days after the last intramuscular dose or at 7, 14, 21, 28 and 35 days after the last subcutaneous dose. At 7 days post treatment mean concentrations (n=4) of 0.17, 0.10, 0.03 and less than 0.025 mg/kg were found in liver, kidney, muscle and fat, respectively, after subcutaneous treatment. After of intramuscular treatment, mean concentrations (n=4) of 0.34, 0.15, 0.08 and 0.04 mg/kg were found in liver, kidney, muscle and fat, respectively.

    In the study with pigs, animals were slaughtered at 3, 7, 14, 21 and 28 days after the last intramuscular dose. At 7 days post treatment mean concentrations (n=4) of 0.22, 0.25, 0.13 and 0.09 mg/kg were found in liver, kidney, muscle and skin plus fat, respectively.

    The results of the determinations of the residue concentrations in edible tissues were consistent and comparable with those obtained in the radiometric studies.

12. For the routine determination of difloxacin in edible tissues of cattle and pigs an HPLC method is proposed. This method has been well described in accordance with ISO 78/2. The validated limits of quantification are 25 µg/kg for all porcine tissues and for bovine liver and muscle, and 104 µg/kg in bovine kidney and fat. The method validation was not satisfactory with respect to the specificity. The analysis of possible interference by other substances was not performed properly.

Conclusions and recommendation

Having considered that:

- a toxicological ADI of 10 µg/kg bw (600 µg for a 60 kg person) was established,
- difloxacin was identified as marker residue and using the ratio of marker to total residue 3 days after treatment for cattle and 2 days after treatment for pigs,
- a well described HPLC method for the routine determination of difloxacin in bovine and porcine tissues was provided, but this method was not fully validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community, with respect to the specificity,
- since residue levels measured in bovine fat were very low an MRL was established at 100 µg/kg, the limit of quantification of the analytical method;

the Committee for Veterinary Medicinal Products recommends the inclusion of difloxacin for bovine and porcine in Annex III 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	Bovine	400 µg/kg 100 µg/kg 1400 µg/kg 800 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.1.2001 Not for use in animals from which milk is produced for human consumption
		Porcine	400 µg/kg 100 µg/kg 800 µg/kg 800 µg/kg	Muscle Skin + fat Liver Kidney	Provisional MRLs expire on 1.1.2001

Based on these MRLs values, the daily maximum intake will represent less than 60% in pigs and approximately 65% in cattle of the ADI.

Before the Committee for Veterinary Medicinal Products can consider the inclusion of difloxacin for bovine and porcine in Annex I of Council Regulation (EEC) No 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. For the routine determination of difloxacin in edible tissues of cattle and pigs an HPLC method is proposed. The method validation was not satisfactory with respect to the specificity and additional information on possible interference by other substances is required.