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

EMEA/MRL/254/97-FINAL
September 1997

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

DANOFLOXACIN

SUMMARY REPORT (2)

1. Danofloxacin is a fluoroquinolone antibacterial and antimycoplasmal drug. It acts by inhibition of bacterial DNA-gyrase (an enzyme which is also referred to as topoisomerase II and which is responsible for maintaining the topography of DNA). However it does not significantly inhibit the corresponding mammalian enzyme. Danofloxacin is administered in the drinking water to broiler chickens and replacement chicks, and to calves, beef cattle and non-lactating dairy cattle by intramuscular injection, for treatment of respiratory disease. In cattle 3 injections of 1.25 mg/kg bw danofloxacin are administered at 24-hour intervals. Birds are given danofloxacin in the drinking water to provide 5 mg/kg bw per day for 3 days. Danofloxacin is currently entered into Annex III of Council Regulation (EEC) No. 2377/90 as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Danofloxacin	Danofloxacin	Bovine	900 µg/kg 500 µg/kg 300 µg/kg 200 µg/kg	Liver Kidney Muscle Fat	Provisional MRLs expire on 1 July 1997
Danofloxacin	Danofloxacin	Chickens	1200 µg/kg 600 µg/kg 300 µg/kg	Liver, kidney Fat+skin Muscle	Provisional MRLs expire on 1 July 1997

2. The pharmacokinetics of danofloxacin were studied in the target species: cattle and chickens, as well as in the laboratory animals, rats and dogs. In chickens, danofloxacin was rapidly absorbed after oral administration in the drinking water and rapidly distributed to the tissues. In cattle, danofloxacin had similar bioavailability by the intramuscular, intravenous and subcutaneous routes of administration. Following oral administration to cattle, plasma concentrations were at or below the limit of detection of the analytical method at all time points and so no pharmacokinetic parameters could be calculated. The oral bioavailability in pigs was estimated to be 89%.

3. In all species, tissue residues at all time points were highest in the liver. Residues in the liver of rats, dogs, cattle and chickens consisted mostly of the unmetabolised parent compound and the N-desmethyl metabolite.

4. Residues in the urine of rats, dogs and cattle consisted mostly of unmetabolised danofloxacin, accounting for at least 50% of the radioactivity in all species. Another major component of urine was the N-desmethyl metabolite. Urine from treated dogs contained a significant amount (corresponding to 25% of total radioactivity) of the N-oxide metabolite; this was a minor metabolite in other species. Residues in the faeces of cattle, rats, dogs and chickens consisted mainly of unmetabolised danofloxacin.

5. Both danofloxacin and its N-desmethyl metabolite were of low acute oral toxicity. In laboratory animals, danofloxacin produced mild effects on the gastrointestinal system. Therapeutic doses of other quinolones have been reported to cause gastrointestinal disturbances in humans. Danofloxacin produced only slight transient skin and eye irritation.

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

6. Repeated-dose toxicity studies were carried out in rats (1-month, 3-months) dogs (1-month, 3-months) with danofloxacin and in rats (3-months) and dogs (1-month, 3-months) with the N-desmethyl metabolite. All of the 3-month rat studies incorporated an *in utero* phase. In female rats, danofloxacin produced a dose-related increase in proteinurea, the severity of which correlated with tubular nephropathy; the NOEL was 2.5 mg/kg bw/day. Administration of up to 6.25 mg/kg bw per day of the N-desmethyl danofloxacin metabolite to rats for 3 months caused no adverse effects. In repeat-dose studies with immature dogs, typical quinolone-type lesions were observed with both danofloxacin and the N-desmethyl-metabolite. The NOEL was 2.4 mg/kg bw/day for danofloxacin and 0.25 mg/kg bw/day for the N-desmethyl-metabolite.

7. Danofloxacin was not teratogenic in the mouse when dose levels of up to 200 mg/kg bw/day were administered to pregnant dams. 200 mg/kg bw/day was maternally toxic causing reduced bodyweight gain. A NOEL for foetotoxicity of 100 mg/kg bw/day was established based on reduced foetal weight and delayed ossification.

8. In rats, doses of 100 and 200 mg/kg bw/day were maternally toxic, producing reduced bodyweight gain and food consumption. The incidences of foetal delayed ossification and dilatation of the cerebral ventricles were also increased at both these dose levels. Dilatation of the cerebral ventricles often precedes hydrocephaly and so must be considered a teratogenic effect. The NOELs for teratogenicity and foetotoxicity were 50 mg/kg bw/day.

9. Several rat multigeneration studies were carried out with both danofloxacin and its N-desmethyl metabolite. Pregnancy rate was adversely affected at high doses; this appeared to be due to a failure to copulate. Dams given high doses produced fewer live pups per litter and pup weight and survival were adversely affected. The adverse effects on reproduction increased in severity with succeeding generations. The NOEL for both danofloxacin and the N-desmethyl metabolite was 6.25 mg/kg bw/day.

10. The mutagenic properties of danofloxacin were investigated in five *in vitro* and one *in vivo* assays which covered all the required end-points. All assays gave negative results apart from an *in vitro* cytogenetics assay in human lymphocytes. It was thought that the clastogenicity *in vitro* was due to the cation chelating properties of the compound because the clastogenicity was reduced or eliminated by addition of magnesium sulphate to the culture medium and/or washing the cells after the treatment period to remove danofloxacin. There was no evidence of clastogenicity *in vivo*.

11. The N-desmethyl metabolite induced significant increases in the nuclear labelling of primary rat hepatocytes in two independent assays but gave negative results in an *in vivo/in vitro* UDS assay and in a micronucleus test.

12. No carcinogenicity studies with danofloxacin were provided. The absence of such studies was justified by :

    - the lack of any structurally-alerting features;
    - the lack of carcinogenicity of most other fluoroquinolones;
    - the results of the mutagenicity assays;
    - the very low potential of danofloxacin for inhibition of the mammalian enzyme, topoisomerase II (in comparison with the considerable potential for inhibition of corresponding bacterial enzyme).

13. A toxicological ADI of 0.024 mg/kg bw/day was calculated by applying a safety factor of 100 to the NOEL of 2.4 mg/kg bw/day for arthropathy which was demonstrated for danofloxacin in a 3-month repeated-dose study in immature dogs. The 100-fold safety factor is justified by the evidence that immature dogs are the species most susceptible to quinolone-induced arthropathy and that humans are relatively insensitive to this effect.

14. It was noted that the WHO/FAO Joint Expert Committee on Food Additives (JECFA) had established a toxicological ADI of 0-0.02 mg/kg bw per day for danofloxacin. This ADI was also based on the NOEL of 2.4 mg/kg bw per day for arthropathy in the 3-month repeated-dose toxicity study in immature dogs and a safety factor of 100. However in accordance with the current JECFA practice, the ADI had been rounded to one significant figure. The CVMP saw no need to round the ADI.

15. *In vitro* MIC data were provided for both danofloxacin and N-desmethyl danofloxacin against several bacterial strains which are representative of those present in the normal gut flora of humans. From these data it was concluded that 0.25 µg/ml (for *Proteus* spp) was the MIC_{50} value for most sensitive bacterial species.

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

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

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

$$\text{ADI} = \frac{\dfrac{0.25 \times 1}{1}}{\dfrac{0.11 \times 150}{60}} \times 100 = 600 \text{ µg/kg bw i.e.36. mg/person}$$

    The following assumptions were made:

- CF1 = 1 because the MIC value for the most sensitive bacterial strain was used (rather than a geometric mean of all the species tested);
- CF2 = 1 because no data were available to correct for differences in growth conditions between the *in vitro* and *in vivo* situation;
- 150 ml was the volume of the daily faecal bolus;
- 0.11 for the fraction of the oral dose available to the distal part of the gastrointestinal tract (based on a bioavailability of 89% following oral administration to pigs);
- Danofloxacin was shown to bind tightly to faeces. The K_{ads} adsorption constant was 540.7. Therefore less than 1% of the danofloxacin present in faeces would be desorbed or "available". The fraction of the oral dose "available" to the distal part of the gastrointestinal tract could therefore be divided by 100;
- 60 kg for the adult bodyweight.

16. It was noted that JECFA had calculated a microbiological ADI of 37 µg/kg bw per day based on the mean MIC_{50} value for *Eubacterium* spp, *Bifidobacterium* spp and *Peptostreptococcus* spp and using a new formula employing a mass of colonic contents value of 220g. The microbiological ADIs calculated by both JECFA and the CVMP were higher than the corresponding toxicological ADIs calculated by these Committees. Consequently the toxicological ADIs were used in assessing the risks to consumers.

17. After oral administration of radiolabelled danofloxacin to chickens and intramuscular administration to cattle, residues of total danofloxacin-related residues in all tissues declined rapidly with time. In both species, total residues were highest in the liver at all time points. Unmetabolised danofloxacin was the major component in chicken liver and constituted 47-61% of pooled liver residues at 6-24 hours after treatment. Residues of N-desmethyl danofloxacin over the same period were 14-20% of the total liver residues. Residues in cattle liver consisted of 14-32% danofloxacin. Residues of N-desmethyl-danofloxacin declined from 30-40% of the total residue at 12-hours, to 14% at 72 hours.

18. In a residue study in which unlabelled danofloxacin was administered to chickens in the drinking water at a rate equivalent to 5 mg/kg bw for 3 days, residues of danofloxacin in muscle declined from 36-90 µg/kg 6 hours after withdrawal of treatment to less than 25 µg/kg 18 hours after the withdrawal of treatment. Residues of the N-desmethyl metabolite were less than 25 µg/kg at all time points. Residues of danofloxacin in liver declined from 157-319 µg/kg 6 hours after the withdrawal of treatment, to 18-66 µg/kg 36 hours after the withdrawal of treatment. Residues of the N-desmethyl metabolite were 35-193 µg/kg and less than 10 µg/kg over the same time points.

19. In a residue study in cattle given 5 daily intramuscular injections of unlabelled danofloxacin at a dose rate of 1.25 mg/kg bw, residues of danofloxacin in liver declined from 372 ± 63 µg/kg 12 hours after the last treatment to 13 ± 3 µg/kg, 5 days after the last treatment. Over the same time period, residues at the injection site declined from 669 ± 364 µg/kg to less than 10 µg/kg, residues in kidney declined from 426 ± 79 µg/kg to 5 µg/kg and residues in muscle declined from 112 ± 27 µg/kg to less than 10 µg/kg. Residues in most fat samples were below the limit of detection. Except for the liver samples taken 12 hours after the last treatment, residues of the N-desmethyl metabolite in all samples were lower than the residues of danofloxacin.

20. An analytical method for the determination of residues of danofloxacin in the edible tissues of cattle and chicken was based on HPLC with fluorescence detection. The method was validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community. The limit of quantification for all tissues was 40 µg/kg. The limits of detection were 1.5, 4.6, 1.4 and 4.0 µg/kg for chicken liver, kidney, muscle and skin/fat and 3.3, 3.6, 5.0 and 2.9 µg/kg for bovine liver, kidney muscle and fat respectively. The method was described in the ISO 78/2 format. A confirmatory LC/MS/MS method was also available.

21. Taking into account the pattern of residues depletion, it was considered that the MRLs for danofloxacin which were currently entered into Annex III of Council Regulation (EEC) 2377/90 were unnecessarily high and did not properly reflect the pattern of distribution. The percentage of the ADI which remained for further development of the substance was very small and unlikely to accommodate possible future MRLs for food commodities such as milk and eggs. For these reasons, it was agreed to adopt as final MRLs, the MRLs which had been agreed at the 48th meeting of JECFA.

Conclusions and recommendation:

Considering that

- a toxicological ADI of 0.024 mg/kg bw per day was established for danofloxacin,
- danofloxacin was the marker residue,
- in cattle and chickens, the percentage of total residues present as danofloxacin varies between tissues; residues in muscle and fat consisted of almost 100% danofloxacin; residues in kidney were approximately 50% danofloxacin, while residues in liver were approximately 20% danofloxacin in cattle and 55% danofloxacin in chicken,
- residues of N-desmethyldanofloxacin were found only in liver, together with residues of danofloxacin and unidentified metabolites; a 10-fold lower NOEL for N-desmethyldanofloxacin had been established, compared with the NOEL for danofloxacin based on the same parameter (arthropathy in juvenile dogs) - this had to be taken into account in elaborating MRLs,
- a validated analytical method for residues monitoring purposes is available;

the Committee recommends the inclusion of danofloxacin in 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
Danofloxacin	Danofloxacin	Bovine	200 µg/kg 100 µg/kg 400 µg/kg	Muscle Fat Liver, kidney	Not for use in animals from which milk is produced for human consumption
		Chicken	200 µg/kg 100 µg/kg 400 µg/kg	Muscle Fat+skin Liver, kidney	Not for use in animals from which eggs are produced for human consumption

Based on these MRLs, it was estimated that the consumer intake of danofloxacin-related residues in bovine tissues (300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat) would represent 43% the toxicological ADI calculated in paragraph 13.