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

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

DANOFLOXACIN

SUMMARY REPORT (1)

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 has been proposed for administration 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.

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 bioavailabilily 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.

6. Repeat dose toxicity studies were carried out in rats (one-month, three-months) dogs (one-month, three-months) with danofloxacin and in rats (three-months) and dogs (one-month, three-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 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 desmethyl danofloxacin metabolite to rats for three months caused no adverse effects. In repeat-dose studies with immature dogs, typical quinolone-type lesions were observed with both danofloxacin and the desmethyl-metabolite. 2.4 mg/kg bw/day was a NOEL for danofloxacin and 0.25 mg/kg bw/day for the 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

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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. 6.25 mg/kg bw/day was a NOEL for both danofloxacin and the N-desmethyl metabolite.

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. Toxicological ADIs of 0-0.024 mg/kg bw/day and 0-0.0025 mg/kg bw/day can be calculated by applying a safety factor of 100 to the NOELs of 2.4 mg/kg bw/day and 0.25 mg/kg bw/day for arthropathy which were demonstrated for danofloxacin and the desmethyl-metabolite respectively, in 3-month repeat-dose studies 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. *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. Based on the JECFA formula, the Working Party calculated a microbiological ADI based on the following assumptions :

    - 1 for the factor CF1 because MIC value for the most sensitive bacterial strain was used (rather than a geometric mean of all the species tested);
    - 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 GI tract could therefore be divided by 100;
    - 150 g for the human daily faecal bolus;
    - 60 kg for the adult bodyweight.

$$ADI = \frac{\frac{0.25}{1} \times 150}{\frac{0.11}{100} \times 60}$$

= 0-0.6 mg/kg bw/day

15. After oral administration 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 it 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 desmethyl-danofloxacin declined from 30-40% of the total residue at 12-hours, to 14% at 72 hours.

16. In a "cold" residue study in which 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 0.036-0.09 mg/kg 6 hours after withdrawal of treatment to <0.025 mg/kg 18 hours after the withdrawal of treatment. Residues of the N-desmethyl metabolite were <0.025 mg/kg at all time points. Residues of danofloxacin in liver declined from 0.157-0.319 mg/kg 6 hours after the withdrawal of treatment, to 0.018-0.066 mg/kg 36 hours after the withdrawal of treatment. Residues of the N-desmethyl metabolite were 0.035-0.193 and <0.01 mg/kg over the same time points.

17. In a "cold" residue study in cattle given 5 daily intramuscular injections of danofloxacin at a dose rate of 1.25 mg/kg bw, residues of danofloxacin in liver declined from 0.372±0.063 mg/kg 12 hours after the last treatment to 0.013±0.003 mg/kg, 5 days after the last treatment. Over the same time period, residues at the injection site declined from 0.669±0.364 mg/kg to <0.010 mg/kg, residues in kidney declined from 0.426±0.079 mg/kg to 0.005 mg/kg and residues in muscle declined from 0.112±0.027 mg/kg to <0.010 mg/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.

18. An analytical method for the determination of residues of danofloxacin in poultry and bovine tissues was proposed, based on HPLC with fluorescence detection. The lowest fortification level which had been analysed with acceptable accuracy and precision was 0.04 mg/kg. The LOQ for cattle tissues was therefore 0.04 mg/kg. Samples of chicken muscle and skin/fat had been analysed with acceptable accuracy and precision at a fortification level of 0.025 mgtkg. The LOQ for chicken tissues was therefore 0.025 mg/kg. The specificity of the proposed routine analytical method was not satisfactory because enrofloxacin co-eluted with danofloxacin. The substances were separated satisfactorily in the proposed confirmatory LC/MS/MS method.

19. Taking into account the different patterns of residue depletion in the bovine and chickens, the following provisional MRLs were elaborated :

Substance	Marker residue	Animal species	MRLs	Target tissues	other provisions
Danofloxacin	Danofloxacin	bovine	300 µg/kg 900 µg/kg 500 µg/kg 200 µg/kg	Muscle Liver Kidney Fat	provisional MRLs expire on 31.03.97
Danofloxacin	Danofloxacin	chickens	300 µg/kg 1200 µg/kg 1200 µg/kg 600 µg/kg	Muscle Liver Kidney Fat/skin	provisional MRLs expire on 31.03.97

At these MRLs, consumer intake of residues of both danofloxacin and the total residues was calculated to be well below the ADIs established for danofloxacin. In addition, the consumer intake of residues of the metabolite desmethyldanofloxacin was calculated to be below the ADI for desmethyldanofloxacin.

20. It was agreed that the above MRLs should be provisional until 31 March 97. Answers to the following questions should be provided before 31 September 1996:

    - The specificity of the routine HPLC analytical method was not satisfactory because enrofloxacin co-elutes with danofloxacin. Although a satisfactory confirmatory LC/MS/MS method had been proposed, the specificity of the proposed routine analytical method needed to be improved.

    - A final version of the routine analytical method for cattle and chicken tissues should be provided (individual data and chromatograms to support the validation parameters). The method should be described in an internationally recognized standard layout (e.g. ISO 78/2).