

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

DANOFLOXACIN (extension to pigs)

SUMMARY REPORT (1)

1. Danofloxacin is a fluoroquinolone antibacterial and antimycoplasmal drug, which acts by inhibition of bacterial DNA-gyrase.

An ADI of 24 µg/kg bw (1440 µg/person) was previously established by the Committee for Veterinary Medicinal Products (CVMP) for danofloxacin by applying a safety factor of 100 to the NOEL of 2.4 mg/kg bw per day for arthropathy which was established in a 3-month repeated-dose study in immature dogs. The 48th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) had established an ADI on the same end-point and using the same safety factor but had rounded the ADI to one significant figure, in line with the JECFA practice.

Based on this ADI, the CVMP proposed provisional MRLs which are currently entered into 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
Danofloxacin	Danofloxacin	Bovine	900 µg/kg 500 µg/kg 300 µg/kg 200 µg/kg	Liver Kidney Muscle Fat	Provisional MRLs expire on 1.1.1999
Danofloxacin	Danofloxacin	Chicken	1200 µg/kg 600 µg/kg 300 µg/kg	Liver, kidney Skin + fat Muscle	Provisional MRLs expire on 1.1.1999

The Committee recently considered the additional information requested for entry of danofloxacin for bovines and chicken into Annex I of the Regulation. On re-evaluating the substance, the Committee considered that the above MRLs were unnecessarily high and did not properly reflect the pattern of distribution. In addition, 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, the Committee recommended that the MRLs which were agreed at the 48th meeting of the JECFA should be adopted as final MRLs and danofloxacin be entered into Annex I as follows:

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
Danofloxacin	Danofloxacin	Chicken	200 µg/kg 100 µg/kg 400 µg/kg	Muscle Skin + fat Liver, kidney	Not for use in animals from which eggs are produced for human consumption

An application for the extension of the MRLs for danofloxacin to pigs has now been submitted. Three intramuscular injections of 1.25 mg/kg bw per day, at 24 hour intervals, would be recommended. In support of this application, some new toxicity studies were provided, which had also been considered at the 48th meeting of JECFA, together with a residue file for pigs.

- Several pharmacokinetic studies were carried out in pigs using the proposed 2.5% danofloxacin prototype commercial formulation. Following 3 daily intramuscular injections of 1.25 mg/kg bw per day danofloxacin, a mean plasma C_{max} value of around 0.6 ± 0.5 µg/ml was attained 0.6 + 0.4 hours after the first dose and after the third dose. The pharmacokinetic parameters for the first and third doses were not statistically different. In a cross-over design study in which pigs were administered 1.25 mg/kg bw danofloxacin by the intramuscular or the intravenous route, a comparison of the $AUC_{0-\infty}$ values indicated that bioavailability for the intramuscular route was around 100%.
- The metabolism of danofloxacin in pigs was similar to that in other species. In pigs given 5 daily intramuscular injections of 1.25 mg/kg bw 3H -danofloxacin, the mean radioactivity recovered in urine and faeces for 2 days before and one day after the last dose accounted for approximately 60% and 20% respectively, of the daily dose. Unmetabolised danofloxacin accounted for 81% and 72% of the radioactivity present in faeces from males and females, respectively, excreted over the 5-day dosing period. Of the radioactivity in faeces 5% (males) and 7% (females) was identified as N-desmethyl danofloxacin. Over the same time period, 78% and 80% of the radioactivity in urine from males and females was identified as unchanged danofloxacin. Of the radioactivity in urine 2 to 3% was N-desmethyl danofloxacin, 10 to 14% was danofloxacin-N-oxide and 3% was the β -glucuronide. The same metabolites were found in bile samples from pigs. The pigs were killed 12 hours after the last treatment. In liver 58% and 68% of the residues were present as danofloxacin and 23% and 29% as N-desmethyl danofloxacin, in males and females respectively.
- Tolerance studies in pigs indicated that danofloxacin was well tolerated at up to 10 times the proposed dose. No gross lesions were observed in any of the joints.
- In a new teratogenicity study, groups of 29 to 39/dose female New Zealand White rabbits were given daily oral doses of 0, 2.5, 7.5 or 15 mg/kg bw per day of danofloxacin from days 6 to 20 of gestation. At 15 mg/kg bw, food consumption was reduced, some does lost body weight and there was a high incidence of abortions. There was no evidence of teratogenicity at any dose level. The dose of 7.5 mg/kg bw per day was a NOEL for both maternal toxicity and embryoletality.
- In a new carcinogenicity study, groups of 50/sex ICR mice were fed diets containing the equivalent of 10, 50 or 100 mg/kg bw per day danofloxacin for up to 2 years. Two groups of 50/sex were fed untreated diets and served as controls. Survival at termination was below 50% in all groups except for the males receiving 10 mg/kg bw. There was no evidence of carcinogenicity.

7. A new carcinogenicity study was carried out in which groups of 50/sex Long-Evans rats were fed diets containing 10, 50 or 100 mg/kg bw danofloxacin for up to 2 years. Two groups of 50/sex were fed untreated diets and served as controls. Survival at termination was below 50% in all groups except for the females receiving 100 mg/kg bw and one of the female control groups. In the 100 mg/kg bw group some haematology parameters were depressed in females and there was an increase in aspartate aminotransferase activity in males. At termination, caecal enlargement (a common finding in rodents fed large doses of antibiotics) was observed in all treated male groups. In males given 100 mg/kg bw, there was an increased incidence of kidney papillary oedema and oligospermia and abnormal content of the epididymides. In females, there were increasing trends in the incidence of pituitary adenomas and granular cell tumours of the uterus and vagina. However, the statistical significance was lost on adjustment for multiple comparisons. The incidence of pituitary adenomas was within the historical control range. The granular cell tumours appeared to be merely enlarged foci and no increased trend was observed when the incidences of foci and tumours were combined. It was concluded that danofloxacin was not carcinogenic in this assay.
8. The Committee considered that the results of the new toxicity studies did not affect the previous ADI calculation.
9. Swine were given 5 daily intramuscular injections of 1.25 mg/kg bw per day of ³H-danofloxacin. The animals were killed, 3 per time-point, 12, 24, 48 or 168 hours (7 days) after the last dose. Mean total residues in liver, kidney, fat and muscle were 988, 833, 74 and 339 µg/kg, 12 hours after the last dose and depleted to 408, 106, 17 and 36 µg/kg, 48 hours after the last dose. Mean total residues at the 5th injection site were 287 µg/kg, 12 hours after the end of treatment and 30 µg/kg, 48 hours after the end of treatment. Seven days after the last dose, mean total residues were 177 µg/kg in liver, 5 µg/kg in kidney, and were undetectable (i.e. below 2 or below 4 µg/kg) in other tissues. Bound, non-extractable residues after hydrolysis of liver samples accounted for around 14% of the total residues in liver, 12 hours to 7 days after dosing.
10. The residues in liver samples from the above study were characterised. Twelve hours after the last dose, 44% of the residues in liver were present as N-desmethyl danofloxacin and 46% were danofloxacin. Twenty-four hours after the last dose, 58% were present as N-desmethyl danofloxacin and 26% as danofloxacin. Forty-eight hours after the last dose, 85% were present as N-desmethyl danofloxacin and 17% as danofloxacin. Seven days after the last dose, 100% of the residues were present as N-desmethyl danofloxacin.
11. In another radiometric study, swine were given 3 daily intramuscular injections of 1.25 mg/kg bw per day of ³H-danofloxacin. The animals were killed, 3 per time-point, 6, 10, 14 and 18 days after the last dose and samples of liver were taken for analysis. Mean total residues in liver 6 days, 10 days and 14 days after the end of dosing were 136 µg/kg, 84 µg/kg, and 35 µg/kg respectively. Approximately 100% of the residues at all 3 time-points were N-desmethyl danofloxacin. Mean total residues in liver 18 days after the last dose were 4 µg/kg and were too low for characterisation.
12. A residue depletion study was carried out in which residues of danofloxacin and N-desmethyl danofloxacin were determined using HPLC with fluorescence detection. Swine were given 3 daily intramuscular injections of 1.25 mg/kg bw danofloxacin. The animals were killed, 4 per time-point, 2, 6, 10, 14 or 18 days after the last dose. Residues of N-desmethyl danofloxacin were found only in liver and declined from a mean value of 622 µg/kg 2 days after the last dose, to 221 µg/kg 6 days after the last dose, to 79 µg/kg 18 days after the last dose. Mean residues of danofloxacin in liver were 27 µg/kg 2 days after the last dose and were undetectable (below 10 µg/kg) at later time-points. Mean residues of danofloxacin in kidney declined from 36 µg/kg 2 days after the last dose, to 5.5 µg/kg 6 days after the last dose and were undetectable (below 5 µg/kg) at later time-points. Two days after the last dose, mean residues of danofloxacin in muscle, fat and at the injection site were 15 µg/kg, below 5 µg/kg, and 17 µg/kg respectively; residues in other tissues were undetectable (below 5 or below 10 µg/kg) at later time-points.

13. To estimate the ratio of marker to total residues, the residues determined at the 48-hour time-point in the HPLC study using 3 daily intramuscular injections of 1.25 mg/kg bw danofloxacin were compared with those determined at the 48-hour time-point in the radiometric study using 5 daily intramuscular injections of 1.25 mg/kg bw per day of ³H-danofloxacin. Based on the mean residues, it was calculated that danofloxacin represented 6%, 34% and 42% of the total residues in liver, kidney and muscle, 48 hours after the last dose. Residues of danofloxacin in fat at 48 hours were too low for quantification. It was agreed to follow the approach adopted by the Joint FAO/WHO Expert Committee on Food Additives and assume that 100% of the residues in fat were danofloxacin.
14. No total or marker residue depletion data were presented for pig skin.
15. Analytical methods for the determination of danofloxacin in swine muscle, liver, kidney and fat were presented in the ISO 78/2 format. The methods were essentially the same with minor differences in procedure and were based on HPLC with fluorescence detection. The methods were of acceptable specificity and chromatograms were provided showing that both danofloxacin and N-desmethyl danofloxacin were clearly separated from ofloxacin, norfloxacin, ciprofloxacin and enrofloxacin. The limits of quantification for danofloxacin appeared to be 12 µg/kg for muscle, kidney, liver and fat, but insufficient data were provided concerning accuracy and precision. The limits of detection for danofloxacin were 2.7 µg/kg for liver, 3.0 µg/kg for kidney, 0.7 µg/kg for muscle and 0.6 µg/kg for fat.
16. The Committee noted that the 48th meeting of the Joint FAO/WHO Expert Committee on Food Additives had proposed MRLs of 100 µg/kg for muscle, 100 µg/kg for fat, 50 µg/kg for liver and 200 µg/kg for kidney, based on danofloxacin as the marker residue. The Committee agreed that these MRLs could not be adopted because they did not reflect the distribution of residues at the 48-hour time-point.

Conclusions and recommendation

Having considered that:

- a toxicological ADI of 0.024 mg/kg bw per day was established for danofloxacin,
- the percentage of marker : total residues was 6%, 34%, 42% and 100% for liver, kidney, muscle and fat, 48 hours after the last dose,
- analytical methods for the monitoring of residues are available but not fully validated;

the Committee recommends the inclusion of danofloxacin in Annex III to 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	Porcine	100 µg/kg 50 µg/kg 200 µg/kg 200 µg/kg	Muscle Skin + fat Liver Kidney	Provisional MRLs expire on 1.1.2000

Based on the above MRLs, the theoretical maximum daily intake of total residues, from the consumption of 300 g porcine muscle, 100 g liver, 50 g kidney and 50 g skin + fat was estimated to be 436 µg per day, i.e. around 30% of the ADI.

Before the Committee can consider the inclusion of danofloxacin for 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. The accuracy and precision of the proposed routine analytical method for the target tissues muscle and kidney should be presented in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community.
2. The proposed routine analytical method should be validated in accordance with Volume VI for the target tissue skin + fat in natural proportions, and not separately in skin and in fat.