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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

DANOFLOXACIN (extension to milk)

SUMMARY REPORT

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

   An Acceptable Daily Intake of 24 µg/kg bw (1440 µg/person) was established 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 and taking into account the pattern of distribution as well as reserving part of the ADI for development of further uses, the Committee for Veterinary Medicinal Products (CVMP) proposed MRLs in line with the MRLs agreed by the 48th meeting of JECFA, which are currently included into Annex I of Council Regulation (EEC) 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 400 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption
		Chickens	200 µg/kg 100 µg/kg 400 µg/kg 400 µg/kg	Muscle Skin + fat Liver Kidney	Not for use in animals from which eggs are produced for human consumption

   The Committee recently considered further information submitted with an application for the extension of the MRLs to porcines and proposed provisional MRLs 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

2. **An Application for the extension of the MRLs for danofloxacin to bovine milk has now been submitted in relation with the treatment of bovine respiratory disease and enteric infections caused by sensitive organisms in dairy cows. Up to five intramuscular injections of 1.25 mg/kg bw per day, at 24 hour intervals, would be recommended. New data concerning the effects of danofloxacin on dairy starter cultures were provided, together with a residue file for bovine milk.**

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3. ***In vitro*** **MIC values were determined for both danofloxacin and N-desmethyldanofloxacin against 5 different dairy starter cultures. Three of these cultures are used in cheese production, one for the production of yoghurt and one for fermented milk. The MICs for danofloxacin were in the range 16 to 32 µg/ml when cultured in the presence of broth and more than or equal to 32 µg/ml when cultured in the presence of sterilised skimmed milk. The MICs for N-desmethyldanofloxacin were higher or equal to those obtained for danofloxacin. In another study, the effect of danofloxacin on acid production was investigated using the same 5 dairy starter cultures. Concentrations of up to 400 µg/L danofloxacin were without effect on acid production.**

4. **Eight lactating dairy cows were given 5 daily intramuscular injections of 1.25 mg/kg bw per day of ^{3}H-danofloxacin. Four cows were in early lactation and 4 were in late lactation. The cows were milked twice daily and total residues in milk were determined using liquid scintillation counting. Mean total residues in milk were highest at the first milking after each dose (mean 1028 to 1195 µg equivalents/kg). Total residues rapidly depleted to between 124 to 190 µg equivalents/kg by the second milking after each dose. Total residues were undetectable in most samples by 72 hours after the last dose. Residues of danofloxacin and N-desmethyldanofloxacin in the milk samples were determined simultaneously using the proposed routine analytical method based on HPLC with fluorescence detection. The limit of quantification for both analytes was 12 µg/kg. Residues of both danofloxacin and N-desmethyldanofloxacin were highest at the first milking after each dose with mean values in the ranges 685 to 947 µg/kg and 150 to 207 µg/kg, respectively. The mean concentrations of both danofloxacin and N-desmethyldanofloxacin depleted rapidly to between 42 to 82 µg/kg and 41 to 53 µg/kg by the second milking after each dose. Twenty four hours after the fifth dose, mean residues of danofloxacin were 42 µg/kg and mean residues of N-desmethyldanofloxacin were 42 µg/kg. Forty eight hours after the fifth dose, residues of both danofloxacin and N-desmethyldanofloxacin were below the limit of quantification in most samples.**

5. **In the above study, danofloxacin accounted for 67 to 79% of the total residues and N-desmethyldanofloxacin accounted for 14 to 19% of the total residues in the milk samples taken 8 hours after each dose. In milk samples taken 24 hours after each dose, the percentage of residues present as danofloxacin had fallen to approximately 35%. N-desmethyldanofloxacin accounted for a similar percentage of the residues at these time points. At 24 and 32 hours after the last (5th dose), danofloxacin represented approximately 34% of the total residues.**

6. **A residue depletion study was carried out in which 8 lactating cows were given 5 daily intramuscular injections of a commercial 2.5% formulation at a dose of 1.25 mg/kg bw per day of danofloxacin. Although the study was not GLP-compliant, it was well conducted and reported and full raw data were provided. Four of the cows were in early lactation and 4 were in late lactation. The cows were milked twice daily and residues of danofloxacin and N-desmethyl danofloxacin in milk were determined simultaneously using HPLC with fluorescence detection. The method was similar to that proposed as the routine analytical method but used a slightly different extraction procedure and different chromatographic conditions. The limit of quantification was reported to be 15 µg/kg for both analytes. Residues of danofloxacin and N-desmethyldanofloxacin were highest at the first milking after each dose with mean values in the ranges 727 to 1042 µg/kg and 211 to 256 µg/kg, respectively. The mean concentrations of both danofloxacin and N-desmethyldanofloxacin depleted rapidly to between 44 to 83 µg/kg and 40 to 55 µg/kg by the second milking after each dose. Twenty four hours after the fifth dose, mean residues of danofloxacin were 55 µg/kg and mean residues of N-desmethyldanofloxacin were 46 µg/kg. Forty eight hours after the fifth dose, residues of both danofloxacin and N-desmethyldanofloxacin were below the limit of quantification in all samples (12 µg/kg).**

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7. A proposed routine analytical method for the determination of residues of danofloxacin in bovine milk was presented in the ISO format. The method was based on HPLC with fluorescence detection and was capable of measuring residues of both danofloxacin and the major metabolite N-desmethyldanofloxacin which eluted with retention times of approximately 19 minutes and 21 minutes, respectively. Inter- and intra-day data on accuracy and precision were provided for milk samples spiked at concentrations of 12, 100, 400 and 2000 μg/kg. The limit of quantification for both danofloxacin and N-desmethyldanofloxacin in bovine milk was 12 μg/kg. The limits of detection for danofloxacin and N-desmethyldanofloxacin in bovine milk were 1.8 μg/kg and 1.0 μg/kg, respectively.

Conclusions and recommendation

Having considered that:

- **a toxicological ADI of 24 μg/kg bw per day (1440 μg/day) was established for danofloxacin,**
- **danofloxacin was already established as the marker residue for bovine (and chicken) tissues; for the sake of consistency it was desirable to use the same marker residue for bovine milk,**
- **danofloxacin accounted for approximately 35% of the total residues in milk taken from cows 24 to 32 hours after the end of treatment,**
- **the proposed routine analytical method based on HPLC with fluorescence detection was fully validated for bovine milk;**

the Committee recommends the establisment of maximum residue limits for danofloxacin in bovine milk and therefore the amendment of the current entry for danofloxacin for bovine 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 400 μg/kg 30 μg/kg	Muscle Fat Liver Kidney Milk	

Based on the above MRL the theoretical daily intake of total residues from 1.5 litres of milk was estimated to be 129 μg/day of total residues, i.e around 9% of the ADI.

It was previously established that, based on the above MRLs the theoretical maximum daily intake of total residues, arising from the consumption of 300 g bovine muscle, 100 g liver, 50 g kidney and 50 g fat, accounted for 43% of the ADI.

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