The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Inspections

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CEFTIOFUR
(Modification of MRLs for bovine species)

SUMMARY REPORT (3)

1. Ceftiofur (CAS Number 80370-57-6) is a third generation cephalosporin antibiotic, which is administered to cattle and pigs for control of bacterial infections of the respiratory tract. It is used in veterinary medicine as the sodium salt (CAS Number 104010-37-9), and the hydrochloride (CAS Number 103980-44-5 intramuscularly in cattle, including lactating cows, at doses of to up to 2 mg/kg bw/day for up to 5 days and in pigs at doses of up to 5 mg/kg bw/day for up to 3 days. It is also used used as crystalline free acid for intramuscular and subcutaneous administration in cattle and pigs.

   A microbiological ADI of 20 µg/kg bw/day (i.e. 1200 µg/person) for ceftiofur was previously established by the Committee for Veterinary Medicinal Products (CVMP) based on the MIC_{50} of 2.0 µg/ml for the most sensitive strains of human gut flora (*Escherichia coli, Lactobacillus* spp and *Clostridium* spp).

   Currently, ceftiofiur is included in Annex I of Council Regulation (EEC) No 2377/90 as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Ceftiofur	Sum of all residues retaining the betalactam structure expressed as desfuroylceftiofur	Bovine	1000 µg/kg 2000 µg/kg 2000 µg/kg 6000 µg/kg	Muscle Fat Liver Kidney	
			100 µg/kg	Milk	Not for intramammary use
		Porcine	1000 µg/kg 2000 µg/kg 2000 µg/kg 6000 µg/kg	Muscle Fat Liver Kidney	

   An application has now been submitted for the modification of the current MRLs to allow for intramammary use in dairy cows. The proposed indication is for intrammary use in the treatment of clinical mastitis in lactating dairy cattle with a formulation containing 125 mg ceftiofur equivalents per 10 ml syringe and to treat under infections at dry off containing 500 mg ceftiofur equivalents per 10 ml syringe.

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2. Dairy cows were given two intramammary infusions of 125 mg/quarter of ^{14}C-ceftiofur, 12 hours apart, following a morning and evening milking. The 2 intramammary infusions of ceftiofur resulted in a plasma C_{max} of 0.7 µg/ml, a T_{max} of 17 hours, an $AUC_{0\text{-}36h}$ of 14 µg.ml/hour, and an MRT of 19 hours. Milk, urine and faeces collected up to 5 days after the last dose contained 70, 15 and 13% of the administered dose respectively. In this study, cows were sacrificed 5 days after the last dose, and residue concentrations in tissues were determined by liquid scintillation counting (LSC; limit of detection of about 2 µg/kg). After the second dose, the mean total residue concentration in milk depleted from 44200 µg equivalents/litre to 75 µg equivalents/litre by 72 hours. The mean total residue was 19 µg equivalents/kg in liver, 75 µg equivalents/kg in kidney, and 5 µg equivalents/kg in muscle and fat.

   In another study, dairy cows were given two intramammary infusions of 125 mg/quarter of ^{14}C-ceftiofur, 24 hours apart, after consecutive morning or evening milkings. The 2 intramammary infusions of ceftiofur resulted in plasma C_{max} values of 0.63 and 0.72 µg/ml, T_{max} values of 8 and 7 hours, $AUC_{0\text{-}< LOQ}$ of 19 µg.ml/hour, and an MRT of 17 hours. Milk, urine, faeces and tissue were collected up to 6 days after the last dose and these contained 58, 22, 13 and 6% of the administered dose respectively. In this study, cows were sacrificed at 0 (12 hours), 2, 4 and 6 days after the last dose administration and residue concentrations in tissues were determined using analytical methods based on LSC (detecting all ^{14}C-labelled residues), HPLC-DCA (detecting acetamide derivatives of ceftiofur and desfuroylceftiofur), HPLC-RAM (detecting all ^{14}C-labelled metabolites), HPLC-DCD (detecting desfuroylceftiofur cysteine disulphide), HPLC-dimer (detecting desfuroylceftiofur dimer), HPLC-DGD (detecting desfuroylceftiofur glutathione disulphide), and HPLC-DC (detecting desfuroylceftiofur). After the second dose, the mean total residue concentration in milk depleted from 49 660 µg equivalents/litre at 12 hours to 88 µg equivalents/litre by 132 hours. Ceftiofur and desfuroylceftiofur related residues (i.e marker residue) concentrations depleted to less than the MRL for milk by 96 hours. Marker residue accounted for about 90% of the total residue in milk from 0 to 24 hours after treatment and about 30% of the total dose at 6 days after treatment. The mean total residue at 12 hours after treatment was 144 µg equivalents/kg in liver, 589 µg equivalents/kg in kidney, 33 µg equivalents/kg in muscle and fat, and 6860 µg equivalent/kg in udder.

   Residue depletion data were presented in cattle at dry off.

   Pregnant dairy cows were given intramammary infusions of 250 or 500 mg/quarter of ceftiofur hydrochloride at dry off. The 250 and 500 mg/quarter intramammary infusions of ceftiofur resulted in plasma C_{max} values of 0.85 and 3.74 µg/ml, T_{max} values of 18 and 9 hours, $AUC_{0\text{-}LOQ}$ values of 50 and 128 µg.ml/hour, and MRTs of 51 and 45 hours. Colostrum and milk samples collected between 0 and 96 hours after parturition contained ceftiofur plus desfuroylceftiofur related residue concentrations less than 10 µg/kg (HPLC-DCA, limit of quantification of 10 µg/kg). In this study, calves were fed colostrum and milk from a treated cow then sacrificed 4 days postpartum. The residue concentrations in calf liver and kidney were less than 50 µg/kg (HPLC-DCA, limit of quantification of 50 µg/kg).

   In another study, pregnant dairy cows were given intramammary infusions of 250 or 500 mg/quarter of ceftiofur hydrochloride at dry off. Half the animals treated at each dosing level were due to calve either 40 or 60 days later. Colostrum and milk samples collected between 0 and 96 hours after parturition contained ceftiofur plus desfuroylceftiofur related residue concentrations less than 50 µg/kg (HPLC-DCA, limit of quantification of 50 µg/kg). In this study, half the male calves were sacrificed at birth and the other half were fed colostrum and milk from a treated cow then sacrificed 4 days postpartum. The residue concentrations in liver and kidney were less than 100 µg/kg (HPLC-DCA, limit of quantification of 100 µg/kg) in all calves.

3. A validated routine analytical method (HPLC-DCA) was presented in an ISO format and based on HPLC with UV detection. Ceftiofur hydrochloride anti-oxime is added to milk (i.e calibration standards quality control and samples) as an internal standard. Dithioerythritol is added to hydrolyse protein-bound residues, freeing all residues retaining a β-lactam ring which are then derivatised to a common desfuroylceftiofur acetamide derivative that can be measured as desfuroylceftiofur equivalents. The limit of quantification of the method was 50 µg/kg for bovine milk and the limit of detection was 15 µg/kg. There was no evidence of interference in this assay from residues of other ß-lactam ring containing compounds.

   As the method for milk was an updated version of the regulatory method adopted by the CVMP for the routine assessment of bovine and porcine meat, new data were submitted to validate the amended method for these tissues. The data submitted demonstrated the use of the modified method to measure ceftiofur in bovine and porcine muscle and kidney.

Conclusions and recommendation

Having considered that:

- a microbiological ADI of 20 µg/kg bw/day, i.e. 1200 µg/person, was previously established for ceftiofur,
- the sum of the metabolites retaining a ß-lactam ring that may be expressed as desfuroylceftiofur equivalents is recommended as the marker residue in all target species and bovine milk,
- the marker residue accounts for all residues with anti-microbiological activity,
- ceftiofur is poorly absorbed from the udder,
- absorbed residues of ceftiofur are rapidly detoxified to metabolites devoid of antimicrobial activity and excreted,
- following intramammary administration of ceftiofur to lactating dairy cattle, the concentration of ceftiofur plus desfuroylceftiofur related residues was below the MRL for milk 96 hours after treatment,
- following intramammary administration of ceftiofur to dairy cattle at drying off, the concentration of ceftiofur plus desfuroylceftiofur related residues was less than the MRL for milk at parturition and 96 hours after parturition; at birth and after 4 days of being fed colostrum and milk from treated cows the concentration of ceftiofur plus desfuroylceftiofur related residues in neonatal calves was less than 100 µg/kg,
- a validated routine analytical method, based on residue derivitisation to an acetamide derivative of desfuroylceftiofur followed by detection by HPLC with ultraviolet detection, is available for determination of residues in porcine and bovine tissues and in bovine milk;

the Committee recommends that the current entry of ceftiofur in Annex I to Council Regulation (EEC) No. 2377/90 regarding bovine species be amended in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Ceftiofur	Sum of all residues retaining the betalactam structure expressed as desfuroylceftiofur	Bovine	1000 µg/kg 2000 µg/kg 2000 µg/kg 6000 µg/kg 100 µg/kg	Muscle Fat Liver Kidney Milk	

The MRL proposed above are the same as those adopted by JECFA.

Based on these MRLs, it was calculated that consumer intake of total residues will represent 87.5% of the ADI.