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

EMEA/MRL/498/98-FINAL
July 1999

# COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

## CEFTIOFUR

## SUMMARY REPORT (2)

1. Ceftiofur is a third generation cephalosporin antibiotic, which is administered to cattle and swine for control of bacterial infections of the respiratory tract. It is used in veterinary medicine as both, the sodium salt, and the hydrochloride. It is administered intramuscularly to cattle, including lactating cows, at doses of to up to 2 mg/kg bw/day for up to 5 days. It is also administered intramuscularly to swine at doses of up to 5 mg/kg bw/day for up to 3 days. It is also intended to be used as crystalline free acid for intramuscular and subcutaneous administration in cattle and swine. Ceftiofur is currently included in 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 |
|---|---|---|---|---|---|
| Ceftiofur | Sum of all residues retaining the beta-lactam structure expressed as desfuroylceftiofur | Bovine | 200 µg/kg<br>600 µg/kg<br>2000 µg/kg<br>2000 µg/kg<br>100 µg/kg | Muscle<br>Fat<br>Liver<br>Kidney<br>Milk | Provisional MRLs expire on 1.1.1999 |
| | | Porcine | 500 µg/kg<br>600 µg/kg<br>3000 µg/kg<br>4000 µg/kg | Muscle<br>Fat<br>Liver<br>Kidney | |

2. Ceftiofur has a wide spectrum of activity against both Gram-positive and Gram-negative bacteria. It exerts its antibacterial action by inhibition of bacterial cell wall synthesis.

3. Ceftiofur was poorly absorbed after oral administration and rapidly absorbed after intramuscular administration. Peak plasma concentrations of approximately 6 µg equivalents/ml were attained approximately 30 minutes after intramuscular administration of 1 mg/kg bw/day to calves and pigs. In *in vivo* studies in rats and cattle and in *in vitro* studies with liver extracts from rats, pigs, cattle and chickens ceftiofur was rapidly metabolised to (initially) desfuroylceftiofur and furoic acid. Maximum blood concentrations of ceftiofur and its metabolites were achieved within 0.5 and 2 hours after dosing. No unmetabolised ceftiofur was detected in blood from 2 to 4 hours after dosing. Blood concentrations declined biphasically.

   More than 95% of the intramuscularly administered dose was excreted within 24 hours of administration; 60 to 80% of this was in the urine and the remainder in the faeces. Most of the excreted material was desfuroylceftiofur and desfuroylceftiofur cysteine disulphide with small amounts of unmetabolised ceftiofur.

4. Ceftiofur was of low acute oral and parenteral toxicity. The acute oral $LD_{50}$ was more than 7760 mg/kg bw/day in rats. The acute intravenous and intramuscular $LD_{50}$ in rats were 2156 mg/kg bw/day and 1250 mg/kg bw/day, respectively.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Switchboard (44-20-7) 418 8400 Fax (44-20-7) 418 8447
E-mail: mail@emea.eudra.org http://www.eudra.org/emea.html

5. Repeated-dose studies of up to 90 days duration were carried out in rats and dogs.

   In a 90-day oral gavage study in rats (0, 30, 100, 300, 1000 or 3000 mg/kg bw per day) gastrointestinal disturbances, reduced erythrocyte counts, reduced serum glucose levels and electrolyte imbalance were observed. Compound-related histopathological alterations were observed at the higher dose levels and reflected the poor nutritional status of these animals. The NOEL was 30 mg/kg bw per day.

   In a 50-day study Beagle dogs ceftiofur was given orally by gavage (0, 300, 1000 or 3000 mg/kg bw/day), adverse effects on the haematopoietic system were observed at all dose levels including extramedullary haematopoiesis in kidneys, liver and spleen. Similar effects were observed at the both highest doses in a 90-day study in which Beagle dogs were given daily oral doses of 0, 10, 30, 100 or 300 mg/kg bw/day. The NOEL in dogs was 30 mg/kg bw/day.

6. In studies in which cattle were given daily intramuscular injections of up to 55 mg/kg bw/day for 5 days and pigs were given daily intramuscular injections of up to 125 mg/kg bw/day for 5 days, transient injection site reactions were the most notable findings.

7. Ceftiofur did not affect male or female fertility, growth or survival of the offspring in a 2-generation study in the rat in which oral gavage doses of 0, 100, 300 or 1000 mg/kg bw/day were administered to the parental animals.

   Ceftiofur was not teratogenic following oral administration of 0, 800, 1600 or 3200 mg/kg bw/ day to Sprague-Dawley rats from days 6 to 15 of gestation; however, maternal toxicity (soft stools) and foetotoxicity (reduced foetal weight) were observed at all dose levels.

   No evidence of teratogenicity or foetotoxicity was observed in CD-1 mice following oral administration of 0, 1000, 2000 or 4000 mg/kg bw/day from days 6 to 15 of gestation.

8. In one *in vitro* cytogenetics assay in Chinese hamster ovary (CHO) cells, ceftiofur increased significantly the number of cells with chromosomal aberrations, but only in the absence of metabolic activation. However, other *in vitro* assays gave negative results on both, ceftiofur and the furoic acid metabolite, as demonstrated in bacterial mutation assays in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 and mammalian cell gene mutation assays at the HPRT locus of Chinese hamster V79 cells.

   In several *in vivo* mutagenicity tests ceftiofur gave negative results as shown in a rat liver unscheduled DNA synthesis (UDS) assay (single oral doses of 1000 to 4000 mg/kg bw/day), a micronucleus test in mouse bone marrow (single intraperitoneal dose of 250 to 1000 mg/kg bw/day) and two chromosome aberration assays with ceftiofur in mouse bone marrow (up to 5 intraperitoneal doses ranging from 350 to 1750 mg/kg bw/day). A radiolabelled study showed that ceftiofur or its metabolites reached the bone marrow following intraperitoneal injection, confirming the validity of the *in vivo* assays which used this tissue. It was concluded that ceftiofur was not mutagenic.

9. No data on carcinogenicity were presented. However, the lack of structural alerts for ceftiofur, the absence of pre-neoplastic lesions in the repeated-dose studies and the evidence from the mutagenicity assays provide adequate reassurance that ceftiofur is not a potential carcinogen.

10. Positive cutaneous anaphylaxis tests in guinea pigs indicated that the desfuroylceftiofur metabolite had the greatest potential to elicit hypersensitivity reactions. However, no positive cutaneous anaphylaxis response was obtained in passively sensitised guinea pigs, which were challenged (intravenously) with 0.3 mg/animal ceftiofur-related residues obtained from injection site muscle and kidney of bovines.

    *In vitro* the serum of 17 penicillin-allergic individuals was tested in a radioallergosorbent test inhibition assay. Although one first generation cephalosporin did partially inhibit the binding of penicillin antibodies in one of these patients, no IgE antibodies were bound to ceftiofur. This suggested that residues of ceftiofur were unlikely to present a significant risk to penicillin allergic persons.

11. A toxicological ADI of 0.3 mg/kg bw was calculated for ceftiofur by applying a safety factor of 100 to the NOEL of 30 mg/kg bw/day in the repeated-dose toxicity studies in rats and dogs.

12. Data on the activity of ceftiofur and its major metabolite desfuroylceftiofur towards 83 strains from 10 genera of bacteria of human gut origin were provided at inoculum densities of $10^5$ and $10^7$ cfu/ml. Data were also provided for the metabolite desfuroylceftiofur cysteine disulphide against 71 strains of bacteria. Ceftiofur was the most microbiologically active substance tested, the metabolites were approximately 16 to 32 times less active. The modal $MIC_{50}$ for the most sensitive strains of human gut flora (*Escherichia coli*, *Lactobacillus* and *Clostridium*) was estimated as 2.0 µg/ml.

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

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

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

$$\text{ADI} = \frac{\dfrac{2 \times 2}{1} \times 150}{0.05 \times 10 \times 60} = 20 \text{ µg/kg bw i.e. } = 1200 \text{ µg/person}$$

The following assumptions were made:

- CF1 = 1 because the modal $MIC_{50}$ of the most sensitive strains was used,
- CF2 = 2 to account for inoculum density effects; no data were provided for possible effects of pH,
- 150 g was the weight of the daily faecal bolus,
- 2.0 µg/ml is the modal $MIC_{50}$ of the most sensitive strains of human gut flora (*Escherichia coli*, *Lactobacillus* and *Clostridium*),
- A factor of 0.05 is used for the assessment of the remaining microbiological activity in the human intestine, based on more than 95% degradation capacity of the human faeces for ceftiofur,
- A factor of 10 covers possible variations in human degradation capacity,
- 60 kg for the human body weight.

14. All milk samples from cattle treated according to the recommended intramuscular dosing regime gave negative results in a number of screening assays including the Delvotest-P. In a special study, milk samples were taken from a cow given 5 intramuscular treatments with 8.8 mg/kg bw per day. The total incurred ceftiofur-related residues (up to 180 µg-equivalents/kg) and milk samples spiked with concentrations of up to 75 µg/kg desfuroylceftiofur cysteine disulphide were examined for their effects on starter cultures. A yoghurt starter culture (based on *Lactobacillus bulgaricus* and *Streptococcus thermophilus*), 3 Italian cheese starter cultures (based on *Streptococcus thermophilus* or *Lactobacillus helveticus*) and 2 buttermilk/sour cream starter cultures were unaffected. However, in a study with parent ceftiofur most of the cultures were adversely affected at the current MRL of 100 µg/kg. Clotting time for one of the cheese starter cultures was inhibited at a concentration of only 7.8 µg/kg of parent ceftiofur.

15. Pharmacokinetic studies were carried out in pigs.

    When pigs were given 3 intramuscular doses of 5.2 mg/kg bw/day $^{14}$C-ceftiofur at 24 hour intervals, the urine and faeces collected up to 12 hours after the last dose contained 62% and 11% of the administered dose, respectively. The metabolites present in urine were: 3,3' -desfuroylceftiofur disulphide dimer (more than 10% of total residues) and unmetabolised ceftiofur (more than 10%), further metabolites were found at concentrations less than or equal to 5% (desfuroylceftiofur, ceftiofur sulphoxide cysteine thioester and unknown polar metabolites B and 1). Desfuroylceftiofur cysteine disulphide was identified as the major unbound metabolite in porcine kidney (12% of total residues). In this study, the pigs were slaughtered 12 hours after the last dose and residues in tissues were determined by liquid scintillation counting (LSC) (limit of detection = 10 µg/kg). The mean total residues were 1550 µg equivalents/kg in liver, 4470 µg equivalents/kg in kidney, 760 µg equivalents/kg in muscle, 1490 µg equivalents/kg in fat and 2900 µg equivalents/kg in injection site muscle tissue, respectively.

    Twenty-four pigs were given intramuscular injections of 3 mg/kg bw per day of ceftiofur on 3 consecutive days. The pigs were slaughtered 12 hours or 2 days after the last dose. In tissues, all residues with an intact β-lactam ring were determined as desfuroylceftiofur equivalents by using a modification of the proposed routine analytical method. Mean residue concentrations were 1510 and less than 200 µg/kg in liver, 2170 and less than 200 µg/kg in kidney, 370 and 200 µg/kg in muscle, 400 and less than 200 µg/kg in fat and 200 and less than 200 µg/kg in injection site muscle, respectively.

    In another study, pigs were given intramuscular injections of 3 mg ceftiofur/kg bw/day for 3 days. The pigs were slaughtered (6 animals/time point) 12 hours and 5 days after the last dose. Mean tissue residue concentrations were 590 and less than 100 µg/kg in liver, 1190 and less than 100 µg/kg in kidney, 250 and less than 30 µg/kg in muscle, 400 and less than 100 µg/kg in skin+fat and 1320 and 40 µg/kg in injection site muscle, respectively.

16. Pharmacokinetic studies were carried out in cattle.

    In cattle intramuscularly dosed with 2 mg/kg bw/day $^{14}$C-ceftiofur 99% of the plasma residues consisted of desfuroylceftiofur and desfuroylceftiofur thioacetone (1:4 w/w, respectively) 0.5 to 8 hours after dosing.

    In cattle given 4 intramuscular injections of 11 mg/kg bw every 4 hours (total dose 44 mg ceftiofur/kg bw/day) and killed 4 hours after the last dose, the majority of the residue content of kidney (78%) and liver (73%) samples was detected as derivatised desfuroylceftiofur acetamide by the proposed routine analytical method.

    In veal calves, intramuscular doses of 1 mg/kg bw/day ceftiofur on 5 days resulted in a plasma $C_{max}$ of 4.34 µg/ml, $T_{max}$ of 2.4 hours, $AUC_{0-24h}$ of 42 µg/ml/hour, a half-life of elimination ($t_{½}$) of 10 hours and a mean residence time (MRT) of 13 hours.

In another study in which lactating cows were given intramuscular doses of 2.3 mg $^{14}$C-ceftiofur/kg bw/day on 5 consecutive days urine, faeces and milk residue elimination accounted for 63, 36 and 0.15% of the dose administered, respectively.

17. Dairy cows were given intramuscular injections of 2.2 mg/kg bw per day of $^{14}$C-ceftiofur on 5 consecutive days after the last dose the cows were slaughtered. Milk was collected at 12 and 24 hours after each dose for up to 5 days after the last dose. Highest residues in milk were found 12 hours after the fifth dose (mean 115 µg equivalents/kg) and depleted to mean values of 60 and 20 µg-equivalents/kg 24 hours and 48 hours after the fifth dose. Approximately 65% of the residues in milk were covalently bound to milk protein, mostly as desfuroylceftiofur. The major free metabolite was desfuroylceftiofur cysteine disulphide. Unmetabolised ceftiofur was not detected in milk samples. Analysis of the milk samples using the proposed routine analytical method indicated that approximately 60% of the $^{14}$C-residues in milk were determined by this method. The mean total residue concentrations in tissues were 380, 2500, 80, 80 and 6720 µg equivalents/kg in liver, kidney, muscle, fat and injection site muscle, respectively.

    In another study, dairy cows were given intramuscular injections of 2.2 mg/kg bw/day of ceftiofur on 5 consecutive days. Milk samples were taken at 2-hour intervals for up to 14 hours after the last dose. Residues in milk were determined using the proposed routine analytical method. The highest residues were found in samples taken 10 hours after the last dose (71 µg/kg) and depleted to approximately 40 µg/kg at 12 hours after the last dose.

    Cattle were given intramuscular injections of 2.2 mg $^{14}$C-ceftiofur/kg bw/day on 3 consecutive days and slaughtered 8 hours, 3, 21 and 39 days, respectively, after the last dose. Total residue concentrations of 1294, 250, 60 and 11 µg equivalents/kg were found in liver, 3508, 953, 159 and 23 µg equivalents/kg in kidney, 208, 20, less than 10 and less than 10 µg equivalents/kg in muscle, 324, 37, less than 10 and less than 10 µg equivalents/kg in fat and 3924, 766, 255 and 30 µg equivalents/kg in injection site muscle, respectively. The limit of detection for the liquid scintillation counting (LSC) method was reported to have been 10 µg/kg for all tissues.

    In another study, six cattle were given intramuscular injections of 2.2 mg $^{14}$C-ceftiofur/kg bw/day for 5 days. All 6 animals were killed 8 hours after the last dose. Mean total residue concentrations were 1350 µg equivalents/kg in liver, 5540 µg equivalents/kg in kidney, 230 µg equivalents in muscle, 550 µg equivalents/kg in fat. Residues at the 5th (last) injection site ranged from 1377 to 10543 µg equivalents/kg.

18. A validated routine analytical method was presented in an ISO format and based on HPLC with UV detection. Free and bound metabolites of ceftiofur, retaining a β-lactam ring, are derivatised to a common desfuroylceftiofur acetamide derivative and measured as desfuroylceftiofur equivalents. The limits of quantification of the method were less than or equal to 100 µg/kg for all bovine and porcine tissues and 50 µg/kg for bovine milk. There was no evidence of interference in this assay from residues of other β-lactam ring containing compounds.

**Conclusions and recommendation**

Having considered that:

- a microbiological ADI of 20 µg/kg bw/day, i.e. 1200 µg/person, was 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,
- a validated routine analytical method is available for determination of residues in porcine and bovine tissues and in bovine milk,
- the ratio of marker to total residue in the relevant tissues of the target species was not determined, however, this information was considered unnecessary due to the marker residue accounting for all residues with anti-microbiological activity,
- following parenteral administration of ceftiofur to dairy cattle, unmetabolised ceftiofur was usually not detected in milk and dairy starter cultures were not affected at the proposed MRL for bovine milk; however, there was no information concerning the composition of residues following intramammary administration;

the Committee recommends the inclusion of ceftiofur in Annex I 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 |
|---|---|---|---|---|---|
| Ceftiofur | Sum of all residues retaining the betalactam structure expressed as desfuroylceftiofur | Bovine | 1000 µg/kg<br>2000 µg/kg<br>2000 µg/kg<br>6000 µg/kg | Muscle<br>Fat<br>Liver<br>Kidney | |
| | | | 100 µg/kg | Milk | Not for intramammary use |
| | | Porcine | 1000 µg/kg<br>2000 µg/kg<br>2000 µg/kg<br>6000 µg/kg | Muscle<br>Fat<br>Liver<br>Kidney | |

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.