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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CEFAZOLIN

SUMMARY REPORT

1. Cefazolin is a first generation cephalosporin intended for treatment of mastitis in lactating cows and for treatment at drying off by intramammary administration. Cefazolin has antimicrobial activity mainly against gram-positive bacteria including the common mastitis pathogens.

2. Oral absorption of cefazolin is poor, i.e. approximately 5% in rats. After intramammary administration absorption also seems to be poor and only low concentrations are seen in plasma and tissues. The volume of distribution for cefazolin is approximately 0.2 l/kg bw.

3. Metabolism of cefazolin is very limited in most of the animal species tested and in man. After parenteral administration of cefazolin nearly 100% is excreted unchanged in urine within 24 hours in man, dog and horse. No major metabolites seem to occur.

4. Cefazolin is of very low acute oral and parenteral toxicity. The acute oral LD_{50} was greater than 11000 mg/kg bw in both rats and mice. The intravenous LD_{50} values in rats and mice were both greater than 2000 mg/kg bw.

5. Repeated-dose toxicity studies of up to six months duration were carried out in rats and dogs in most cases using subcutaneous administration. Apart from local toxicity at the injection site most effects occurred in the intestinal tract resulting in diarrhoea and enlargement of the caecum. In a 90 day subcutaneous study in rat 250 mg/kg bw only caused reaction at the injection site and caecal enlargement (2/20 animals). In a 3 and 6 month subcutaneous study in dogs, 250 mg/kg and 125 mg/kg, respectively, were without effect. An oral 90 day study was performed in rats. Diarrhoea and abdominal swelling was observed together with caecal enlargement. The NOEL in this study was 20 mg/kg bw.

   Cefazolin has been shown not to be nephrotoxic.

6. The effect of cefazolin on reproduction was investigated in segment I, II and III studies in rats, mice and rabbits. Cefazolin administered subcutaneously did not affect male or female fertility, or the postnatal development of the offspring in rats. Cefazolin was not teratogenic in mice and rabbits after subcutaneous dosing. Oral administration of cefazolin to pregnant rats caused maternal toxicity (diarrhoea and caecal enlargement) and reduction in litter size and foetal weight, but showed no specific effects on the foetuses. The NOEL for dams (and foetuses) was 10 mg/kg bw.

7. Cefazolin was shown to be non-mutagenic in the Ames test, the mouse lymphoma test and the mouse micronucleus test.

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

9. In a guinea pig maximisation test cefazolin did not cause sensitization.

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10. Data on the activity of cefazolin towards 10 bacterial species (10-20 strains per species) from the human gut flora were provided. Based on the most sensitive species a NOEL of 2.0 mg/l can be set for the antimicrobial effect of cefazolin on the human gut flora.

11. The inhibitory effect of cefazolin on microorganisms used in the dairy industry was examined using 8 different strains. No significant inhibition was observed on the activity of the most sensitive strain of bacterial cultures commonly used in the dairy industry at concentrations up to 0.05 mg/l.

12. Cefazolin has been used for more than 20 years in the human clinic, including most European countries and the USA. Cefazolin is administered parenterally in doses of 3-4 g per day.

13. For the calculation of the microbiological ADI, the formula recommended by the CVMP was used using the following assumptions:

    - 2.0 mg/l is the modal MIC_{50} for the most sensitive species of human gut flora.
    - a factor of 2 is used to adjust for the effect of bacterial density.
    - all cefazolin ingested stays in the intestinal tract in a microbiologically active form.
    - human faecal bolus = 150 ml; human body weight = 60 kg.

$$ADI = \frac{\dfrac{\text{geometric mean } MIC_{50} \times CF2}{} \text{ (µg/ml) } \times \text{ daily faecal bolus (0.15 l)}}{\dfrac{CF1}{\text{fraction of an oral dose available for microorganisms} \times \text{ weight of human (60 kg)}}} \text{ (µg/kg bw)}$$

$$ADI = \frac{2.0 \times 2}{1} \times \frac{150}{60} = 10 \text{ µg/kg bw} = 600 \text{ µg/person}$$

14. A toxicological ADI of 0.1 mg/kg bw (6000 µg/person) was calculated by applying a safety factor of 100 to the NOEL for maternal toxicity of 10 mg/kg bw in the oral rat teratogenicity study.

15. In depletion studies carried out in lactating cows it was shown that after the recommended treatment with the commercial formulation intended for lactating cows (two treatments of all four quarters at two successive milkings), residues in composite milk samples were below 50 µg/l at the 7th milking and below 25 µg/l at the 8th milking after the last treatment. In tissues cefazolin could be found only in kidney and liver at 3 hours after last treatment and after 24 hours only in the kidney, and at very low concentrations.

16. In depletion studies carried out in cows at drying off it was shown that after the recommended treatment with the commercial formulation intended for dry cow treatment (single treatment of all four quarters at drying off), residues in udder secretion declined from 3500-16500 µg/l at day 7 after treatment to 40-1400 µg/l at day 14. On day 21 the concentrations varied from below the limit of quantification of the analytical method (25 µg/l) to 600 µg/l - except for a concentration of 1400 µg/l in the secrete from one of 12 quarters. No residues were detected in milk sampled at the 1st and 2nd milking after calving from cows treated at drying off 28-40 days before calving. No residues were detected in edible tissues collected 21 days after drying off treatment.

17. Residues of cefazolin in milk and bovine tissues can be monitored using an HPLC analytical method. The method has been appropriately validated for milk (limit of quantification (LOQ) 25 µg/l), for muscle, liver and kidney (LOQ 50 µg/kg), and for fat (LOQ 250 µg/kg). The coefficient of variation at 50 µg per kg was 16%; accuracy of the method was not investigated at intermediate concentrations). The specificity of the method with respect to other cephalosporin-ß-lactam antibiotics is acceptable. The method has been adequately described.

18. Based on the antimicrobial effect of cefazolin on bacteria commonly used within the dairy industry, the MRL for milk can be set at 50 µg/kg.

19. Since the ADI for cefazolin has been set at 0-600 µg/person, an MRL for milk of 50 µg/kg leaves room for a maximum of total residues of 525 µg in the other edible tissues contained in the EU-recognised food package. When composed of tissues sampled from lactating cows 24 hours after recommended treatment the contents in the food package stemming from these tissues will amount to only a few percent of this value. Although data concerning the contents in edible tissues during the first few days immediately following treatment at drying off have not been presented, the general pharmacokinetic characteristics of systemically absorbed cefazolin indicate that the residues in all relevant tissues will be even lower following drying off treatment than after treatment during lactation. Therefore, it is proposed that, as regards edible bovine tissues except milk, cefazolin be placed in Annex II. However, this applies only to intramammary administration.

Recommendations and conclusion

- having set an ADI of 10 µg/kg bw,
- having considered the residue depletion of cefazolin after intramammary administration,
- having checked the availability of a validated analytical method for residues monitoring purposes;

the Committee recommends the establishment of MRLs for cefazolin in milk and its inclusion 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 tissue	Other provisions
Cefazolin	Cefazolin	Bovine	50 µg/kg	Milk	

and for other tissues except milk, the inclusion of cefazolin in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table :

Pharmacologically active substance(s)	Animal species	Other provisions
Cefazolin	Bovine	For intramammary use only and for all tissues except milk