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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CEFOPERAZONE

SUMMARY REPORT (1)

1. Cefoperazone is a third generation semi-synthetic cephalosporin with a broad spectrum of activity against the majority of aerobic and anaerobic gram-positive and gram-negative pathogenic bacteria *in vitro*. Cefoperazone sodium and -dihydrate are used for intramammary treatment of clinical mastitis in lactating cattle. Dose levels used are 250 mg cefoperazone sodium per infected quarter once or 100 mg cefoperazone dihydrate per infected quarter twice with a dosing interval of 24 hours.

2. Pharmacological studies with cefoperazone doses in the range of 250 to 6000 mg/kg bw administered intravenously or subcutaneously in mice, rats, rabbits, dogs and guinea pigs, elicited effects on several pharmacological parameters, such as central nervous system function, electro-encephalogram, autonomic functions (respiration, cardiovascular system), haematological parameters, smooth muscle function, renal function, secretion of gastric juice and bile. Cefoperazone in general had effects comparable to those of other cephalosporins, but its cholinergic effects were minor. Because the extremely high doses at which these effects were found, the reported results had no consequence for the assessment of the ADI.

3. Plasma elimination half-life after parenteral administration in mouse, rat, rabbit, dog, monkey and man was 8 to 120 minutes. No significant differences were seen in plasma elimination half-life between intramuscular, intravenous, subcutaneous and intraperitoneal administration. Large species differences occurred in protein binding (86 to 89% in human, monkey and rabbit versus 15 to 48% in dog, rat and mouse). Cefoperazone (radiolabelled and unlabelled) was distributed rapidly (within 15 minutes in mice) throughout the whole body with short-lived (less than 4 to 6 hours) high concentrations in liver and kidney and longer lasting (greater than 4 to 6 hours) high concentrations in urine and intestinal contents. Cefoperazone was hardly found in the central nervous system. Cefoperazone was eliminated quickly; within one day concentrations in tissues and organs were no longer detectable, with the exception of intestinal content and urine. Bioavailability was low (about 10%) after oral administration of 50 mg/kg bw in rats.

4. Cefoperazone was excreted via urine, faeces and bile. The excretion pattern differed between species. After parenteral administration, rats excreted the major part in bile (60 to 84% versus 14 to 39% in urine), while rabbits, dogs and monkeys excreted a larger part in urine (49 to 57%) than in bile (8 to 21%). After multiple dosage in rats of 50 mg/kg bw during 7 days, 100% was recovered in urine and faeces at two days after the last dosage. After oral administration in rats about 9% was excreted by biliary, 2% by urinary route and about 96% was excreted with faeces.

5. Cefoperazone was little metabolised in mouse, rat, rabbit, dog, monkey and man. In the urine of mice, rats, rabbits, dogs and monkeys, no antibacterial components other than the parent compound were found. In urine and bile the metabolites T-1551-A and T-1551-F were found in small amounts (less than 5%). In faeces, the percentage of metabolites was higher. T-1551-F was present in 9% and 17% for rat and mouse, respectively. Furthermore, the metabolite T 1551-D was only present in faeces, 9% and 29% for rat and mouse, respectively. This metabolite was probably a product of hydrolysis by intestinal bacterial ß-lactamases. This metabolite was shown virtually not to be absorbed after intraduodenal administration of the radiolabelled metabolite in rats.

6. Acute oral toxicity in rats and mice was low (LD_{50} greater than 12 g/kg bw). Parenteral LD_{50} values in these species were approximately 4 to 10 g/kg bw.

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7. No oral repeated dose toxicity studies were available. Five repeated dose toxicity studies were carried out in rats: 1 month and 6 month subcutaneous studies with doses ranging from 125 to 2 000 mg/kg bw, and 1, 3 and 6 month intraperitoneal studies with doses ranging from 500 to 4 000 mg/kg bw. Adverse effects were observed at all examined dose levels. Effects found at the lowest dose levels were slight growth inhibition, increased fluidity of intestinal contents, caecum enlargement and occasional loose stools. Five repeated dose toxicity studies were carried out in dogs: one 35-day subcutaneous study with doses ranging from 250 to 1 000 mg/kg bw, and 3-month and 6-month intramuscular studies with doses ranging from 100 to 500 mg/kg bw. Three intravenous toxicity studies of 1, 3 and 6 months were conducted with doses of 75 to 4 000 mg/kg bw. For intravenously treated dogs, a NOEL of 75 mg/kg bw was retained.

   In a one month intramuscular study in monkeys with doses of 0, 100, 200 and 400 mg/kg bw, mainly local tissue damage at the site of injection and haematological effects, probably related to this tissue damage, were found at doses of 200 and 400 mg/kg bw.

   In general, toxicity found at repeated high parenteral doses in the different experiments included local injection site tissue damage, haemolytic anaemia and positive reaction in a haemagglutination test for antibodies (Coombs reaction) decreased thymus weight, nephrotoxicity and gastrointestinal effects. Caecum enlargement in rats was explained by the antibiotic effect on intestinal microflora.

8. No drug related effects on reproduction were found in a two-generation reproduction study and a peri-/postnatal reproduction study in rats examining subcutaneous doses of 125 to 1 000 mg/kg bw.

9. No oral teratogenicity studies were available. Teratogenicity after intravenous administration was studied in rhesus monkeys (0, 50, 100, 400 mg/kg bw), mice (0, 125, 250, 500, 1 000 mg/kg bw) and rats (0, 125, 250, 500, 1 000 mg/kg bw). No teratogenic or foetotoxic effects were found in these experiments. Maternal toxicity was found at all examined dose levels in the rhesus monkeys (gastrointestinal effects, i.e. loose stools) and rats (caecal enlargement) and not at any dose level in the mouse study.

10. No mutagenicity of cefoperazone and its (separately tested) metabolites was observed in reverse mutation tests in *Escherichia coli* and *Salmonella typhimurium*, in a *Bacillus subtilis* recombination test and in an intramuscular *Salmonella typhimurium* host mediated assay in mice. An intravenous dominant lethal test in mice and an intravenous bone marrow cytogenetic test in male mice were also negative.

11. No carcinogenicity studies were provided. Since there was no indication from the repeated dose toxicity studies that cefoperazone causes preneoplastic lesions, the mutagenicity tests were negative and β-lactams in general are not mutagenic or carcinogenic, carcinogenicity data are not needed.

12. Immunotoxicity tests (rabbits, guinea pigs, mice, rats) showed that cefoperazone was hardly antigenic, alone or in combination with an adjuvant. Specific antibodies were formed in rabbit serum after intracutaneous injection with drug-bovine serum albumin coupled antigen (BSA); haemagglutination tests showed that their titer was lower than that of two other cephalosporins in the same experiment and that there was no cross-reactivity with other tested cephalosporins. Passive cutaneous anaphylaxis could be induced with cefoperazone-BSA complex in guinea pigs injected intracutaneously with rabbit anti-cefoperazone-BSA serum. This reaction was inhibited by cefoperazone but not some other tested cephalosporins. No antibody production was found in mice, no active or passive anaphylactic shock could be induced with intravenously administered cefoperazone in guinea-pigs, a weak Coombs' reaction was found with human serum. From the provided data it can be concluded that cefoperazone is not more antigenic than other β-lactam antibiotics.

13. Repeated dose toxicity studies were provided following parenteral administration. Because of the low oral bioavailability of cefoperazone, it was acceptable to base the toxicological ADI on a NOEL from a parenteral study. The lowest intravenous dose in dogs in which no drug related effect was found was 75 mg/kg bw. A toxicological ADI based on this latter dose and a safety factor of 100 would be 0.75 mg/kg bw (45 mg/person).

1. In vitro MIC_{50} values were available for ten relevant genera/species characteristic for the human gut flora at inoculum levels of 10^6 and 10^9 cfu/ml. The lowest MIC_{50} values at these two densities were 0.031 and 0.063 µg/ml (both for *Bifidobacterium*) and the geometric mean MIC_{50} values were 0.93 and 2.38 µg/ml (tenth percentile: 0.85 µg/ml). These data as well as those in available literature showed that at increasing bacterial density the MIC_{50} values of cefoperazone increased and that an increase with at least a factor 2 of the MIC_{50} found at 10^9 cfu/ml could be expected at the *in vivo* occurring density. The effect of incubation under simulated intestinal conditions on the antimicrobial activity of cefoperazone against three strains of *Escherichia coli* was examined. Only a small decrease of activity was found under these conditions, at most with a factor 2. Available literature data indicated that resistance of bacteria against cefoperazone may be due to chromosomal as well as plasmidic mechanisms.

1. Inhibition of dairy starters by cefoperazone was examined at sub-MIC concentrations in four dairy starter cultures, selected from a range of 22 starter organisms for which the MIC values were determined in standard media and in milk. The effect of cefoperazone on total tritrable acidity, bacterial growth, clotting of milk and pH of the four selected starters incubated in milk were determined. The data showed that at concentrations of 250 µg/l and higher inhibitory effects on dairy organisms could be expected. However, the data were not suitable to derive a clear concentration without effect. Extrapolation of the relation between the effect on final pH and cefoperazone concentration resulted in an estimated concentration with negligible effect of 50 µg/l.

16. Literature on observations in humans was provided. Known side effects (after parenteral use of doses of 1 to 8 g per 12 hours) are skin rash, fever, diarrhoea, transient elevation of serum transaminases and transient eosinophilia. The general pattern of side effects of cefoperazone is similar to that of other cephalosporins, but intestinal effects like diarrhoea occur more frequently with cefoperazone than with other cephalosporins. The latter effect can be explained by its high excretion in bile and faeces and little metabolism resulting in effects on the intestinal flora.

17. The microbiological ADI was calculated as follows:

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

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

$$\text{ADI} = \frac{\dfrac{0.85 \times 4}{3} \times 150}{1 \times 60} = 2.8 \text{ µg/kg bw i.e. } 170 \text{ µg/person}$$

The following assumptions were made:

- CF1 = 3 (the intermediate value between the values 1 and 5 for chromosomal and plasmidic resistance mechanisms);
- CF2 = 4 (a factor 2 to correct for inoculum density and a factor ot 2 to correct for the effect of intestinal conditions on the microbiological activity of cefoperazone);
- 150 g was the weight of the daily faecal bolus;
- the fraction of an oral dose available for microorganisms was assumed to be 1;
- geometric mean MIC_{50} (10^{th} percentile) = 0.85 µg/ml.

Since the microbiological ADI was lower than the toxicological ADI the microbiological ADI was established as the overall ADI.

18. In residue studies serum and urinary concentrations were measured. After intramammary administration of 250 mg cefoperazone as the sodium salt in four quarters of lactating cattle, low blood serum levels (0.03 to 0.68 µg/ml, using a microbiological method with a detection limit of 0.02 µg/ml) were found around 8 to 9 hours after administration. In a four day period after administration about 10% of the dose was excreted by urinary route.

18. A radiolabel residue study was provided in 28 lactating cattle, treated once with 250 mg ^{14}C-Cefoperazone per quarter. Four cows per time point were slaughtered at 12, 24, 72, 96 and 120 hours after administration and eight cows at 168 hours after administration. Total radioactivity in muscle, fat tissue, liver, kidney and mammary tissue was determined. Cefoperazone concentrations were determined by HPLC-MS/MS (the proposed monitoring method). Mean total radioactive residues in tissues were 123 µg-equivalents/kg in liver, 426 µg-equivalents/kg in kidney, 24 µg-equivalents/kg in muscle, 50 µg-equivalents/kg in fat and 10 816 µg-equivalents/kg in udder tissue at 12 hours post dose, and at 24 hours post dose 34 µg-equivalents/kg in liver, 278 µg-equivalents/kg in kidney, 9 µg-equivalents/kg in muscle, 21 µg-equivalents/kg in fat and 1 421 µg-equivalents/kg in udder tissue. At later time points residue concentrations were lower. Ranges of cefoperazone concentrations were less than 42 to 145 µg/kg in liver, 165 to 320 µg/kg in kidney, less than 18 µg/kg (limit of quantification) in muscle and less than 16 µg/kg (limit of quantification) to 17 µg/kg in fat at 12 hours post dose, and at 24 hours post dose, less than 39 µg/kg (limit of quantification) to less than 61 µg/kg (liver), 33 to 73 µg/kg (kidney), much less than 18 µg/kg (limit of quantification) in muscle and less than 16 µg/kg (limit of quantification) in fat. At later time points only incidentally low detectable residue concentrations were found. Mean cefoperazone concentrations in udder tissue were 17 500 µg/kg at 12 hours and 1 890 µg/kg at 24 hours.

20. A radiolabel residue study was provided in 28 lactating cattle, treated once with 250 mg ^{14}C-Cefoperazone per quarter. Milk samples were collected up to 144 hours post dose. Concentrations of total radioactivity were measured at all milkings in all animals. At 12 hours post dose total residue concentrations were in the range of about 55 000 to 116 000 µg-equivalents/kg, at 24 hours they were about 3 000 to 30 000 µg-equivalents/kg, thereafter they decreased to 6 to 204 µg-equivalents/kg at 60 hours and less than 1 to 2 µg-equivalents/kg at 144 hours. In general, concentrations in low yielding cows were higher than those in high yielding cows. Concentrations of the marker cefoperazone (HPLC-MS/MS, proposed monitoring method) were determined only up to 60 hours post dose in four low yielding and four high yielding cows. The limit of quantification was 17 µg/kg. At 12 hours post dose a range of about 62 000 to 138 000 µg/kg was found, at 24 hours 44 000 to 19 000 µg/kg, decreasing to less than the limit of quantification to 92 µg/kg at 60 hours post dose and less than the limit of quantification at 84 hours. The ratio of marker to total residue was variable, but high (up to 100%) during the first four milkings after administration. At the 5th milking (mean marker residue concentration about 30 µg/kg) it was in the range 55 to 80%, and at later milkings it could not be determined due to too low marker residue concentrations.

21. A non-radiolabelled residue study was provided in which no concentrations of cefoperazone detectable with bioassays were found (less than 25 µg/kg) in liver, kidney, muscle and fat tissue of lactating cows slaughtered 1, 3 and 5 days after intramammary administration of 250 mg cefoperazone as the sodium salt in each quarter. In udder tissue only on the first day detectable concentrations (250 to 400 µg/kg) were found.

22. In milk the concentration in the first milking after intramammary administration of 250 mg non-radiolabelled cefoperazone as the sodium salt in each quarter as determined with bioassay methods was in the range 8 000 to 330 000 µg/kg (the high value was found in a quarter producing 250 ml of milk). Hereafter, concentrations decreased to about 30 µg/kg at around the 5th to the 8th milking and to concentrations of 10 µg/kg at around the 7th to the 9th milking. Similar concentrations were found after one or two doses of 100 mg cefoperazone in all quarters as the dihydrate.

23. For monitoring of the marker cefoperazone in milk an HPLC-MS/MS method was provided. It was described in ISO 78/2 format. The proposed limit of quantification was 7.0 μg/kg. The limit of detection was 5.6 μg/kg. The method was not satisfactorily validated. In particular, accuracy and precision were very variable from day to day, possibly because of signal suppression caused by interaction with matrix components

Conclusions and recommendation

Having considered:

- the microbiological ADI of 2.8 μg/kg bw,
- the microbiological activity of the parent compound,
- the ratio of marker to total residues,
- that compared to the ADI (170 μg/person) the maximum residue from edible tissues at 12 hours after administration (30 μg/person/day) was very low,
- the parent compound as the marker residue,
- that the validation of the analytical method proposed for residues monitoring purposes in milk is not yet completely satisfactory;

the Committee for Veterinary Medicinal Products recommends the inclusion of cefoperazone for bovine milk 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
Cefoperazone	Cefoperazone	Bovine	50 μg/kg	Milk	Provisional MRL expires on 1.1.2001

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

Pharmacologically active substance(s)	Animal species	Other provisions
Cefoperazone	Bovine	For intramammary use in lactating cows only and for all tissues except milk.

Based on this MRL value for milk the maximum daily intake will represent 62% of the ADI.

LIST OF QUESTIONS:

1. The applicant should improve the performance of the method for milk, in particular with respect to accuracy and precision. More data should be provided on the stability of residues during storage and at sample preparation and on the ruggedness of the method. Further validation data should be provided in order to show that the method is in accordance with the recommendations of Volume VI of the Rules Governing Medicinal Products in the European Community.