

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CEFAPIRIN

SUMMARY REPORT (1)

1. Cefapirin is a first generation semi-synthetic cephalosporin with a broad spectrum of activity against both gram-positive and gram-negative bacteria *in vitro*. The benzathine and sodium salt are used for intramammary treatment of mastitis in dry and lactating cows in recommended doses of 200-300 mg per quarter. The benzathine salt is used for intrauterine treatment of subacute and chronic endometritis at a recommended dose of 500 mg/cow. The recommended dose for parenteral (intravenous, intramuscular, subcutaneous) sodium cefapirin treatment of infections in cattle, sheep, goats and pigs is 10 mg/kg. The relative activity of sodium cefapirin is 855 µg/mg and that of benzathine cefapirin is 700 µg/mg.
2. Cefapirin exerts a bactericidal action due to inhibition of bacterial cell-wall synthesis, mediated by binding to one or more Penicillin Binding Proteins located under the cell wall of susceptible bacteria. The resulting high osmotic pressure in the cell causes rupture of the cytoplasmic membrane. Cefapirin's bactericidal potential resembles that of most penicillins and cephalosporins. It is not inactivated by β-lactamase-producing staphylococci. No information on other pharmacodynamic actions was provided. Resistance mechanisms for cephalosporins in general include both chromosomal and plasmid-mediated transference.
3. The pharmacokinetics of sodium cefapirin after parenteral administration has been studied in mice, rats and dogs. Cefapirin was metabolised into desacetylcefapirin, the rate and extent of metabolism decreasing from rodents to dogs. In the species examined, the plasma elimination half life of cefapirin and desacetylcefapirin was 0.4-0.9 hours. In intravenously treated dogs, cefapirin rapidly distributed over the body with a half life of 0.12 hours. Total volume of distribution was approximately 32%, and approximately 98% of the administered dose was recovered within 8 hours in urine as cefapirin and its desacetyl derivative in a 1:2 ratio. In intravenously treated humans less desacetylcefapirin was found in plasma and urine compared to dogs and rodents. The plasma half lives of cefapirin and desacetylcefapirin were 0.5 and 0.43 hours. At 6 hours post injection approximately 48% of the dose had been excreted in urine as parent compound and 45% as desacetylcefapirin.
4. Due to its acid susceptibility, bioavailability of cefapirin after oral administration is claimed to be very low. No supporting data have been submitted, but several studies point to a low oral bioavailability of cefapirin. Cefapirin administered orally at 20-times parenteral dosages in mice, resulted in lower serum-cefapirin levels than after parenteral administration. Cefapirin-induced toxicity after oral administration is low.
5. Cefapirin has low acute toxicity. The oral LD₅₀ in male rats was 14000 mg/kg bw. Parenteral LD₅₀ values ranged from 2500 mg/kg bw, after intravenous administration in male and female dogs, to 8400 mg/kg bw after intraperitoneal administration in rats. The main effects observed following administration were ataxia and convulsions.

6. Intraperitoneal repeated-dose toxicity studies of 9 weeks and 6 months with sodium cefapirin were carried out in rats with doses of 0, 200, 500 and 1000 mg/kg bw/day. In the 9 weeks study, a slight stimulation of male bodyweight was found at all dose levels without a clear dose effect relation. In the 6 month study, a decreased bodyweight gain was observed in all females and in males in the high dose only. In one male and seven female rats a mild dose related anemia associated with reticulocytosis was found.
7. Three parenteral repeated-dose toxicity studies with sodium cefapirin were carried out in dogs. In a one month intravenous study with doses of 0, 200 and 500 mg/kg bw/day, no treatment-related effects were recorded. In a 10-week intramuscular study in dogs with doses of 0, 100, 200 and 400 mg/kg bw/day, the relative kidney weight was elevated in dogs of both sexes receiving 400 mg/kg bw/day. No effects were found at the 2 lower dose levels. In a 6-month intramuscular study with doses of 0, 100, 200 and 400 mg/kg bw/day, severe anemia was observed in some of the animals at all dose levels. This effect may be explained as a direct effect of cefapirin on dog red blood cell membranes. Several lines of evidence indicate that an immunological component is not likely to play a role in the observed anemia.
8. In three months oral toxicity studies in rats and dogs only one dose of sodium cefapirin was tested (20 mg/kg bw). No clear treatment-related adverse effects were observed. Therefore the oral NOEL was 20 mg cefapirin/kg bw/day.
9. Three tolerance studies were carried out in cows. In the first study, lactating cows received 500 mg sodium cefapirin by infusion in each quarter of the udder. In the second study, cows were subjected to intrauterine infusion of 0, 1500, 4500, 7500 mg benzathine cefapirin on three consecutive days. In the third study, the effect of intrauterine infusion of 500 and 1500 mg benzathine cefapirin on a single day was studied. No systemic toxicity relevant for the safety assessment of residues was observed.
10. Sodium cefapirin was tested at subcutaneous doses of 0, 200 and 500 mg/kg bw/day in separate experiments for reproductive toxicity (phase I and III studies in rats), foetotoxicity and teratogenicity (phase II studies in rats and mice). No evidence for reproductive, foetotoxic and/or teratogenic toxicity was observed at doses tested.
11. No two generation study was carried out which is justified because no clear reproduction toxicologic effects were observed in other provided studies.
12. No evidence of mutagenicity was observed in the Ames' Salmonella test, mouse lymphoma assay and oral mouse micronucleus test.
13. No carcinogenicity studies were carried out. This was considered acceptable since no evidence for mutagenicity was observed, no evidence for preneoplastic changes was found in repeated toxicity studies and the cefapirin molecule contains no structural alerts.
14. No special studies concerning immunotoxicity have been carried out. No evidence for immunological effects was found in the repeated dose toxicity studies. In the 6 months intramuscular toxicity study in dogs, evidence of hypersensitivity to cefapirin was specifically examined as a possible explanation for anemia but not found. In humans, the allergic cross-reactivity between penicillins and cephalosporins is low and occurs only in 5% of patients hypersensitive to penicillins. From the information submitted by the applicant, no immunotoxic effects of cefapirin is to be expected. Allergic reactions may occur in patients hypersensitive to penicillins.

15. *In vitro* MIC₅₀ values were available for seven of the ten genera of bacteria considered representative of the human intestinal flora: *Clostridium spp*, *Peptostreptococcus spp*, *Bacteroides spp*, *Fusobacterium spp*, *Proteus spp*, *Escherichia spp*. (*E. coli*), *Streptococcus spp*. For *Clostridium* and *Peptostreptococcus* the tested number of strains was too low. Cefapirin was in particular effective against *Streptococci*, *Fusobacterium* and *Bacteroides* (MIC₅₀-range: 0.032-0.128 æg/ml). Desacetylcefapirin contains less microbiological activity against tested bacteria of normal human gut flora than cefapirin (MIC₅₀-values for *E.coli* and *Proteus mirabilis* were 120 and 34 æg/ml for desacetylcefapirin as compared to 7.6 and 3.9 µg/ml for cefapirin, respectively). Synergistic antimicrobial effects were observed when cefapirin was tested in a number of species of bacteria in combination with its desacetyl metabolite, however synergy was only complete in a minority of tested strains. Although the antimicrobial potency of the metabolite in the examined species was clearly lower than that of the parent compound, the evidence for a synergistic effect, the lack of information about the proportion of the total residue present as the metabolite and the evidence for *in vitro* degradation of the parent compound to the metabolite are reasons to include the metabolite in the marker residue for MRLs based on a microbiological ADI.
16. In the yoghurt inhibitor test, no significant inhibition was observed on the activity of bacterial cultures commonly used in the dairy industry at concentrations up to 0.08 mg/l.
17. The toxicological NOEL of 20 mg/kg bw/day was based on the 3 months rat and dog studies. Because of the inadequate design of these studies, only one dose level was examined, an increased safety factor of 200 was used to derive an ADI of 0.1 mg/kg bw/day, which is equal to 6 mg/day for a 60 kg person.
18. Data were provided on seven strains and genera of bacteria relevant for the establishment of a provisional ADI. The overall geometric mean MIC₅₀ of these bacteria was 0.30 µg/ml.

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

CF1 = 3 (the intermediate value between the values 1 and 5 for chromosomal and plasmidic resistance mechanisms)

CF2 = 4 (based on the effect of inoculum density on MIC values found in a number of bacterial strains)

The fraction of an oral dose available for microorganisms was conservatively set to 1.

$$\text{ADI} = \frac{\frac{\text{geometric mean MIC}_{50} \times (\text{CF2})}{(\text{CF1})} \times \frac{150 \text{ ml}}{60 \text{ kg}}}{\frac{\text{fraction of an oral dose available for microorganisms}}{1}} \quad (\mu\text{g/kg bw})$$

$$\text{ADI} = \frac{0.30 \times 4/3 \times 150}{1 \times 60} = 1.0 \mu\text{g/kg bw} = 0.001 \text{ mg/kg bw} = 0.060 \text{ mg/person}$$

The proposed microbiological ADI is provisional. A final ADI will be set when the applicant has submitted data for the number of strains and bacterial species normally requested by CVMP and for the estimate of CF1, CF2 and the availability of residues to the intestinal flora.

19. A number of plasma and serum pharmacokinetic studies were provided, using different routes of administration. After intramuscular administration of 8.5 mg/kg sodium cefapirin in dairy cows a C_{max} of 14 $\mu\text{g/ml}$ at 30 min and a serum elimination half life of 1 hour were found. Similar results were found for calves. Intramuscular administration of 8.3 mg/kg benzathine cefapirin in dairy cows resulted in a much lower serum C_{max} of 0.33 $\mu\text{g/ml}$ at 11 hours (Area Under the Curve (AUC) was 1 mg/ml/min). Intravenous administration of 8.5 mg/kg sodium cefapirin in dairy cows resulted in an elimination half-life of 1.1 hours and an AUC of 11 mg/l/h. Intrauterine and intramammary administration of benzathine cefapirin in dairy cows caused low plasma cefapirin levels of short duration. Plasma C_{max} was 0.13 $\mu\text{g/ml}$ at 1-3 hours after intrauterine administration of 500 mg cefapirin as benzathine cefapirin. After 24 hours plasma cefapirin levels were below the limit of detection (0.01 $\mu\text{g/ml}$). After intramammary administration of 381 mg benzathine cefapirin in dry cows the plasma C_{max} was 0.025-0.32 $\mu\text{g/ml}$ at 6-12 hours. In foals C_{max} was 21 $\mu\text{g/ml}$ at 10 minutes after intramuscular administration of 20 mg/kg sodium cefapirin; elimination half-life was about 0.7 hours. Intravenous administration of 20 mg/kg sodium cefapirin in adult horses resulted in elimination half-life of 0.9 hours and AUC of 2 mg/ml/min.
20. In dairy cows the average bile concentration was 10.3 $\mu\text{g/g}$ 6 hours after intravenous administration of 8.6 mg/kg sodium cefapirin and 0.3 $\mu\text{g/g}$ at 4.5 hours after intramuscular administration of 8.5 mg/kg benzathine cefapirin. High levels of cefapirin were found in urine. At 6 hours after intravenous administration of 8.6 mg/kg sodium cefapirin in dairy cows, 1700 $\mu\text{g/ml}$ was found in urine. Intrauterine administration of 500 mg cefapirin as benzathine cefapirin resulted in 2-57 $\mu\text{g/ml}$ urine at 1-12 hours; at 72 hours levels were below the limit of detection (0.05 $\mu\text{g/ml}$). Intramammary administration of 381 mg benzathine cefapirin resulted in 2.4-17 $\mu\text{g/ml}$ urine on day 1; from day 7 on levels were below the limit of detection (0.04 $\mu\text{g/ml}$). In horses intramuscular administration of 20 mg/kg sodium cefapirin (5 doses at 12 hour intervals) resulted in a urinary level of 2474 $\mu\text{g/ml}$ after the 5th dose. Inhibition of renal tubular secretion by concurrent administration of probenecid resulted in increased concentrations of cefapirin in blood serum, peritoneal and synovial fluid, endometrium and urine. No cumulative excretion data in bile, faeces and urine were provided. From the available data it can be concluded that in target animals cefapirin is mainly eliminated by the urinary route and to a smaller extent by the biliary route.
21. The tissue distribution of total residues is unknown. Since the available residue concentrations were measured by means of microbiological methods, they represent the microbiologically active residues expressed in terms of cefapirin activity. After intravenous treatment of dairy cows with 7.5 mg/kg sodium cefapirin, residue concentrations at 4.5 hours in kidney were 1.5-6.8 mg/kg, in muscle less than 0.008 to less than 0.024 mg/kg and in liver 0.37 mg/kg. Intramuscular treatment of dairy cows with 8.5 mg/kg benzathine cefapirin, resulted at 4.5 hours in concentrations of 1 to 5 mg/kg in kidney, less than 0.008 to less than 0.024 mg/kg in muscle and less than 0.045 mg/kg in liver. After intrauterine treatment of dairy cows with 500 mg cefapirin as benzathine cefapirin, levels in endometrium were less than 0.10-66 mg/kg at 0-24 hours. In a similar study residue concentrations in kidney, liver, meat, fat and udder tissue cefapirin levels were below the limit of detection on day 2-4. After intramammary administration of 381 mg benzathine cefapirin, cefapirin concentrations in fat, muscle, udder, kidney and liver were below the limit of detection at day 21-42. In piglets after intramuscular treatment with 20 mg/kg sodium cefapirin, no cefapirin levels could be detected in liver, kidney, spleen, lung and muscle (injection site) at 24-120 hours after treatment. In horses after intramuscular treatment with 20 mg/kg sodium cefapirin (4 treatments at 8 hour intervals), synovial and peritoneal tissue levels were 4.6 and 5 mg/kg at 2 hours after 4th treatment; endometrium levels were 2.2 mg/kg at 4 hours after 3rd treatment.
22. Desacetylcefapirin was the most important metabolite of cefapirin in milk. After *in vitro* incubation of cefapirin in milk and in serum desacetylcefapirin was found in milk as well as in serum. Desacetylcefapirin was found *in vivo* in milk at 0-24 hours after intramammary administration (4.6-33.5 mg/l). From 24-60 hours values of 0-3.5 mg/l were found. Evidence was provided that desacetylcefapirin is formed *in vivo* in serum. No studies were done on metabolites in tissues, with other routes of administration and other species.

23. After intramuscular administration of 10 mg/kg sodium cefapirin in lactating cows, 0.03-0.11 mg/l was found in milk at 1-4 hours, levels of 0.01 mg/l were found from 4-8 hours. After subcutaneous administration of 10 mg/kg sodium cefapirin in dairy cows no cefapirin could be detected in milk. After intrauterine treatment of lactating cows with 3 doses of 500 mg cefapirin as benzathine cefapirin, cefapirin concentrations in milk were less than 0.01-0.029 mg/l at first milking; after two or more milkings cefapirin concentrations were below the limit of detection (0.01 mg/l). When a dose of 500 mg was administered, cefapirin concentrations in milk were below the limit of detection at all milkings. After intramammary administration of 500 mg benzathine cefapirin in dry cows, cefapirin levels in milk (after 5-86 days of dry period) were less than 0.02-1.5 mg/l at first to third milking, less than 0.02-0.13 at fourth and fifth milking and less than 0.02 from sixth milking. After intramammary administration of 261 mg cefapirin as sodium cefapirin in lactating cows, cefapirin levels in milk were 5-20 mg/l at first milking, at fourth milking levels were still up to 2.5 mg/l. From the fifth milking, levels were below the limit of detection (value not reported).
24. HPLC methods were proposed as routine analytical methods. These methods were not adequately validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community.

Recommendations and conclusion

Having considered :

- the provisional ADI of 0.001 mg/kg bw,
- the microbiological activity of the parent compound and desacetylcefapirin,
- having identified the sum of cefapirin and desacetylcefapirin as the marker residue,
- that the analytical method proposed for residues monitoring purposes is not fully validated;

the Committee recommends the inclusion of cefapirin 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
Cefapirin	Sum of cefapirin and desacetyl- cefapirin	Bovine	50 µg/kg 50 µg/kg 100 µg/kg 50 µg/kg 10 µg/kg	muscle liver kidney fat milk	Provisional MRLs expire on 1.1.1999

For other animal species (sheep, goats, pigs and horses) no MRL can be proposed at present.

LIST OF QUESTIONS

Safety File

1. The applicant should provide MIC₅₀ data in at least 10 strains per species in the following bacterial species: *Clostridium spp.*, *Peptostreptococcus spp.*, *Bifidobacterium spp.*, *Eubacterium spp.*, *Lactobacillus spp.*
2. A new proposal for a final microbiological ADI supported by a complete set of data, including adequate data to estimate correction factors, should be provided.

Residue File

1. Horses were not mentioned by the applicant as target animal, although pharmacokinetic studies in horses were provided. The applicant mentioned goats and sheep as target animals for which no pharmacokinetic and metabolite data or depletion studies were given. For pigs a tissue depletion study was given but no pharmacokinetic data. The applicant should clarify the proposed target species.
2. Tissue and milk depletion studies including injection site residues after intramuscular administration in all proposed target species should be provided.
3. Additional experimental data are required on tissue depletion after intramammary treatment of dry cows with benzathine cefapirin at time intervals of 0-21 days after treatment.
4. The applicant should provide a tissue depletion study from 0-24 hours after intrauterine administration.
5. The applicant should provide a confirmatory method which measures both cefapirin and desacetylcefapirin.
6. The applicant should show that there are no other microbiologically relevant metabolites than desacetylcefapirin.
7. The applicant should provide adequate data on residue composition and marker residue/total residues ratio in all relevant edible tissues and milk after the recommended use of the compound in relation to time after administration and data on *in vitro* residue degradation.
8. The microbiological method proposed for screening is not specific, not clearly described and not entirely validated. The applicant should provide clear information on modification of the method reported in exp. 91/9113 and 91/9116). If the limits of determination cannot be established, minimal detectable limits should be provided. The method should be described in an internationally recognised format (e.g. the ISO 78/2).
9. The applicant proposed an HPLC method for confirmatory purposes. This method was not adequately validated. The applicant should provide a confirmatory method for the measurement of the sum of cefapirin and desacetylcefapirin, which is validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community. The description of the method should be provided in an internationally recognized format (e.g. ISO 78/2).