COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CEFAPIRIN

SUMMARY REPORT (2)

1. Cefapirin is a first generation semi-synthetic cefalosporin 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 to 300 mg/quarter. The benzathine salt is used for intrauterine treatment of sub-acute and chronic endometritis at a recommended dose of 500 mg/cow. The relative activity of sodium cefapirin is 855 µg/mg and that of benzathine cefapirin is 700 µg/mg.

Currently, cefapirin is included in Annex III of Council Regulation (EEC) No 2377/90 in accordance with the following table:

<table>
<thead>
<tr>
<th>Pharmacologically active substance(s)</th>
<th>Marker residue</th>
<th>Animal species</th>
<th>MRLs</th>
<th>Target tissues</th>
<th>Other provisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephapirin</td>
<td>Sum of cephalin and desacetylcephapirin</td>
<td>Bovine</td>
<td>50 µg/kg</td>
<td>Muscle</td>
<td>Provisional MRLs expire on 1.1.2001</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>50 µg/kg</td>
<td>Liver</td>
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<td></td>
<td>100 µg/kg</td>
<td>Kidney</td>
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<td></td>
<td></td>
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<td>50 µg/kg</td>
<td>Fat</td>
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<td></td>
<td></td>
<td>10 µg/kg</td>
<td>Milk</td>
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</table>

Additional information intended to enable the inclusion of cefapirin for bovine species in Annex I of Council Regulation (EEC) No 2377/90 was provided as a response to the List of Questions adopted at the time of recommending provisional MRLs. Further to the assessment of the response, a CVMP Opinion on cefapirin was adopted concluding that the establishment of maximum residue limits, as referred to in Article 2 of the aforementioned Council Regulation for cefapirin in bovine species, could not be recommended. An intention to appeal against the Opinion and grounds for appeal were submitted to the EMEA.

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 cefalosporins. It is not inactivated by β-lactamase-producing staphylococci. No information on other pharmacodynamic actions was provided. Resistance mechanisms for cefalosporins 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 to 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. Circumstantial evidence indicates that the oral bioavailability of cefapirin is very low. 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 LD$_{50}$ in male rats was 14 000 mg/kg bw. Parenteral LD$_{50}$ 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 intraterine infusion of 0, 1500, 4500, 7500 mg benzathine cefapirin on three consecutive days. In the third study, the effect of intraterine 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 cefalosporins 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 MIC\textsubscript{50} values were available for 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., Bifidobacterium spp., Eubacterium spp., and Lactobacillus spp. Cefapirin was in particular effective against Peptostreptococcus, Lactobacillus, and Clostridium (mean geometric MIC\textsubscript{50} range: 0.4 to 1.4 µg/ml, at an inoculum density of 10\textsuperscript{8} CFU/ml). Desacetylcefapirin exerts less microbiological activity against tested bacteria of normal human gut flora than cefapirin: the overall mean geometric mean MIC\textsubscript{50} is 24.1 µg/ml for desacetylcefapirin as compared to 11.9 µg/ml for cefapirin. To cover the range of MIC\textsubscript{50} values, the one-tailed 10% lower confidence limit of the overall geometric mean MIC\textsubscript{50} for cefapirin was calculated to be 4.53 µg/ml. 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 an in vitro gut model, the effect of simulated gastrointestinal conditions on cefapirin residues was studied in the presence of meat, low fat milk and artificial saliva. It was estimated that 89% of the oral intake will pass in active form the stomach and duodenum of humans under average gastrointestinal conditions.

17. 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.

18. 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.

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

\[
\text{ADI} = \frac{\text{geometric mean MIC}_{50} \times \text{CF2}}{\text{CF1}} \times \frac{\text{daily faecal bolus (150 ml)}}{\text{fraction of an oral dose available for micro-organisms}} \times \text{weight of human (60 kg)}
\]

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

\[
\text{ADI} = \frac{4.53 \times 1}{5} \times \frac{x}{0.89 \times 60} = 2.54 \text{ µg/kg bw i.e. } = 153 \text{ µg/person}
\]
The following assumptions were made:

- CF1 = 5 to adjust for development of chromosomal and plasmidic resistance; correction for the width of the MIC\textsubscript{50} range and the number of MIC\textsubscript{50} determined was not considered necessary, because the overall geometric mean MIC\textsubscript{50} was calculated taking into account 10 bacterial strains considered representative of the human gut flora,
- CF2 = 1; since the MIC determinations were carried out employing an appropriate inoculum density of 10\textsuperscript{7} CFU/ml, no adjustment for differences between \textit{in vitro} and \textit{in vivo} growth conditions was considered necessary; potential effects of pH on MIC values were not studied, but are considered to be minimal,
- 150 g was the weight of the daily faecal bolus;
- the 10\% lower confidence limit of the overall geometric mean MIC\textsubscript{50} of cefapirin is 4.53 \mu g/ml,
- The fraction of an oral dose available to microorganisms was set at 0.89, based on the results of a study on the breakdown of cefapirin under simulated human gastrointestinal conditions.

20. The microbiological ADI (153 \mu g/person) is considerably lower than the toxicological ADI (6 mg/person). Therefore, the microbiological ADI was considered to be the relevant ADI for the safety assessment of cefapirin.

21. 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\textsubscript{max} of 14 \mu 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\textsubscript{max} of 0.33 \mu 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. Intraterine and intramammary administration of benzathine cefapirin in dairy cows caused low plasma cefapirin levels of short duration. Plasma C\textsubscript{max} was 0.13 \mu g/ml at 1 to 3 hours after intraterine administration of 500 mg cefapirin as benzathine cefapirin. After 24 hours plasma cefapirin levels were below the limit of detection (0.01 \mu g/ml). After intramammary administration of 381 mg benzathine cefapirin in dry cows the plasma C\textsubscript{max} was 0.025 to 0.32 \mu g/ml at 6 to 12 hours.

22. In dairy cows the average bile concentration was 10.3 \mu g/g 6 hours after intravenous administration of 8.6 mg/kg sodium cefapirin and 0.3 \mu 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 g/ml was found in urine. Intraterine administration of 500 mg cefapirin as benzathine cefapirin resulted in 2.57 \mu g/ml urine at 1 to 12 hours; at 72 hours levels were below the limit of detection (0.05 \mu g/ml). Intramammary administration of 381 mg benzathine cefapirin resulted in 2.4 to 17 \mu g/ml urine on day 1; from day 7 on levels were below the limit of detection (0.04 \mu g/ml). From the available data it can be concluded that in cows cefapirin is mainly eliminated by the urinary route and to a smaller extent by the biliary route.

23. Total residue is defined as the antimicrobially active residue. As cefapirin is degraded to desacetylcefapirin in all edible tissues except for fat and milk, and because the total residues reported were very low, the ratio of marker (cefapirin plus desacetylcefapirin) to total antimicrobially active residues was estimated to be 1. Since the available residue concentrations were measured by means of microbiological methods (unless indicated otherwise), they represent the microbiologically active residues expressed in terms of cefapirin activity. In some residue studies employing the intramammary and intraterine routes of administration, HPLC with microbiological detection was used. The applicability of the latter method for muscle, fat and milk was not proven, because very low recoveries were found. Furthermore, the linearity of the method was not proven observing high coefficients of variation. As a consequence, the data obtained by HPLC with microbiological detection may not properly reflect incurred residue levels.
24. 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 8 to less than 24 µg/kg and in liver 370 µg/kg. Intramuscular treatment of dairy cows with 8.5 mg/kg benzathine cefapirin, resulted at 4.5 hours in concentrations of 1000 to 5000 µg/kg in kidney, less than 8 to less than 24 µg/kg in muscle and less than 45 µg/kg in liver.

25. After intrauterine treatment of dairy cows with 1000 mg cefapirin as benzathine cefapirin, high residue levels were reported in endometrium (6.4 to 113.9 mg/kg) at 6 hours after treatment, while at that time in kidney residue levels ranged from 20 to 94 µg/kg and residue levels in liver were less than 20 µg/kg. In liver and kidney samples derived from this study, HPLC with microbiological detection revealed only measurable concentrations of desacetylcefapirin, and no detectable concentrations of parent compound were reported (15 µg/kg). After intrauterine treatment of dairy cows with 500 mg cefapirin as benzathine cefapirin, residue levels in endometrium were less than 0.10 to 66 mg/kg at 0 to 24 hours. In a similar study residue concentrations in kidney, liver, meat, fat and udder tissue cefapirin levels were below 15 µg/kg at day 2 to 4.

26. After intramammary administration of 300 mg/quarter benzathine cefapirin, cefapirin concentrations in fat, muscle, kidney and liver as measured by HPLC with microbiological detection were below 20 µg/kg at day 14 to 21. After intramammary administration of 381 mg/quarter benzathine cefapirin, cefapirin concentrations in fat, muscle, udder, kidney and liver were below the limit of detection (ranging from 20 to 50 µg/kg) at day 21 to 42. In lactating cows, where two quarters were treated with 300 mg cefapirin each, residues were below 20 µg/kg in muscle, liver and fat. Residues ranged from 20 to 51 µg/kg in kidney samples at 6 hours after treatment, and from 21 to 93 µg/kg at 15 hours after treatment. From 24 hours after treatment onwards all samples were below 20 µg/kg. HPLC with microbiological detection showed that in all samples from this study cefapirin concentrations were below 15 µg/kg. Desacetylcefapirin was observed in kidney ranging from 88 to 286 µg/kg, and 19 to 61 µg/kg, at 6 and 15 hours after administration, respectively. At 24 hours after treatment, all kidney samples were 15 µg/kg.

27. 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 to 24 hours after intramammary administration (4.6 to 33.5 mg/l). From 24 to 60 hours values of 0 to 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.

28. After intramuscular administration of 10 mg/kg sodium cefapirin in lactating cows, 30 to 110 µg/l was found in milk at 1 to 4 hours, levels of 10 µg/l were found from 4 to 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 10 to 29 µg/l first milking; after two or more milkings cefapirin concentrations were below 10 µg/l. When a dose of 500 mg was administered, cefapirin concentrations in milk were below 20 µg/l at all milkings. After intramammary administration of 500 mg/quarter benzathine cefapirin in dairy cows, cefapirin levels in milk (after 5 to 86 days of dry period) were less than 20 to 1500 µg/l at first to third milking, less than 20 to 130 µg/l at fourth and fifth milking and less than 20 µg/l from sixth milking. After intramammary administration of 261 mg/quarter cefapirin as sodium cefapirin in lactating cows, cefapirin levels in milk were 5 to 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).
29. Further information provided during the appeal against the CVMP opinion showed that LC-MS-MS methods for monitoring residues in bovine tissues and milk are available. The methods were described in ISO 78/2 format and adequately validated, in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community, for cefapirin and desacetylcefapirin (milk and fat), or for desacetylcefapirin (muscle and kidney). As cefapirin is degraded to desacetylcefapirin in all edible tissues except for fat and milk, it was considered acceptable that the method for muscle and kidney was validated for desacetylcefapirin only. Although the reported data show that the proposed method may applicable for liver also, the method was not fully validated for this matrix. Given the known instability of cefapirin, the fact that the method was validated for kidney, as well as the fact that liver is not a target organ for cefapirin, this was considered acceptable.

Conclusions and recommendation

Having considered that:

• the microbiological ADI is 2.54 µg/kg bw, (i.e. 153 µg/person),
• the sum of the parent compound cefapirin and its metabolite desacetylcefapirin was identified as a suitable marker residue for milk and tissues (the estimated ratio marker to total antimicrobially active residues is 1),
• in kidney and muscle only desacetylcefapirin will be found,
• liver is not a target organ for cefapirin and residues of cefapirin in this tissue are unstable, and therefore it was not considered necessary to establish an MRL for liver,
• LC-MS-MS methods, validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community, are available for cefapirin and desacetylcefapirin (milk and fat), or for desacetylcefapirin (muscle and kidney);

the Committee for Veterinary Medicinal Products, having considered the grounds for appeal, recommends the inclusion of cefapirin in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

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Based on these MRL values, the daily intake from bovine tissues and milk will represent approximately 73% of the ADI.