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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CEFALONIUM

SUMMARY REPORT (2)

1. Cefalonium (CAS Number: 5575-21-3) is a first generation semi-synthetic cephalosporin with a broad spectrum of activity against both Gram-positive and Gram-negative bacteria. The dihydrate of cefalonium is administered *via* the intramammary route, to cattle during the dry period, at a recommended dose of 250 mg per quarter to treat existing sub-clinical infections and to prevent new infections. In addition, cefalonium is used in eye ointment to treat cefalonium-sensitive bacterial ocular infections in cattle including keratoconjunctivitis. Cefalonium is instilled into the conjuctival sac at a dose of 80 mg per eye repeated 48 to 72 hours later if necessary.

   Currently, cefalonium is 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 tissue	Other provisions
Cefalonium	Cefalonium	Bovine	10 µg/kg	Milk	Provisional MRL expires on 1.1.2003

   For other tissues except milk, cefalonium is included in Annex II to Council Regulation (EEC) No 2377/90 in accordance with the following table:

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

   Additional data were provided in response to the list of questions adopted at the time of recommending the extension of the expiry date of the provisional MRL for milk intended to enable the inclusion of cefalonium for bovine milk in Annex I of Council Regulation (EEC) No 2377/90. Further to the assessment of the response, a CVMP Opinion on cefalonium was adopted in May 2002. The Opinion concluded that the establishment of a maximum residue limit, as referred to in Article 2 of the aforementioned Council Regulation for cefalonium in milk could not be recommended.

   An intention to appeal against the Opinion and grounds for appeal were subsequently submitted to the EMEA.

2. The bactericidal activity of cefalonium is a result of its inhibitory action on bacterial cell wall synthesis due to binding to one or more penicillin binding proteins located under the cell wall of susceptible bacteria. The resulting high internal osmotic pressure leads to rupture of the cytoplasmic membrane.

Resistance against cephalosporins may be due to inactivation by β-lactamases, decreased permeability of the bacterial cell wall or alteration of penicillin binding proteins. β-lactamases against cephalosporins may be encoded both in chromosomes and plasmids. No data on other pharmacodynamic effects were provided.

3. The only pharmacokinetic data on cefalonium reported in laboratory species were serum levels in two oral repeated dose toxicity studies in dogs and plasma levels in rats used for a mutagenicity study. In the first dog study, after administration by gavage in dogs, peak serum cefalonium concentrations were less than 0.03 to 0.33 µg/ml after 2 hours (dose of 10 mg cefalonium/kg bw), 0.38 to 0.57 µg/ml after 2 hours (dose of 50 mg cefalonium/kg bw), 0.30 to 1.76 µg/ml after 4 hours (dose of 100 mg cefalonium/kg bw) and 1.63 to 2.86 µg/ml after 8 hours (dose of 1000 mg cefalonium/kg bw).

   In the second dog study, one hour after the first oral administration of cefalonium dihydrate at dose levels of 10 and 1000 mg/kg bw, the serum concentrations were 0.41 to 0.64 and 0.34 to 1.26 µg/ml. Two hours after the 85^{th} daily dose they were 0.38 to 1.06 and 1.32 to 1.78 µg/ml.

   Two to 4 and 12 to 14 hours after administration by gavage of 2000 mg cefalonium/kg bw in rats, cefalonium plasma peaks were in the ranges of 0.38 to 0.675 and 0.094 to 0.995 µg/ml respectively. The relatively low blood levels indicate that cefalonium is poorly absorbed from the gastrointestinal tract in these species. No information on metabolism and excretion of cefalonium in laboratory animals was provided.

4. Cefalonium serum concentrations after intramammary treatment with 250 mg unlabelled cefalonium in each quarter in dry cows were 0.21 to 0.42 µg/ml, 0.15 to 0.27 µg/ml and less than 0.1 µg/ml at, respectively 8, 12 and 24 to 72 hours after treatment. After administration of the same dose of radiolabelled cefalonium, a mean peak plasma concentration of 0.268 µg equivalents/ml was found at 36 hours after administration.

   After intramammary infusion of the commercial formulation at a dose of 250 mg cefalonium per quarter, high cefalonium concentrations were found in urine, up to 23 µg/ml on the first day, and gradually decreasing to less than 0.08 µg/ml at respectively 8 and 15 days after treatment in two examined cows.

   In another study cefalonium excretion in urine was 4.55 to 24.1 µg/ml at 12 hours after intramammary treatment with 250 mg cefalonium in each quarter and slowly decreased to 0.26 µg/ml at day 19 and less than 0.08 µg/ml thereafter.

   In a radiolabel experiment, cefalonium was excreted in urine and in faeces after intramammary infusion in dry cows of 250 mg radiolabelled cefalonium in each quarter. During the first 3 days after treatment, about 29% of the total radioactive dose was excreted in urine and 2% in faeces. At days 7, 14 and 21 radioactivity in urine was, respectively, 0.7, 0.4 and 0.4% of the dose and radioactivity in faeces was, respectively, 0.3, 0.08 and 0.03%. The sum of urinary and faecal excretion and cage wash on days 1 to 3, 7, 14 and 21 was 49.43% of the dose. Since no measurements were carried out on days 4 to 6, 8 to 13 and 15 to 20, it cannot be excluded that more than 50% of the total dose had been absorbed from the udder.

   Radiolabelled cefalonium (250 mg/quarter) was infused into the udder of 8 dry cows. The animals were 51 ± 3 days from the expected calving at the time of infusion. In plasma, C_{max} was 0.015 ± 0.038 µg equivalents/g at 48 hours after administration. The plasma radioactivity then declined slowly to 0.085 µg equivalents/g at 96 hours.

5. Acute toxicity of cefalonium was low. The oral LD_{50} values in male and female mice were greater than 12 000 mg/kg and greater than 5000 mg/kg bw in both sexes of rats. The subcutaneous LD_{50} values in both sexes of mice and rats were greater than 2000 mg/kg bw. The intraperitoneal LD_{50} values in female rats and mice were greater than 2680 and 3400 mg/kg bw, respectively. The main effects observed in both species after intraperitoneal administration were inhibition of autonomic motion and sedation and in rats ptosis of the abdominal region and pallor. At necropsy almost no intestinal contents were found in orally and subcutaneously treated fatal cases but there was local tissue damage in intraperitoneally treated animals. Little evidence was found for systemic toxicity.

6. Two oral repeated dose toxicity studies of 4 and 13 weeks were carried out in rats. In the 4-week study, dietary cefalonium intake was 0, 43, 234, 1194 and 6014 mg/kg bw/day in males, and 0, 47, 247, 1208 and 5834 mg/kg bw day in females. An increase in the size of the caecum was observed in males and females receiving more than 234 and 247 mg/kg bw/day, respectively. At all dose levels increased water and food intake were found. However, since no other adverse effects were observed at the two lowest dose levels, the increases at these levels were considered not relevant for human safety.

   In the 13-week study, dietary intake of cefalonium in males was 0, 4, 39, 440 and 4434 mg/kg bw/day and 0, 4, 44, 462 and 4674 mg/kg bw/day in females. An increase in the size of the caecum was observed in males and females receiving more than 440 and 462 mg/kg bw/day. Blood urea nitrogen levels were decreased in males at the two highest dose levels. Serum ketone bodies were increased in males and females at the highest dose. Serum globulin levels were decreased in males at the two highest dose levels (no difference in effect between these two levels) and in females at the three highest dose levels (dose related). Water and food intake were increased at all dose levels, however, since no other adverse effects were observed at the lowest dose level, these increases at this level were considered not relevant for human safety.

   Although the 4 week and 13 week rat studies were pre-GLP and no raw data were available, they do indicate that no toxic effects are expected at 4 mg/kg bw/day and therefore the dose of 4 mg/kg bw/day could be accepted as a toxicological NOEL.

7. Two oral repeated-dose toxicity studies of 7 days and 13 weeks were carried out in beagle dogs. In the 7-day study, 2 dogs per sex per dose group received 10, 50, 100 or 1000 mg cefalonium/kg bw/day as cefalonium dihydrate by gavage. In this dose-range finding study, the small intestine of high-dose animals exhibited pathological changes probably resulting from the administration of large amounts of a relatively insoluble suspension. The oral NOEL was 100 mg/kg bw/day. In the 13-week study, two dogs per sex per dose group received 0, 10 or 1000 mg cefalonium (as the dihydrate)/kg bw/day. In this study, no treatment related effects were observed up to the highest tested dose of 1000 mg/kg bw/day. However the number of dose levels and test animals were too low, to allow any conclusion.

8. No tolerance studies in target species were provided. It is stated that the incidence of adverse reactions is extremely low but this was not supported by data.

9. In an oral teratogenicity study in rats no teratogenic effects were observed at doses of 20, 200 and 2000 mg cefalonium/kg bw. The NOEL for embryotoxicity and teratogenicity therefore was 2000 mg/kg, the highest dose tested. Food and water intake were, respectively, statistically significantly lower and higher in both mid and high dose treated dams. Caecum weight was increased in drug treated dams at all dose levels. The study was reported in too little detail (i.e. complete raw data were lacking) to reliably establish the NOEL for maternal toxicity.

   A review containing results of teratogenicity tests of fifteen cephalosporins showed little evidence for teratogenicity of members of this group in general. In addition, studies were provided on parenteral reproductive toxicity of cefuroxime and ceftazidime. In these studies no teratogenic effects were found and no evidence was found for general reproductive toxicity (fertility and peri and post natal development). The main effects found were changes of food and water intake of dams and increased caecum weight of dams and pups. Taking into consideration the provided information on the general absence of teratogenic effects and reproduction toxicity in cephalosporins, no further teratogenicity and reproduction data were considered necessary.

10. No evidence for mutagenic potential was found in the following mutagenicity tests in prokaryotes (all with and without metabolic activation): the *Salmonella* microsomal assay (strains TA 1535, TA 1537 and TA 1538), fluctuation tests in *Escherichia coli* (strains WP2, WP2 uvra, 343/113 lys 60, WP2 pKM101 and PW2 uvra pKM101, at doses up to 10 and 20 µg/ml) and *Salmonella* (strains TA98, TA100, TA1535 and TA1537, at doses up to 10 and 20 µg/ml) and the gene conversion test in *Saccharomyces cerevisiae* (strain JD1, up to a dose of 500 µg/ml).

Cefalonium was not mutagenic in the mouse lymphoma assay (TK locus) at a dose range of 263 to 1138 µg/ml and 250 to 1081 µg/ml in the absence or presence of metabolic activation, respectively. Cefalonium induced a dose-dependent increase in structural chromosomal aberrations (chromatid deletions and gaps) in cultured human peripheral blood lymphocytes at a dose-range of 585 to 900 µg/ml in the absence of metabolic activation.

Cefalonium was not mutagenic in two micronucleus tests in two different strains of rats receiving, respectively, a single oral dose of 5000 mg/kg bw and two daily oral doses up to 2000 mg/kg bw. Cefalonium did not induce unscheduled DNA synthesis in cultured liver cells from rats exposed to maximally 2000 mg/kg bw by gavage. There was no evidence for *in vivo* genotoxicity of cefalonium.

11. No carcinogenicity studies were carried out. This was considered acceptable because no evidence for mutagenicity was observed, no evidence for preneoplastic changes was found in the repeated dose toxicity studies and the cefalonium molecule does not contain structural alerts.

12. No immunotoxicity studies were carried out. This is acceptable because no evidence for immunological effects was found in the repeated-dose toxicity studies.

13. Based on the NOEL of 4 mg/kg bw/day retained in the 13-week dietary toxicity study in rats and applying a safety factor of 200 to take into account the insufficient quality of the data package on toxicity, a toxicological ADI of 0.02 mg/kg bw, i.e. 1.2 mg/person was established.

14. *In vitro* MIC_{50} data were determined for all 10 genera considered representative for human intestinal flora: *Peptostreptococcus* spp, *Clostridium* spp, *Bifidobacterium* spp, *Bacteroides* spp, *Fusobacterium* spp, *Eubacterium* spp, *Lactobacillus* spp, *Enterococcus* spp, *Proteus* spp and *Escherichia coli*. The geometric mean MIC_{50} at an inoculum level of about 10^8 CFU as 4.6 µg/ml and the lowest MIC_{50} value was 0.125 µg/ml. Dilution of the inoculum by a factor of 100 resulted in a decrease of the MIC_{50} value of about a factor 2. Medium pH had little or no effect on the MIC values found. In an *in vitro* gut model the effect of simulated intestinal conditions on survival of two isolates of *Clostridium*, *Peptostreptococcus*, *Bacteroides*, *Escherichia coli* and *Lactobacillus* in the presence of cefalonium was examined. Because of the design of the experiment no conclusion on the effect of intestinal conditions on cefalonium activity was possible. Ninety isolates belonging to 10 genera representative of human intestinal flora were semi quantitatively assayed for intrinsic and cefalonium-induced β-lactamase production.

    Significant β-lactamase activity was observed in 8 bacterial strains. Co-incubation of cefalonium-sensitive and -insensitive strains resulted in protection of the sensitive strain against cefalonium as evidenced by an increase in the minimum bactericidal concentration in 3 out of 10 co-cultures.

15. Sufficient data were provided to establish a microbiological ADI, based on the geometric mean MIC_{50} of 4.6 µg/ml.

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

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

(μg/kg bw)

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

$$\text{ADI} = \frac{\dfrac{4.6 \times 4}{3} \times 150}{1 \times 60} = 15.3 \ \mu g/kg \text{ bw i.e.} = 920 \ \mu g/\text{person}$$

The following assumptions were made:

- CF1 = 3, because resistance factors may be transferred by chromosomal and plasmidic mechanisms;
- CF2 = 4 considering a factor 2 for the effect of density of the microflora and a factor 2 for the effect of the presence of lactamase producing bacteria;
- the fraction of an oral dose available for intestinal gut flora was conservatively set at 1.

16. The microbiological ADI (15.3 µg/kg bw) is slightly lower than the toxicological ADI (20 µg/kg bw), therefore the microbiological ADI was considered the relevant ADI for the safety assessment of cefalonium.

17. The effect of concentrations of 0.01 and 0.1 µg/ml cefalonium on acid production by 34 cheese and yoghurt starter cultures was examined. A small number of starters were inhibited at the lowest tested concentration and more than 50% at the highest concentration. Additional data on effects of incurred cefalonium residues in milk showed that at a residue concentration of 0.01 µg/ml effects on acid and coagulum production by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were found in about half of the examined samples. The MICs of cefalonium against 10 pure starter cultures (isolated from mixed commercial starter cultures) were determined in the presence and absence of milk. The tested strains were representative of cultures for the production of yoghurts, cheese and fermented milk products. The most sensitive strain was inhibited by cefalonium at a concentration of 0.031 µg/ml in both the absence and presence of milk. None of the organisms was inhibited at a concentration of 0.016 µg/ml.

    Six mixed commercial dairy starter cultures showed decreased sensitivity to cefalonium as compared to pure cultures in terms of growth. There was insufficient evidence to suggest the sensitivity of commercial cultures to cefalonium is significantly affected by the presence or absence of milk.

    Continuous pH measurement was used to monitor the production of acid from 6 mixed commercial dairy starter cultures. The cultures were exposed to cefalonium concentrations of 0.05, 0.1 and 0.2 µg/ml in milk and the resulting pH profiles were compared with those of uninhibited controls. Two out of 6 cultures were not significantly affected by cefalonium at any of the concentrations used; three cultures were affected at one or both of the higher concentrations; the remaining culture was affected at all three test concentrations.

    The reproducibility of the effects of cefalonium on the pH profiling of 2 commercial dairy starter cultures was demonstrated. A concentration of 0.1 µg/ml cefalonium was used. While one of the cultures was not affected at this concentration, the other exerted a significant inhibitory effect.

    The inhibitory effect of cefalonium on the acid production by two commercial dairy starter cultures was studied using 4 test concentrations of cefalonium in milk (0.05, 0.10, 0.15 and 0.20 µg/ml). Both cultures displayed a concentration-related sensitivity to the inhibitory effects of cefalonium.

    A thermophilic yoghurt culture mix was selected for further study on the basis of its close similarity to another commercial starter culture, which was shown previously to be highly sensitive to cefalonium, but was no longer commercially available. At 4 hours of incubation, the mean pH of samples exposed to 0.02 µg/ml cefalonium was 0.4 pH units higher as compared to cefalonium-free samples. The time required to reach pH 5.0 was 39 minutes longer. However, at 5½ hours of incubation the difference in pH between cefalonium-fortified and cefalonium-free samples was less than 0.3 pH units. At a concentration of 0.01 µg/ml, the difference between cefalonium-exposed and cefalonium-free samples was -.01 pH units at 4 hours of incubation and 9 minutes in terms of time to reach pH 5.0. These differences were considered to be negligible. At 0.015 µg/ml, the mean pH of cefalonium-exposed samples was 0.24 pH units higher than that of cefalonium-free samples. This difference was considered to be not relevant. The mean difference in time required for inoculated milk to reach pH 5.0 differed by 25 minutes between cefalonium-exposed samples and cefalonium-free samples. This difference was not statistically significant.

It was concluded that the overall NOEL for the inhibitory action of cefalonium on the acid production of commercial dairy starter cultures was 0.020 µg/ml.

18. Tissue distribution of residues following intramammary administration of 250 mg radiolabelled cefalonium per quarter was studied in dry cows which calved 11 to 42 days after infusion. The animals were slaughtered 7 days after calving. After the shortest examined withdrawal period of 18 days the total residue concentrations in kidney, liver, muscle and fat were 265, 43, less than 13 and less than 23 µg equivalents/kg. In the animal slaughtered after the longest studied withdrawal period of 49 days the total residue concentrations were 146, 28, less than 13 and less than 23 µg equivalents/kg. Total residue concentrations in udder tissue were less than 14 up to 644 µg equivalents/kg. In 3 animals slaughtered 18, 36 and 49 days after administration the ratio of parent compound to total residue in kidney was low, between 5 and 8%, and in one animal slaughtered after 29 days it was higher (27%). Most of the total residue in kidney consisted of metabolites, which were not identified. The composition of residues in other tissues was not examined.

In another study in dry cows, intramammary administration of 250 mg unlabelled cefalonium to each quarter resulted in residues in kidney, liver, heart, leg muscle and fat which were less than 80 µg/kg, after 3 weeks. Residues in udder tissue ranged from less than 80 to 4490 µg/kg.

Radiolabelled cefalonium (250 mg/quarter) was infused into the udder of 8 dry cows. The animals were 51 ± 3 days from the expected calving at the time of infusion. Four cows were killed at 36 and 96 hours after infusion, respectively. At 36 hours after administration, the mean total cefalonium-derived residues were 673 µg equivalents/kg in kidney, 60 µg equivalents/kg in liver, 15 µg equivalents/kg in fat, and 9 µg equivalents/kg in muscle. At 96 hours after administration the total residue levels in the tissues were somewhat lower, although no decline was observed in liver. The kidney was shown to be the major target tissue. Attempts to study the residue composition by radio-HPLC and HPLC-MS/MS were not satisfactory. Attempts to measure antimicrobially active residues in tissues were not successful either. Consequently, the ratio of proposed marker (i.e. unchanged cefalonium) to total (antimicrobially active) residues could not be defined.

19. Milk residue depletion after infusion of 250 mg radiolabelled cefalonium per quarter in dry cows was studied in 7 cows which calved either 11 to 17 (n = 3) or 29 to 37 (n = 3) days after infusion. In the first milking after calving the concentration of total radiolabelled residue was 666 to 6544 µg equivalents/kg, the cefalonium concentration (measured by HPLC-MS/MS) was less than 10 to 448 µg/kg and total antibiotic activity (measured by microbiological test) was less than 15 to 269 µg/kg. The data were not suitable to determine a reliable ratio of parent compound to total residues. However, after the first few days after calving the proportion of parent compound was likely to be variable and low.

20. Radiolabelled cefalonium was administered by the intramammary route at a dose of 250 mg/quarter in dry cows, which calved 41 to 71 days later. The dry period in this study (41 to 71 days) was considerably longer than the ones in the previous study (i.e. either 11 to 17 or 29 to 37 days). The prolonged dry period more adequately reflects the commercial use of cefalonium. Except for one animal killed due to an emergency, all cows were sacrificed after the 14th milking. About 40% of the dose was excreted in the first 7 days after infusion: 33 to 36% in urine and 3 to 5% in faeces. Less than 3% of the total dose was excreted in milk. Unchanged cefalonium was the only major residue present in milk. Based on HPLC-MS/MS data, the marker (i.e. parent compound) to total radioactive residue ratios was 0.88, 1.39, 1.15 and 1.18 after the 3rd, 5th, 7th and 9th milking. Based on radio-HPLC, these ratios were 0.61, 0.64, 0.61 and 0.58, respectively. Attempts to measure antimicrobial activity were unsuccessful.

In another milk residue study, the concentration of microbiologically active residues of cefalonium in milk after intramammary treatment of 20 dry cows with 250 mg unlabelled cefalonium in each quarter was examined. Duration of the dry period was 29 to 97 days. Cefalonium concentrations in milk from the cow with the shortest dry period of 29 days decreased from 180 µg/kg at the 5th milking to less than 10 µg/kg at the 22nd milking.

Cefalonium concentrations and the incidence of detectable cefalonium residues decreased with length of dry period. In one of two cows with a dry period of 97 days one detectable cefalonium residue of 10 µg/kg was found at the second milking, in the other animal no detectable residues were found.

In this experiment the effect of the incurred residues on acid and coagulum production by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* was examined in addition. It was found that in about half of the cases a residue concentration of 10 µg/kg had an inhibiting effect on one or more of the tested parameters in starter cultures.

21. For routine monitoring of the parent compound in bovine milk initially a reversed phase HPLC method with electrospray MS/MS detection was proposed. The method was described in the ISO 78/2 format. The method was specific and validated for the determination of cefalonium in bovine milk in the concentration range of 10-200 µg/kg. The limit of detection (LOD) was 5 µg/kg and the limit of quantification (LOQ) was 10 µg/kg. The analytical method was validated according to Volume VI of the Rules Governing Medicinal Products in the European Union. The CVMP recommends this method for routine monitoring purposes.

22. Subsequently, a reversed phase HPLC method with tandem mass spectrometric detection using cephaloridine as an internal standard was proposed for the routine monitoring of the parent compound in bovine milk. The method was described in the ISO 78/2 format. Validation data were provided regarding the determination of cefalonium in pasteurised bovine milk in the concentration range of 1.25 to 20 µg/kg. The limit of quantification was 1.25 µg/kg. Although this analytical method was also validated according to Volume VI of the Rules Governing Medicinal Products in the European Union, the CVMP does not recommend this method for routine monitoring purposes because of the limited concentration range validated as compared to the initially proposed method.

Conclusions and recommendation

Having considered that:

- a microbiological ADI of 15.3 µg/kg bw (i.e. 920 µg/person) was established,
- the parent compound was identified as the marker residue,
- a NOEL of 0.020 µg/ml was established for the acid inhibitory effects of milk residues on dairy starter cultures,
- a validated routine analytical method for the determination of cefalonium in bovine milk is available

The Committee for Veterinary Medicinal Products, further to the consideration of the grounds for appeal, recommends the inclusion of cefalonium into 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
Cefalonium	Cefalonium	Bovine	20 µg/kg	Milk	

Based on the above MRL value, and tentatively considering 0.60 as the ratio of marker : total antimicrobially active residues, the daily intake will represent approximately 5.4% of the microbiological ADI.