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

EMEA/MRL/499/98-FINAL
September 1998

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CEFACETRILE

SUMMARY REPORT (1)

1. Cefacetrile is a first generation cephalosporin. The sodium salt of cefacetrile is included in an oily formulation recommended for the intramammary treatment of mastitis in lactating cows. A dose of 250 mg cefacetrile sodium per day per udder quarter is administered by the intramammary route by injector once or twice at an interval of 24 hours. Cefacetrile is also used in human medicine in intramuscular and intravenous formulations at daily doses of 2 to 6 g for treatment of bacterial infections.

2. Cefacetrile exerts high activity (MIC values below 2 µg/ml) against Gram-positive bacteria such as *Staphylococci* and *Streptococci*, in experimentally infected laboratory animals but is less active (MIC values close to 5 µg/ml) against Gram-negative bacteria including *E. coli*, *Klebsiella* and *Salmonella* spp. Cefacetrile treatment is not or only weakly effective in infections provoked by *Pseudomonas* spp., *Listeria* spp. and penicillin- or cephalosporine-resistant strains. Besides the parent compound, the major metabolite desacetylcefacetrile was found to show some microbiological activity. MIC values for this metabolite were 2.5 to 7.5 times higher than those for the parent compound.

3. Pharmacokinetics following parenteral administration of cefacetrile to laboratory animals (rats, rabbits), ruminants (cattle, sheep, goat) and man are characterised by low body distribution of the substance (V_d of 0.2 to 0.5 l/kg), short serum/plasma half-lives of elimination (approximately 1 hour or less) and poor metabolisation with desacetylcefacetrile and its lactone being the only metabolites detected. The substance is rapidly and almost exclusively excreted via the urine. In rats and rabbits given ^{14}C-labelled cefacetrile (50 mg/kg bw, intravenously or subcutaneously) the majority of the dose (80%) was recovered in urine within 4 hours post dose already and excretion was complete within 72 hours. The urinary radioactivity was accounted for by unchanged cefacetrile and desacetylcefacetrile. Plasma elimination half-lives for the intravenous route were approximately 20 minutes. In goats (10 mg/kg bw, intramuscularly) about 80% of the dose was recovered in urine as antimicrobially active substance within 12 hours and more than 90% within the first day after treatment. Elimination half-lives in cows and ewes treated intravenously with cefacetrile (8.5 to 12 mg/kg bw) were reported to be less than 1 hour. In humans, nearly 100% of a dose of ^{14}C-labelled cefacetrile (approximately 1 g/person, intravenously) was excreted within 48 hours *via* urine as unchanged cefacetrile (76%) and as desacetylcefacetrile or its lactone (21%). The serum half-life was 0.7 to 1.3 hours.

   Oral bioavailability of cefacetrile appears to be rather low. As observed in calves only about 3% of an oral dose (20 mg/kg bw) was absorbed from the gastrointestinal tract. The intestinal absorption rate in humans has not been determined. Absorption from the udder was only moderate. In cows tested after a single intramammary dose (250 mg sodium cefacetrile/udder quarter), maximum plasma concentrations of about 170 µg equivalents/l were obtained approximately 4 hours post treatment (measured by bioassay). Thereafter, plasma concentrations declined rapidly. At 23.5 hours post treatment, the plasma concentrations were below 10 µg equivalents/l. After intramammary administration of ^{14}C-labelled cefacetrile to a cow (210 to 216 mg/udder quarter) 54.6% of the dose was recovered in milk, 21% in urine and faeces and 2.5% from tissues within 5 days.

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It was shown that the microbiologically active residue in milk and udder tissue consisted mostly of parent cefacetrile (more than 90%). Concentrations of the antibiotic in other cow tissues were too low for metabolite analysis.

4. The acute toxicity of cefacetrile is low with LD_{50} values of 11600 mg/kg bw in rats (subcutaneously), greater than or equal to 3700 mg/kg bw in mice (intravenously) and greater than or equal to 7500 mg/kg bw in mice (subcutaneously). Guinea pigs are more susceptible with a LD_{50} of 240 mg/kg bw (subcutaneously). The acute renal toxicity of cefacetrile after intravenous or subcutaneous injection in rats, dogs and rabbits is low compared to the nephrotoxic congener cephaloridine. Only minor renal changes were detected even after the administration of high doses of cefacetrile (up to 4500 mg/kg bw in rats, 6000 mg/kg bw in rabbits). In dogs no effects were noted at an intravenous dose of 330 mg/kg bw.

5. Repeated dose toxicity studies with parenterally administered cefacetrile were performed in rats and dogs. Rats (10 females and 10 males/group) received daily subcutaneous injections at doses of 0, 100, 300 or 1000 mg/kg bw for 30 days. Apart from local reactions at the injection sites, no substance related effects were recorded. Dogs (2 to 3 females and 2 to 3 males/group) were injected daily intravenously with doses of 0, 150 or 400 mg/kg bw for 4 weeks and with doses of 0, 250, 500 or 750 mg/kg bw for 90 days. In all experiments, salivation and/or vomiting and pruritus at the head were observed in connection with the intravenous injections. However, no effects including clinical-chemical and pathological-histopathological changes, which could be attributed to the substance, were observed in the repeated dose toxicity studies at the doses tested. A NOEL of 750 mg/kg bw was established on the basis of the 90-day intravenous toxicity study in dogs. No data on the repeated dose toxicity of cefacetrile following oral administration were available.

6. The effect of cefacetrile on fertility of laboratory animals was not investigated. However, so far cephalosporin antibiotics have not been associated with reproductive toxicity.

   The potential embryo-foetotoxicity and teratogenicity of cefacetrile was investigated in mice, rats and rabbits. Unfortunately, only summary reports of the studies were provided. The mice teratogenicity study (intravenous doses of 0, 200, 400 or 600 mg/kg bw from day 6 to 15 of pregnancy) revealed maternotoxic effects (increased mortality) at 400 mg/kg bw and above and a significantly decreased number of implantations and live foetuses in the offspring of dams treated with 600 mg/kg bw. In rats (intravenous doses of 0, 200, 400 or 800 mg/kg bw from day 6 to 10 or day 11 to 15 of pregnancy), maternotoxicity as indicated by temporary apathy was noted at 800 mg/kg bw and the average body weight of the foetuses from these dams was reduced. In rabbits (intravenous doses of 0, 100, 300 or 1000 mg/kg bw from day 6 to 15 of pregnancy), cefacetrile caused maternotoxicity in dams (reduced weight gain) at 1000 mg/kg bw and reduced average bodyweights in the offspring of this dose group. Provided data suggested that intravenous doses of 400 mg/kg bw in mice and rats and 300 mg/kg bw in rabbits appear to be devoid of embryo-foetotoxic or teratogenic potency in these species. Effects observed in the offspring at higher doses were paralleled or even preceded by maternotoxic effects in all experiments. However, definite NOELs could not be derived due to incomplete reporting of the studies.

7. The mutagenic potential of cefacetrile was investigated in pro- and eukaryotic cells using different endpoints. The substance was tested for gene mutation activity in the *Salmonella typhimurium* assay in the presence and absence of microsomal activation (TA 1535, TA 1537, TA 1538, TA 100 and TA 98 strains; up to 2000 µg/plate) and in strains of *Saccharomyces* (up to 5000 µg/ml). Gene mutation activity in yeast cells was also investigated following metabolic activation with urine of cefacetrile treated mice and in a host mediated assay in mice. Chromosomal aberration was tested *in vivo* in bone marrow cell micronucleus assays in mice (intravenously, up to 100 mg/kg bw) and in a cytogenetic assay in Chinese hamsters (intraperitoneally, up to 5000 mg/kg bw). *In vitro* tests for DNA repair were conducted with and without metabolic activation using human WI-38 fibroblasts (up to 5000 µg/ml). None of the experiments did reveal any evidence for a significant genotoxic potential of cefacetrile. The molecule does appear to be related to known mutagenic substances.

8. No carcinogenicity studies have been carried out. However, the molecule does not appear to possess alerting molecular structures. Based on this and in view of the negative results for mutagenicity testing no carcinogenicity studies are deemed necessary.

9. Studies on local tolerance in dairy cows using a commercial cefacetrile formulation revealed minor to moderate udder irritation.

10. The eye and skin irritating potential of cefacetrile in laboratory animals is minimal to moderate. In pre-sensitised guinea pigs, cefacetrile exerts a mild skin sensitising effect following dermal application.

11. Considering that the toxicological studies were conducted before GLP-requirements have been established and results were exclusively based on parenteral dosing of cefacetrile a definite toxicological ADI was not defined. Nevertheless, the data clearly suggested that intravenous doses greater than 200 mg/kg bw are needed to produce first discernible effects in experimental animals (maternotoxicity in mice). Because of this relatively low systemic toxicity and the poor gastro-intestinal absorption of the substance the antimicrobial properties of cefacetrile are the determining factor in establishing an appropriate ADI.

12. MIC data were provided on microorganisms representative of the human gut flora (in total 80 strains). MIC_{50} values ranged from 40 µg/ml for *Proteus* and *Bifidobacterium* to 0.5 µg/ml for *Fusobacterium* and *Peptostreptococcus* at pH 7.1 and a bacterial density of 10^8 cfu/ml.

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

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

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

$$\text{ADI} = \frac{\dfrac{2.1 \times 2}{3} \times 150}{1 \times 60} = 3.50\ \mu g/kg\ bw \text{ i.e. } = 210\ \mu g/person$$

    The following assumptions were made:

    - CF1 = 3, to account for both chromosomal and plasmidic resistance mechanisms;
    - CF2 = 2, because MIC-determinations at a bacterial density more representative of the *in vivo* situation, although rather limited, do indicate an increase of MIC values;
    - 150 g was the weight of the daily faecal bolus;
    - 2.1 is the 10% lower confidence limit of the geometric mean MIC_{50} values for all strains tested;
    - 1 is the dose available to bacteria of the colon because oral absorption was only poor.

13. No adequate studies were provided on the effect of cefacetrile on dairy starter organisms. However, bacteria of the genera *Bifidobacterium, Streptococcus* and *Lactobacillus* commonly used in food and dairy industry were found to be of low to intermediate susceptibility to cefacetrile with lowest MIC values of 5 µg/ml.

14. The risk of immunological effects in humans following ingestion of cefacetrile residues in foodstuffs from treated animals is not known but is supposed to be low due to the poor absorption after oral administration.

15. Residue distribution of radiolabelled ^{14}C-cefacetrile was investigated in one cow (210 to 216 mg/udder quarter). The highest residues in organs were found in kidney and udder tissue. At slaughter, 5 days post treatment, kidney, udder and liver showed total drug related residues equivalent to 232 µg/kg, 227 µg/kg and 33 µg/kg, respectively.

    In a new non-radiometric GLP study, tissue residue depletion was investigated in 12 cows treated intramammary (250 mg sodium cefacetrile/udder quarter) twice at an interval of 24 hours. Cows were slaughtered at 1, 3 and 5 days post dose. Samples of liver, kidney, fat, muscle and udder were analysed by bioassay as well as by LC-MS/MS (limit of quantification being 25 µg/kg). Residues above the limit of quantification were found in udder tissue only. The mean concentrations of microbiologically active residues and parent cefacetrile as determined by bioassay and by LC-MS/MS at day 1 after treatment were 221 µg/kg and 295 µg/kg, respectively.

    In a new non-radiometric GLP study for milk, 8 lactating cows were treated intramammary with 250 mg sodium cefacetrile per udder quarter twice at an interval of 24 hours. The mean residue concentration in milk of the first milking post dose as determined by bioassay was 14257 µg/l and 1860 µg/l of the second milking. Thereafter, concentrations decreased to about 208 µg/l at the 5th milking, to about 20 µg/l at the 7th milking and to less than 5 µg/l at the 9th milking. The milk samples were also analysed by LC-MS/MS up to the third milking post dose. The concentrations found were comparable within experimental error, suggesting that most of the microbiologically active residue was parent cefacetrile.

    It can be concluded from the two pivotal residue depletion studies that the parent compound cefacetrile is the most suitable marker residue because more than 90% of the microbiologically active residue in milk and in udder tissue could be attributed to this substance. Residues in other edible tissues were too low to be quantified.

16. A bioassay for milk and tissues was provided. The limits of quantification were 10 µg/kg for milk and 25 µg/kg for tissues. However, due to the general lack of specificity of microbiological methods, this is no validated routine analytical method in accordance with the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community. A confirmatory routine analytical method for cefacetrile based on HPLC-MS/MS was provided. However, the method is not fully validated with respect to all relevant parameters.

Conclusions and recommendation

Having considered that:

- a microbiological ADI of 3.5 µg/kg (210 µg/person) was set for cefacetrile,
- cefacetrile is the marker residue accounting for almost all of the microbiologically active residues in milk,
- no microbiologically active residues were detected in the target tissues at day 1 post treatment,
- the analytical method for milk is not fully validated;

the Committee recommends the inclusion of cefacetrile in Annex III of Council Regulation (EEC) No. 2377/90 as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	Target tissue	MRLs	Other provisions
Cefacetrile	Cefacetrile	Bovine	Milk	125 µg/kg	For intramammary use only. Provisional MRL expires on 1.1.2001

In addition, it is recommended to include cefacetrile in Annex II of Council Regulation (EEC) No. 2377/90 as follows:

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

Based on the MRL for milk and taking into account that microbiologically active residues in edible tissues were below the limit of quantification of 25 µg/kg, the theoretical maximum daily intake of total microbiologically active residue can be estimated being around 90 % of the ADI.

Before the Committee can consider the inclusion of cefacetrile in Annex I to Council Regulation (EEC) No. 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The applicant should provide adequate studies allowing to assess the effect of cefacetrile on starter cultures used in food and dietary industry.

2. The applicant should provide a fully validated routine analytical method for the analysis of cefacetrile in milk. This method should be described in an internationally recognised format (e.g. ISO 78/2), and validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community. In addition, the documentation should provide data on the stability of cefacetrile in the milk extracts.