The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CEFALEXIN

SUMMARY REPORT

1. Cefalexin is a first generation cephalosporin with a broad spectrum of activity against both Gram positive and Gram negative bacteria. Cefalexin sodium is used for the intramuscular treatment of cefalexin-sensitive infections in cattle, sheep and pigs at a recommended dose of 7, 10 and 10 mg/kg bw, respectively, for up to 5 days. Cefalexin-monohydrate is used for the intramammary treatment of mastitis in lactating cows at a recommended dose of 200 mg/quarter for up to 4 consecutive milkings and for the intramuscular treatment of infections in veal calves at a dose of 15 mg/kg bw twice a day for up to 3 days. Cefalexin-benzathine is used for the intramammary treatment and prevention of infections in dry cows at a dose of 375 mg/quarter.

   The information requested by the Committee of Veterinary Medicinal Products was provided concerning cattle only.

   Cefalexin is also used in human medicine, predominantly by oral route but parenteral treatment is also possible.

2. The bactericidal activity of cefalexin is the 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. As a result, the high internal osmotic pressure leads to bacterial lysis. Inactivation of cephalosporins by β-lactamases constitutes the most prevalent mechanism of bacterial resistance against cephalosporins. Beta-lactamases against cephalosporins may be encoded both in chromosomes and plasmids.

   A number of pharmacodynamic studies in laboratory animals was provided. The lowest (single) oral dose with pharmacological action (decreased spontaneous locomotor activity and grip strength, interpreted as sedative or relaxant effect) was 30 mg/kg bw in mice. At 30 mg/kg bw this effect was only shortlasting, however, its duration and the number of animals in which it was observed increased dose dependently at 100 and 300 mg/kg bw. At single oral doses of 100 to 300 mg/kg bw also effects were found on pentobarbital sleeping time (increase) in mice, intestinal motility (decrease) in rats and renal function (increased potassium excretion and at the high dose increased urinary chlorine concentrations and decreased urine output).

3. Pharmacokinetic data of cefalexin were available for several laboratory animal species.

   In mice 30 minutes after an oral radiolabelled dose of 16 mg/kg bw, 6 µg equivalents/ml were measured and about 90% of the radioactivity excreted in urine within 24 hours.

   In rats, within the first 24 hours after an oral radiolabelled dose of 16 mg/bw, 84% of the radioactivity was recovered in urine, 15% in faeces. The t_{max}, C_{max} and $t_{½}$ were 1 hour, 3.8 µg/ml and 1.5 hours, respectively. The oral bioavailability in adult rats was 90%.

   In dogs, 2 hours after oral administration of 10 mg/kg bw, 17 µg equivalent cephalexin/ml were measured in blood and more than 50% of the dose was recovered within 6 hours as antibiotic activity in the urine. In orally treated cats (13 to 15 mg/kg bw) serum t_{max}, C_{max} and $t_{½}$ were about 1.5 to 2.5 hours, 13 µg/ml and 1.5 hours, respectively.

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After oral administration cefalexin the highest concentrations of radioactivity or antibiotic activity were found in liver and kidney in mice, rats and dogs. Cefalexin was excreted in rat and dog milk.

4. Pharmacokinetic data were provided for cattle, pigs and sheep.

In non-lactating cattle, after single intravenous administration of 20 mg ^{14}C-cefalexin lysinate/kg bw, the radioactivity in plasma declined from 205 µg equivalents·h/ml, 1 minute after the injection to 4.50 and to 0.20 µg equivalents·h/ml, 3 and 48 hours post injection, respectively. In 10 non-lactating cattle receiving intramuscularly 7 mg cefalexin–sodium /kg bw (in oily vehicle) for 5 consecutive days the following results were seen after the last dose: mean serum C_{max} 9.8 µg/ml, t_{max} 1 hour, $AUC_{0\text{-}96h}$ 22.3 µg·h/ml and elimination half life 1.3 hours.

In lactating cows, after a single intramuscular administration of 7 mg ^{14}C-radiolabelled cefalexin sodium/kg bw in an oily vehicle the mean peak plasma concentration of total radioactivity was 11.8 µg equivalents/ml at 0.5 hour after injection. Single intramammary administration of 200 mg ^{14}C-cefalexin monohydrate into each quarter of lactating cows resulted in a plasma C_{max} of 0.252 to 0.387 µg equivalents/ml, t_{max} of 3 to12 hours, $AUC_{0\text{-}72h}$ of 4.278 to 5.387 µg equivalents·h/ml.

In pre-ruminant calves (n=6), after a single oral administration of 25 mg/kg bw cefalexin the plasma C_{max} was 3.75 µg/ml, t_{max}, 5.33 hours and $AUC_{0\text{-}24h}$, 37.6 µg·h/ml. After repeated intramuscular administrations at 12 hours intervals of 15 mg cefalexin monohydrate/kg bw the mean serum concentrations ranged from 7.94 to 11.6 µg/ml 1 to 2 hours after the injections, respectively.

After a single intravenous administration of 20 mg ^{14}C-cefalexin lysinate/kg bw to non-lactating cattle about 68% of the radioactivity was excreted via urine and about 16% via faeces within 48 hours. The major compound measured in urine and faeces was cefalexin lysinate (measured by HPLC): 78% and 95% in urine samples collected between 0 and 36 hours after dosing, and 53% to 71% in faeces samples collected at respectively 8 to 12 hours and 36 to 48 hours after dosing.

Single intramammary administration of 200 mg ^{14}C-cefalexin monohydrate in each quarter of 3 lactating cows resulted in cumulative excretion of radioactivity of 63% in urine and about 6% in faeces at 72 hours after administration. The parent compound (measured by HPLC) represented more than 83 and 59% of the urinary and faecal radioactivity, respectively.

In 10 sheep, which received intramuscular injections of 10 mg cefalexin sodium/kg bw/day for 5 days the mean serum C_{max} was 14.6 µg/ml, t_{max} 0.5 to 1 hour, $AUC_{0\text{-}96}$ 27.1 µg·h/ml and the elimination half life of 1.3 hours after the last injection.

In 10 pigs, which received intramuscular injections of 10 mg cefalexin sodium/kg bw/day for 5 days the mean serum C_{max} was 13.4 µg/ml, t_{max} 0.5 hour, $AUC_{0\text{-}54h}$ 16.7 µg·h/ml and the elimination half life of 1.3 hours after the last injection.

In humans, oral bioavailability of cefalexin is high. After oral administration of a single dose of 500 mg/person, 87% was eliminated as the parent compound in urine. The t_{max}, C_{max} and $t_{½}$ were 1 hour, 18 µg/ml and 0.7 hours, respectively. In humans, protein binding was 6 to 15%. Cefalexin crossed the placental barrier in humans. Oral dosing of 6 lactating mothers with 1 g of cefalexin resulted in a peak milk concentration of 0.50 ± 0.23 µg/ml after 4 hours. In humans, cefalexin did not enter the cerebrospinal fluid in significant quantities.

5. Acute toxicity data were provided for several animal species. Oral LD_{50} values for mice ranged from 1600 to more than 6200 mg/kg bw and for rats from more than 3000 to more than 12 000 mg/kg bw. In guinea pigs and rabbits a single oral dose of 1000 mg/kg bw killed 1 out of 2 tested males and none of 2 tested females. In cats and dogs no deaths were found at oral doses up to 500 to 1000 mg/kg bw and emesis prevented the testing of larger doses. In monkeys, oral LD_{50} values of more than 450 and more than 1000 mg/kg bw were reported. Parenteral (intraperitoneal, intravenous and subcutaneous) LD_{50} values for mice and rats were in the range of 400 to 1370 mg/kg bw and of more than 3700 to more than 12 000 mg/kg bw. Intraperitoneal LD_{50} values for rats and rabbits were more than 3700 mg/kg and more than 4000 mg/kg bw, respectively. Mice were more susceptible than rats. The main effects observed in mice were polyuria, dehydration, ptosis, hypoactivity and anorexia. Polyuria was also observed in rats, but at higher doses than in mice.

6. Two 3-month oral (gavage) repeated dose studies in rats and dogs were available, with dose levels of 0, 160, 400 and 1000 mg cefalexin (as monohydrate)/kg bw/day.

   In rats, toxicity was found at the high (mortality, kidney toxicity, water intake, adrenal effects, haematology, blood biochemistry) and mid (adrenal effects, haematology, blood biochemistry) dose level. The effects on blood biochemistry might be related to renal and/or adrenal toxicity and/or the effect on water consumption. Effects found at the low dose level were minor (salivation, haemoglobin levels in females, potassium in females, blood protein in males). This level can be considered to be close to the NOEL. However, a clear NOEL could not be derived from the rat study.

   In dogs, slight effects were found on blood biochemistry at the mid and high dose level, salivation and emesis were observed at all dose levels. Considering the salivation and emesis as reactions to the unpleasant taste of the compound, the NOEL in this study was 160 mg/kg bw/day.

   Compilations of summarised data from old oral repeated dose studies were also available.

   In a 35-day and a 6-month gavage study in rats with 0, 1000, 2000 and 4000 mg cefalexin/kg bw/day, treatment related adrenal and renal toxicity was found at the mid and high dose levels. Increased water intake, changes in blood and urinary parameters and increased caecum volume were observed at all dose levels.

   Two oral (gavage and capsules) 3-month studies in rats and dogs with doses of 0, 200, 400, 600 and 800 mg cefalexin/kg bw/day revealed renal toxicity at doses of 400 mg/kg bw/day and higher in both species. Because of the lack of detailed results, NOELs could not be established for these studies.

   From a publication summarising a 380-day dietary study in rats with dose levels of 0, 150 to 250, 300 to 500 and 600 to 1000 mg cefalexin/kg bw/day, a 1-year oral (capsule) study in dogs with dose levels of 100, 200 and 400 mg cefalexin/kg bw/day and a 1-month gavage study in Rhesus monkeys with dose levels of 200 and 400 mg cefalexin/kg bw/day it could be concluded that cefalexin caused haematological effects at all dose levels in the rat study, salivation at the mid and high dose level in dogs and at the high dose level in monkeys and diarrhoea in monkeys at both dose levels. The reports were too little detailed to establish NOELs.

   From the overall set of data in rats, dogs and monkeys it was concluded that an overall NOEL for repeated dose toxicity is expected to be lower than 160 mg/kg bw.

7. Intramuscular tolerance studies in cattle, sheep and pigs and an intramammary tolerance study in cattle were available. The main relevant finding was that cefalexin caused local irritation, and that in sheep and cattle visible amounts of an oily cefalexin sodium formulation may remain at the injection site for at least 1 to 2 weeks after administration.

8. In a 2-generation rat reproduction study with oral (gavage) dose levels of 0, 250, 500 and 1000 mg cefalexin (as monohydrate)/kg bw/day parental effects (salivation, effects on food intake and body weight) were found at all dose levels. A dose of 1000 mg/kg cefalexin/day caused adverse effects on fertility (increased length of gestation, decreased fertility index and decreased live birth index). Fertility index was also affected in the F1-generation at the mid dose (significantly, fertility index equal to 75%) and at the high dose (not significantly, but fertility index equal to 76%, the fertility index for negative control group was 95.8%) females. For this reason the mid dose was no clear NOEL. A NOEL of 250 mg cefalexin/kg bw/day was established for reproductive toxicity in this study.

9. Two oral (gavage) teratogenicity studies in rats and mice were available, with dose levels of 0, 300, 600 and 1200 mg cefalexin (as monohydrate)/kg bw/day in rats and 0, 100, 200 and 400 mg/kg bw/day in mice.

   In the rat study maternal effects (decreased food intake and loose faeces) were found at all dose levels and no evidence for foetotoxicity and teratogenicity was found up to 1200 mg/kg bw/day.

   In the mouse study the NOEL for maternal toxicity (decreased food intake and body weight) and foetotoxicity (decreased body weight) was 200 mg/kg bw/day. No evidence of teratogenicity was found up to 400 mg/kg bw/day.

A compilation of teratology studies contained a summary of a rabbit teratogenicity study with (oral) doses of 0, 100, 200, 400, 600 and 800 mg cefalexin/kg bw/day. Maternal deaths were seen at 400 to 800 mg/kg bw/day, and abortion at 600 to 800 mg/kg bw/day. Foetotoxicity (retarded foetal maturation) occurred at 400 mg/kg bw and higher. From the report it was not clear whether effects on maternal food intake and bodyweight also occurred at the lower dose levels.

Summaries of old oral teratogenicity studies in mice with doses of 0, 200, 400, 800 and 1600 mg cefalexin/kg bw /day (effects: maternal and foetotoxicity at 800 and 1600 mg/kg bw/day, no teratogenicity was observed), 0, 100 and 800 mg cefalexin/kg bw/day (effects: decreased litter size at the high dose level, maternal and postnatal pup toxicity, i.e. dose related effects on body and organ weights, at both dose levels), and 250 and 500 mg cefalexin/kg bw/day (no effects reported) supported the conclusion from the complete study that cefalexin was not teratogenic in mice, but indicated that in mice cefalexin could induce maternal toxicity, foetotoxicity and postnatal toxicity at doses of 100 mg/kg bw and higher.

Summaries of old oral teratogenicity studies in rats with doses of 0, 500 and 4000 mg cefalexin/kg bw /day (effects: maternal and foetotoxicity, effects on body and organ weights at both dose levels, no teratogenicity observed) and 250 or 500 mg/kg bw/day (no effects reported) supported the previous conclusion that in rats cefalexin was not teratogenic, but might elicit maternal toxicity and foetotoxicity at 500 mg/kg bw/day and higher doses.

Overall, it was concluded that in rats and mice cefalexin was not teratogenic up to doses of at least 400 mg/kg bw/day in mice and 1200 mg/kg bw/day in rats, but could elicit maternal and foetotoxicity at all tested dose levels, the lowest of which was 100 mg/kg bw/day in mice.

10. No evidence for mutagenic potential was found in 2 reverse mutation assays in prokaryotes, with and without metabolic activation: one in 5 *Salmonella* strains at concentrations up to 40 µg/plate and the other in 4 *Salmonella* and 2 *Escherichia coli* strains at concentrations up to 1 and 2 µg/ml. Cefalexin did not increase mutation frequency in two mammalian point mutation tests with and without metabolic activation: one at the HPRT locus in Chinese hamster ovary (CHO) cells up to a concentration of 5000 µg/ml and the other at the TK locus in the mouse lymphoma assay up to a concentration of 3700 µg/ml. Evidence for clastogenicity in the absence and not in the presence of metabolic activation was found in 2 *in vitro* cytogenicity tests, one in Chinese hamster ovary (CHO) cells (concentrations up to 2000 and 2500 µg/ml) and the other in cultured human peripheral blood lymphocytes (concentrations 618.3 to 3474 µg/ml). However, no evidence for *in vivo* mutagenicity was found in a micronucleus test in CD-1 mice receiving single oral doses up to 1250 mg/kg bw. Therefore, it was concluded that the potential mutagenic effects of residues of cefalexin were of no toxicological concern.

11. No carcinogenicity studies were carried out. Since cefalexin is considered to be not mutagenic, no evidence for pre-neoplastic changes were found in the repeated dose toxicity studies and the cefalexin molecule does not contain structural alerts, carcinogenicity studies were not considered necessary.

12. No immunotoxicity studies were provided. No evidence for immunological effects was found in the repeated dose toxicity studies. In general, anaphylactic reactions due to cephalosporins are rare and cross-hypersensitivity with penicillins occurs in less than 5% of the patients.

13. In 2 studies *in vitro* MIC_{50} data were determined for 10 genera of bacteria considered representative for human intestinal flora: *Peptostreptococcus* spp, *Clostridium* spp, *Bifidobacterium* spp, *Eubacterium* spp, *Bacteroides* spp, *Fusobacterium* spp, *Lactobacillus* spp, *Enterococcus* spp, *Streptococcus* spp, *Proteus* spp and *Escherichia coli*. In 1 study, the geometric mean MIC_{50} and the lowest MIC_{50} at an inoculum level of 10^7 cfu/ml were 4.0 and 0.25 µg/ml, respectively. In this study, dilution of the inoculum by a factor 10^2 resulted in a decrease of the MIC_{50} value of about a factor 2. In another study, the geometric mean MIC_{50} and the lowest MIC_{50} at an inoculum level of 10^7 cfu/ml were 5.9 and 1.0 µg/ml, respectively. The geometric mean MIC_{50} was based on the geometric mean of 2 values of 4.0 and 5.9 found in these 2 experiments, resulting in an overall estimate of 4.9 µg/ml.

14. In 1 study, the effect of 0.01 and 0.1 µg/ml cefalexin on acid production by 39 dairy starter cultures was examined. Acid production was inhibited by 10% in 1 starter culture at the lowest concentration. Two cultures exhibited a 10 to 11% inhibition in acid production at the highest concentration. In another study, the effect of a cefalexin concentration range on acid production was tested in 7 dairy starter cultures. For 1 starter culture the concentration causing a pH effect of 0.1 unit was 0.043 µg/ml, for the other cultures this concentration was 0.6 µg/ml and higher.

    The MIC values of cefalexin against 10 pure dairy 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 yoghurt, cheese and fermented milk products. With the exception of one strain, the sensitivity of the isolates to cefalexin was not affected by the presence or absence of milk. The most sensitive strains were inhibited by cefalexin at a concentration of 0.5 µg/ml in the presence of milk. Continuous pH measurement was used to monitor the production of acid from 6 mixed commercial dairy starter cultures exposed to different concentrations of cefalexin. Hardly any inhibitory effects of cefalexin upon starter culture performance was found, except for 1 culture which was clearly inhibited when exposed to 0.2 µg/ml cefalexin, while no inhibition occurred at 0.1 µg/ml.

    At 0.2 and 0.4 µg/ml cefalexin did not inhibit the acid production of the two starter cultures investigated.

    From all the set of data provided, it was concluded that milk residues of 100 µg/l is unlikely to adversely affect the growth of commercial starter cultures.

15. Cefalexin is used in human medicine at (divided) oral doses of 1 to 4 g/person/day in adults and of 25 to 50 mg/kg bw/day in children. Side effects at these doses were found only in small percentages of patients (3 to 6%). Most commonly reported were gastrointestinal effects (diarrhoea) and hypersensitivity (skin rash and urticaria).

16. It was concluded that the dose of 100 mg/kg bw/day from a teratogenicity experiment in mice (i.e. the lowest tested dose) should be used as a basis for the establishment of a toxicological ADI. Since still some effects were observed at this dose level, an increased safety factor of 200 was used to derive an ADI of 0.5 mg/kg bw i.e. 30 mg/person.

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

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

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

$$\text{ADI} = \frac{\dfrac{4.9 \times 2}{3} \times 150}{0.15 \times 60} = 54.4 \text{ µg/kg bw i.e. } = 3265 \text{ µg/person,}$$

    which can be rounded to 3300 µg/person

    The following assumptions were made:

    - CF1 = 3 because resistance against cephalosporins can be transmitted by plasmidic and chromosomal transfer;
    - CF2 = 2 to account for the effect of bacterial density, while a factor for potential effects of ß-lactamase production could not be justified by the provided data;
    - 150 g was the weight of the daily faecal bolus;
    - geometric mean MIC_{50} was based on the geometric mean of 2 values of 4.0 and 5.9 found at an inoculum density of 10^7 cfu/ml in 2 different experiments, resulting in an overall estimate of 4.9 µg/ml;

- as in man at least 85% of cefalexin is eliminated by urinary excretion, the fraction of the oral dose available for intestinal gut flora was considered to be 0.15.

18. The microbiological ADI is lower than the toxicological ADI. Therefore it was considered appropriate to retain the microbiological ADI for the safety assessment of cefalexin.

19. No specific investigation of the metabolism of cefalexin has been performed in cattle.

20. Tissue distribution of radiolabelled cefalexin was studied in cattle after intravenous, intramammary and intramuscular administration.

    Single intravenous administration of 20 mg ^{14}C-cefalexin (as lysinate) /kg bw to non-lactating cattle (n=3 per slaughter time) resulted in mean concentrations of radioactivity in kidney, liver, subcutaneous fat, perirenal fat and muscle of respectively 75173, 6130, 4530 and 5297 µg equivalents/kg at 3 hours, and respectively 3397, 333, 187 µg equivalents/kg and below the limit of quantification (30 µg equivalents/kg) for perirenal fat and muscle at 48 hours after administration.

    After a single intramammary administration in three lactating cows of 200 mg ^{14}C-cefalexin (as monohydrate)/quarter the mean concentrations of radioactivity in kidney, liver, subcutaneous fat and muscle were 46, 10, 4, 6 µg equivalents/kg at 72 hours after administration.

    After a single intramuscular administration of 7 mg ^{14}C-cefalexin/kg bw (as sodium salt) to 6 lactating cows the mean-concentrations of total radioactive residue were 42, 228 and 2575 µg equivalents/kg in liver, kidney and the injection site at 4 days after dosing. At this samplig time, the levels of radioactivity in fat, muscle and udder were below the limit of quantification (13 to 40 µg equivalents/kg according to the tissue). The microbiological activity in the edible tissues was below the sensitivity of the microbiological method (less than 62 µg equivalents/kg) except for the injection site. The parent compound could only be quantified by HPLC-MS (mean value of 52 µg/kg).

21. Parent compound concentrations measured by HPLC in kidney, liver, muscle and fat samples of 3 cattle slaughtered 3 hours after single intravenous administration of 20 mg ^{14}C-cefalexin/kg bw (as lysinate) represented about 84%, 56%, 57% and 74% of total radioactivity. At 48 hours after the intravenous injection, 19% of radioactivity in kidney consisted of parent compound, and residues in the other tissues were too low for further analysis. In tissue and milk samples from cattle slaughtered 4 days after a single intramuscular injection of 7 mg/kg bw radiolabelled cefalexin sodium, only very small percentages (less than 5 to 15%) of the radioactive residue co-eluted with parent compound.

22. Total residue depletion studies in other target animals than cattle were not available.

23. A number of non-radiometric tissue residue depletion studies were provided.

    After intramammary treatment of lactating cows with 200 mg cefalexin (as monohydrate) per quarter for 4 consecutive milkings, the mean cefalexin concentrations were 790, 1072, 60, 163 and 65 µg/kg in mammary tissue, kidney, liver, fat and muscle, respectively at 12 hours. Then, they declined to 79 µg/kg in mammary tissue and were below or close to the limit of quantification in the other edible tissues at 4 days. Significant amounts of cephalexin (69 µg/kg) could be measured in mammary tissue at 9 days after last treatment.

    Intramuscular administration of 15 mg cefalexin monohydrate/kg bw on 5 consecutive days to 18 ruminating calves resulted in concentrations below the limit of quantification of the microbiological assay (reported to be 100 µg/kg) in kidney, liver, fat, muscle and injection site after withdrawal periods of 5 and 10 days (the report of this study was not complete).

    No residue concentrations above the limit of quantification were found by HPLC-UV (reported limit of quantification 45 µg/kg) in edible tissues of 12 non-ruminating calves slaughtered 7, 14, 21 and 28 days after intramuscular administration of 15 mg cefalexin (as monohydrate) for 3 days at 12 hours intervals.

No detectable residues were found by bioassay (reported limit of quantification: 60 μg/kg) in tissues of 5 non-lactating cattle slaughtered 4 days after receiving intramuscularly 7 mg cefalexin/kg bw/day for 5 days (as the sodium salt in an oily formulation).

Single intramammary administration of 200 mg ^{14}C-cefalexin monohydrate in each quarter of 3 lactating cows resulted in cumulative excretion in milk from 1st to 6th milking as 5.45 to 13.21% of the dose. The mean total radioactive residue decreased from 5575 μg equivalents/kg at the 1st milking to 52 μg equivalents/kg at the 6th milking after treatment. The parent compound concentration in milk samples collected up to 72 hours post dose was 80 to 100% of total radioactivity.

Milk residue depletion was studied in 6 lactating cows that were intramuscularly treated with 7 mg ^{14}C-cefalexin /kg bw (as sodium salt). Total radioactive residue decreased from 74 μg equivalents/kg at the 1st milking to 10 and to less than 4 μg equivalents/kg at the 4th and 8th milking after treatment. With a bioassay (limit of quantification: less than 62 μg/l) residue concentrations were not detectable. The concentration of parent compound (measured by HPLC-MS) was less 10 μg/kg in milkings 1 to 4.

Milk residue depletion was studied in 10 lactating cows intramammarily treated with 200 mg cefalexin monohydrate/quarter for 4 consecutive milkings. Cefalexin concentrations in milk (determined by HPLC) up to 37 320 μg/l were found during administration. Cefalexin concentrations ranging from 1181 to 37 061 μg/l at the 1st milking after the last dose decreased to less than 10 μg/l at the 13th to 15th milkings after the last dosing.

During and after treatment in milk from 10 cows receiving intramuscular injections of 7 mg/kg bw/day cefalexin sodium (in oily vehicle) for 5 days, the residues of cefalexin detected by bioassay (Delvotest) during the treatment period.

In another experiment in 10 cows, which received daily intramuscular injections of 15 mg cefalexin monohydrate/kg bw/day for 5 days, only traces of antimicrobial activity were found with a number of different bioassays, but these occurred also in milk collected before the first treatment.

24. In 3 sheep and 3 pigs treated intramuscularly with 7 mg/kg bw/day cefalexin (as sodium salt in oily formulation) for 5 days and slaughtered at 10 days after the last injection, no detectable residues were found in edible tissues by bioassay (reported limit of quantification: 60 μg/kg). In 5 sheep and 5 pigs treated for 5 days with 10 mg cefalexin sodium/kg bw/day and slaughtered 3 (sheep) and 2 (pigs) days later, no detectable residues were found in edible tissues by bioassay (reported limit of quantification: 60 μg/kg).

25. A reverse phase HPLC-MS method proposed for the routine monitoring of residues of cefalexin in bovine tissues and bovine milk, described in ISO 78/2 format, with limits of quantification of 10 μg/kg for bovine tissues and milk, and limits of detection of 5 μg/kg for bovine tissues and 1 μg/kg for bovine milk, was validated in accordance with the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community.

Conclusions and recommendation

Having considered that:

- a microbiological ADI of 54.4 µg/kg bw (i.e. 3300 µg/person) was established,
- cefalexin as the free acid was identified as the marker residue as it was concluded to be the major compound with antimicrobial activity,
- insufficient data are available to allow the establishment of MRLs for sheep and pigs,
- a validated HPLC-MS method is available for monitoring of cefalexin in bovine milk and tissues;

the Committee recommends the inclusion of cefalexin in 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 tissues	Other provisions
Cefalexin	Cefalexin	Bovine	200 µg/kg 200 µg/kg 200 µg/kg 1000 µg/kg 100 µg/kg	Muscle Fat Liver Kidney Milk	

Based on these MRL values, the daily intake will represent about 9% of the microbiological ADI.