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

EMEA/MRL/887/03-FINAL
November 2003

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

NAFCILLIN
(Extension to all ruminants)

SUMMARY REPORT (3)

1. Nafcillin is a derivative of 6-amidinopenicillanic acid, i.e. semisynthetic penicillinase-resistant penicillin; other members of the family include methicillin and the isoxazolyl penicillins, e.g. oxacillin, cloxacillin, and dicloxacillin. The compound is an active ingredient in intramammary preparations for treatment of subclinical mastitis and prevention of mastitis in cows during the dry period (single treatment) and for treatment of mastitis in lactating cows (recommended treatment regimen: 1 dose per affected quarter per day for 3 successive days). The products currently marketed contain nafcillin (100 mg) as the sodium monohydrate salt, penicillin (300 000 IU) and dihydrostreptomycin (100 mg) per dose of 3 gram.

   Currently, nafcillin is included in Annex I of Council Regulation (EEC) No. 2377/90 as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Nafcillin	Nafcillin	Bovine	300 µg/kg 300 µg/kg 300 µg/kg 300 µg/kg 30 µg/kg	Muscle Fat Liver Kidney Milk	For intramammary use only

   An application for the extension of the MRLs for nafcillin to sheep after intramammary administration of a slow release product has now been submitted. The nafcillin-containing veterinary medicinal product (100 mg nafcillin as sodium monohydrate and 100 mg dihydrostreptomycin) is intended for treatment of subclinical mastitis and prevention of mastitis in sheep at drying off.

2. Ninety-four pregnant ewes with clinically normal udders received 1 tube (3 grams) of the above intramammary preparation (100 mg nafcillin as sodium monohydrate) per teat immediately after the last milking prior to drying-off. Individual udder milk samples from each ewe were collected at 2, 3, 4, 5 and 6 days after lambing, and at weaning for quantitative microbiological assay of nafcillin (confirmed with penicillinase and beta-lactamase). The limit of detection was 20 µg/l. The average length of the dry period was 112 days (range 85 to 223 days). Nafcillin concentrations were below 20 µg/l in milk from all ewes irrespective of the length of the dry period and the interval between lambing and sampling.

3. Ten dairy ewes received 1 tube (3 grams) of the above intramammary preparation (100 mg nafcillin as sodium monohydrate) into each of the two udder halves immediately after the last milking before drying-off. Plasma nafcillin concentration was determined at 2, 4, 6, 8, 24, 30, 48, 56, 72, 96, 120, 144 and 168 hours after treatment. Nafcillin residues were determined using validated microbiological assay (limit of quantification 0.150 µg/ml).

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 84 47
E-mail: mail@emea.eu.int http://www.emea.eu.int

Nafcillin was only detectable (0.153 µg/ml) in 1 sample 30 hours after treatment. All other plasma samples were below the limit of quantification. Thus, nafcillin is absorbed to a very limited degree from the udder to the blood stream after treatment with the intramammary preparation at drying off.

4. Twenty non-pregnant ewes received 1 tube (3 grams) of the above intramammary preparation into each of the two teats immediately after the last milking before drying-off. Two ewes remained untreated (negative controls). Four ewes were slaughtered at each time point: 21, 35, 49, 70 and 84 days. Plasma, kidney, liver, muscle and fat samples were collected and quantitatively assayed for nafcillin using a microbiological method. The limits of detection in the various tissues ranged from 15 to 20 µg/kg. Nafcillin was not detected in any plasma, kidney, liver, muscle and fat sample at any time point. However, in this depletion study the first sampling point was 21 days after treatment.

5. Ten dairy ewes received 1 tube (3 grams) of the above intramammary preparation into each of the two udder halves immediately after the last milking before drying-off. Five ewes were slaughtered at 3 and 7 days after treatment. Nafcillin concentration in muscle, fat, liver and kidney samples were determined using a validated microbiological assay (the limit of quantification was 19.9 µg/kg for fat and 39.9 µg/kg for muscle, liver and kidney). Nafcillin concentrations were below the limit of quantification in all liver, muscle and fat samples from ewes slaughtered at 3 or 7 days after treatment, but was detectable in kidney samples of 4 of 5 ewes slaughtered at 3 days after treatment (ranging from 45.7 to 46.7 µg/kg) and 3 of 5 ewes slaughtered at 7 days after treatment (ranging from 48.9 to 347 µg/kg).

6. Two hundred and seventeen goats with clinically normal udders were included in the study. The two teats were infused with one tube of the above intramammary preparation per teat immediately after the last milking prior to drying-off. One composite milk sample was collected per animal in the period between 1 and 19 days *post partum*. Milk samples were assayed for nafcillin with a microbiological method. The limit of detection was 15 µg/kg. The length of the dry period was 61.0 ± 14.3 days (range 23 to 156 days). Nafcillin was only detected in a milk sample from 1 goat (concentration: 20 µg/kg) 3 days after kidding (dry period 60 days).

7. The applicant has developed a routine analytical method based on a coupled HPLC/microbiological assay for determination of extractable nafcillin-residues in muscle, liver, kidney and fat tissue. The method is fully validated and sufficiently described in an international recognised format (e.g. ISO 78/2). The limit of quantification-values for nafcillin in tissue samples are 150 µg/kg for muscle, liver, kidney and fat. The limit of detection-values for nafcillin in tissue samples are 19 µg/kg for muscle, 22 µg/kg for liver, 14 µg/kg for kidney, and 17 µg/kg for fat.

   An analytical method was also provided for ovine milk. The method is identical to the method for the determination of nafcillin in cows milk and is based on HPLC succeeded by microbiological assay by agar diffusion as quantification of nafcillin. The method was presented in ISO 78/2 format and validated in accordance with the requirements of Volume 8 of the Rules Governing Medicinal Products in the European Union and the Note for Guidance on the establishment of maximum residue limits for minor animal species (EMEA/CVMP/153a/97-FINAL. The limit of detection of the method was 0.5 µg/kg and the limit of quantification 5 µg/kg.

   Applicability of these methods to other species should not be problematic and therefore from this aspect extrapolation to the tissues and milk of other ruminants would be possible.

8. In application of the guideline "Risk Analysis Approach for Residue of Veterinary Medicinal Products in Food of Animal Origin "(EMEA/CVMP/187/00-Final)" it was considered possible to extrapolate the MRLs for bovine and ovine to further ruminants.

Conclusions and recommendation

Having considered that:

- a microbiological ADI of 4.4 µ/kg bw (i.e. 264 µg/person) was previously established for nafcillin,
- the parent compound is considered to be an appropriate marker residue for milk and all edible tissues,
- a validated routine analytical method for monitoring residues for bovine and ovine including milk is available and that the method is also considered to be applicable to other ruminants,
- nafcillin is intended to be for intramammary administration only;

the Committee for Veterinary Medicinal Products recommends the modification of the current entry of nafcillin in Annex I of Council regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance	Marker residue	Animal species	MRL	Target tissues	Other provisions
Nafcillin	Nafcillin	All ruminants	300 µg/kg 300 µg/kg 300 µg/kg 300 µg/kg 30 µg/kg	Muscle Fat Liver Kidney Milk	For intramammary use only

Based on these MRL values the maximum daily intake of total residues was estimated to be 195 µg per day, equivalent to 74% of the microbiological ADI.