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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

BACITRACIN

SUMMARY REPORT (2)

1. Bacitracin is an antibiotic from the group of peptide antibacterial compounds. In veterinary medicine, bacitracin is used in combination with tetracyclin, neomycin and prednisolone for intramammary treatment of mastitis in lactating cows. The recommended dosage is 2000 IU bacitracin (standard potency 74 IU/mg, i.e. 1 IU/13.5 µg) per infected quarter, to be repeated after 12 or 24 hours if necessary. In the past, bacitracin has been used as a feed additive for poultry, pigs, calves, lambs and kids, but in 1998 the authorisations for this use were withdrawn in accordance with Council Regulation (EC) No. 2821/98.

   In human medicine, (zinc) bacitracin is used in the topical treatment of local infections, often in conjunction with other antibiotics.

   Currently, bacitracin in milk is entered 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
Bacitracin	Bacitracin	Bovine	150 µg/kg	Milk	Provisional MRL expires on 1.7.2001

   and for other tissues except milk, bacitracin is included in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

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

   Additional data on the validation of the analytical were provided in response to the list of questions, further to the establishment of provisional MRLs for bacitracin in milk.

2. Bacitracin is produced by *Bacillus licheniformis* and *Bacillus subtilis*, and is a mixture of several closely related polypeptides, mainly consisting of bacitracin A, B1, B2, C, and a small portion of bacitracin F (with relative ratio's of approximately 53%, 22%, 13%, 8%, and 4%, respectively, although this may vary according to producing species and/or production conditions). The zinc salt adds stability particularly during storage of the product.

   Bacitracin is mainly active against Gram-positive microorganisms. The activities of each of bacitracins B1, B2 and C constitute about 90% of the antibacterial activity of bacitracin A. Bacitracin F is almost inactive.

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3. After oral application to rats, chickens and pigs, bacitracin is hardly absorbed from the gastro-intestinal tract, and the distribution to organs and tissues is negligible. In rats, chickens and pigs, approximately 95% of an oral dose is excreted via faeces, and only 3% or less via urine. Bacitracin is metabolised to amino acids and smaller peptides via the main metabolite desamidobacitracin, which is microbiologically inactive. Main metabolites in faeces are bacitracin A, B1, B2, F, desamidobacitracin and catabolic peptides. In urine and bile only hydrolytic cleavage products (di- and tripeptides) are present.

4. The oral acute toxicity of (zinc)bacitracin is low, with LD_{50} values greater than 1200 mg/kg bw for rabbits, greater than 3750 mg/kg bw for mice and greater than 9500 mg/kg bw for dogs.

5. In two adequately performed studies, rats received feed-grade and/or pure zinc bacitracin by gavage at doses of 0, 36, 72, 144, 250, 500, and 1000 mg/kg bw/day for 28 days (range-finding study), or 0, 11, 34, 150, 250, and 500 mg/kg bw/day for 13 weeks. In these studies the most relevant effects were post-dosing salivation, loose faeces, decreased food utilisation and (13-week study only) minor pathological changes in the stomach. No overall NOEL could be established, as in the 13-week study post-dosing salivation (with brown facial staining) was observed at all dose levels, as well as over-excitation in females from all treated groups. Therefore, 11 mg zinc bacitracin/kg bw/day is regarded as LOEL.

6. In a 1-year study (non-GLP), rats received feed-grade zinc bacitracin in their diet at doses equivalent to 0, 1, 10, and 50 mg/kg bw/day. At the end, the rats that were not sacrificed received control feed and their fertility and reproduction was examined. No toxic effects were observed up to the highest dose tested, although clinical signs were not recorded. There were no signs of nephrotoxicity, which is known to occur after systemic administration of bacitracin. Compared to controls, there was no increase in neoplasms, and the ability to reproduce was not adversely affected.

7. Apart from the limited reproduction data in the 1-year rat study, no data on reproductive toxicity have been provided. This is not considered necessary, as bacitracin is not structurally related to compounds known to have an effect on reproduction, and bacitracin is hardly absorbed after oral administration.

8. In a teratogenicity study, rats received feed-grade and/or pure zinc bacitracin by gavage at dose levels of 0, 11, 34, 150, 250 and 500 mg/kg bw on days 7 to 17 of pregnancy. Zinc bacitracin had no adverse effects on embryo-foetal development, and did not produce irreversible structural malformations up to the highest dose tested. In dams, post-dosing salivation, soft faeces, increased water intake, and slightly decreased body weight gain were noted, resulting in a LOEL of 11 mg/kg bw /day.

9. Zinc bacitracin was negative in *in vitro* tests for gene mutations in *Salmonella typhimurium*, gene mutations in mouse lymphoma cells, chromosomal aberrations in human peripheral lymphocytes and in *in vivo* tests for chromosomal aberrations in rat bone marrow cells, unscheduled DNA synthesis (UDS) in rat spleen cells. It was concluded that zinc bacitracin is non-genotoxic.

10. No carcinogenicity studies were provided. This is not considered necessary, as bacitracin is not genotoxic, has no structural alerts, and there is no indication for carcinogenic potential from repeated dose studies. Besides, bacitracin is not absorbed after oral administration.

11. In an *in vitro* test the MIC-values for bacitracin were determined in a range of bacterial species isolated from the human gut (Gram-positive and Gram-negative anaerobes and aerobes/facultative anaerobes). It was demonstrated that Gram-negative bacteria were not susceptible to bacitracin. The MIC_{50} values for the Gram-positive strains tested ranged from 0.5 µg/ml (*Bifidobacterium spp.*) to 64 µg/ml (*Clostridium spp.*), with a geometric MIC_{50} of 5.7 µg/ml.

12. In the yoghurt inhibition test, the no effect level for bacitracin on the growth (detected by acid production) of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was 540 µg/l.

13. Based on the overall toxicological LOEL of 11 mg/kg bw/day in the 13-week rat study and the rat teratogenicity study and applying a safety factor of 200, a toxicological ADI of 0.055 mg/kg bw (i.e. 3.3 m/person) was established for bacitracin.

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

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

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

$$\text{ADI} = \frac{\dfrac{1.57 \times 1}{1} \times 150}{1 \times 60} = 3.9 \text{ µg/kg bw i.e.} = 234 \text{ µg/person}$$

The following assumptions were made:

- Geometric mean MIC_{50} = 1.57; the variability in MIC_{50}-values for the most sensitive strains tested is calculated as the one-tailed 10% lower confidence limit of the geometric mean MIC50;
- CF1 = 1 because resistance against bacitracin hardly occurs;
- CF2 = 1 because as no information on effects of pH and inoculum sizes on the MIC determinations was provided;
- 150 g was the weight of the daily faecal bolus;
- 1 for the fraction available to gut microorganisms, as bacitracin is not absorbed from the gastro-intestinal tract;

15. As the microbiological ADI is lower than the toxicological ADI, the former is most relevant for the safety assessment of bacitracin.

16. After intramammary treatment of cows with the commercial formulation in two quarters per cow at 4 consecutive milkings, no bacitracin was detected in plasma during and after treatment (limit of detection 40.5 µg/l). Hence, bacitracin is not absorbed from the udder into plasma.

17. After intramammary treatment of lactating cows with the commercial formulation in two quarters per cow at 4 consecutive milkings, residues of bacitracin were determined in muscle, liver, kidney, fat and udder after withdrawal periods of 14, 28, 42, 56, and 84 days after last treatment (2 animals/time point). Bacitracin was not detectable in any tissue at any time point (limit of detection 40.5 to 135 µg/kg). Although no information on tissue residues was provided at shorter withdrawal periods, it is not likely that residues of bacitracin in bovine tissues will occur at earlier time points than 14 days because pharmacokinetic data with the commercial formulation demonstrated that during and after intramammary treatment bacitracin is not absorbed from the udder into the systemic circulation.

18. Residues of bacitracin in milk were studied in 12 cows (daily milk yields 10 to 32 kg) after intramammary treatment with the commercial formulation in 2 quarters per cow at 4 consecutive milkings. Bacitracin was not detectable in milk from untreated quarters (limit of detection 73 µg/l). In milk from treated quarters, however, bacitracin was detected during treatment (1620 to 76950 µg/l), declining to approximately 540 µg/l at the 8th milking after last treatment.

19. Residues of bacitracin A, B, and C were studied in 3 lactating cows following intramammary treatment with the commercial formulation in one quarter per cow at 4 consecutive milkings. Milk samples were taken at the first, third and sixth milking after the last infusion and analysed using LC-MS/MS, the method proposed as routine analytical method. The sum of bacitracin A, B, and C averaged 5091 µg/l at the first milking after last treatment, and declined to 887 µg/l at the third milking and 167 µg/l at the sixth milking. The ratio of the 3 bacitracin components A, B, and C in milk remained constant over time, with mean relative percentages of 55.2%, 35.0% and 9.8%, respectively.

20. Considering that, if metabolised at all, bacitracin is metabolised in microbiologically inactive compounds only, the choice of the parent compound as marker residue is justified. As bacitracin consists mainly of bacitracin A, B and C, the marker residue is defined as the sum of bacitracin A, bacitracin B and bacitracin C. It was not possible to identify only one of these components as the marker residue, because the relative ratio's between the compounds may change depending on the producing bacterial species used and production conditions and the ratio marker to total residues will change accordingly.

21. No pharmacokinetic and residue data were available on the intramammary use of bacitracin in dry cows and on the topical use of bacitracin as wound powder.

22. An LC-MS/MS method was proposed as routine analytical method for the determination of bacitracin A, B and C in bovine milk. The method was described in ISO 78/2 format and fully validated with limits of quantification of 39.8 µg/l for bacitracin A, 26.3 µg/l for bacitracin B, and 6.0 µg/l for bacitracin C.

Conclusions and recommendation

Having considered that:

- the microbiological ADI is 3.9 µg/kg bw (i.e. 236 µg for a 60 kg person),
- bacitracin was the only microbiologically active compound and therefore retained as the marker residue, and since bacitracin consists mainly of bacitracin A, B, and C, the marker residue is defined as the sum of bacitracin A, B, and C,
- bacitracin is hardly absorbed after oral administration,
- the intramammary use of bacitracin in lactating cows resulted in undetectable bacitracin residues in plasma and tissues,
- after intramammary use in lactating cows, the marker residue bacitracin is detectable in milk,
- a fully validated LC-MS/MS method for the routine determination of bacitracin A, B, and C in bovine milk is available,

the Committee recommends the inclusion of bacitracin in milk 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 tissue	Other provisions
Bacitracin	Sum of bacitracin A, bacitracin B, and bacitracin C	Bovine	100 µg/kg	Milk	

Based on the MRL value for milk, the daily intake will represent about 64% of the ADI.