



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

NAFCILLIN

SUMMARY REPORT (2)

1. Nafcillin is a derivative of 6-amidinopenicillanic acid, i.e. a 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 intended 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: one dose per affected quarter per day for three 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 grams.

Currently nafcillin 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 tissues	Other provisions
Nafcillin	Nafcillin	Bovine	300 µg/kg	Muscle	For intramammary use only Provisional MRLs expire on 1.1.2001
			300 µg/kg	Fat	
			300 µg/kg	Liver	
			300 µg/kg	Kidney	
			30 µg/kg	Milk	

Further information has now been received in support of an entry of nafcillin for bovine in Annex I of Council Regulation (EEC) No 2377/90.

2. Nafcillin is active against various Gram positive species, e.g. staphylococci, streptococci and pneumococci. Like the other penicillinase-resistant penicillins, it shows little or no activity against Gram negative microbes. The antibacterial spectrum and the potency of nafcillin, oxacillin and cloxacillin against bovine mastitis pathogens are comparable. On a weight-to-weight basis nafcillin is 4 to 8 times more bactericidal than methicillin.
3. Administered intravenously to dogs in doses from 250 to 500 mg/kg bw nafcillin produced hypotension and bradycardia without affecting the pressor responses induced by epinephrine or norepinephrine. Doses of 500 to 1000 mg/kg bw caused irreversible cardiovascular collapse in some animals, while 1000 mg/kg bw was 100% lethal. Cats were somewhat more sensitive to the pharmacological effects of nafcillin than dogs. Methicillin in comparable doses showed less pharmacological activity than nafcillin.

4. In dogs, absorption following oral administration tends to be poor. Maximum concentration in serum is reached within 30 minutes following intramuscular administration. At similar oral doses peak serum levels are lower and plasma levels are less persistent than observed for methicillin and benzylpenicillin. In contrast to methicillin and especially benzylpenicillin, the liver is the main excretory pathway for nafcillin, the 0 to 24 hour bile accounting for 97%, 56% and 30% of the total dose following intravenous, intramuscular, and oral administration, respectively. Corresponding figures for methicillin and benzylpenicillin following intravenous administration are 22% and 9%, respectively. Concentrations of nafcillin in organs/tissues tended to be higher and more persistent following parenteral administration than is the case for methicillin and benzylpenicillin, apparently due to enterohepatic recirculation for nafcillin.
5. No data on the biotransformation of nafcillin have been provided. However, the fact that all determinations of nafcillin in the kinetic studies were carried out by means of microbiologically based methods coupled with the observed relatively high 0 to 24 hours excretion rates via the liver makes it seem plausible that, like most other penicillins, the substance undergoes biotransformation only to a small extent.
6. The presence of detectable levels in plasma and urine of treated cows shows that nafcillin is absorbed systemically following intramammary administration. After intramammary use in lactating cows the major part of the nafcillin is excreted in the milk, while a higher proportion is absorbed from the udder when nafcillin is administered at drying off. Obviously, systemic absorption in connection with intramammary administration depends on time of treatment as well as product formulation. The proportions of the doses actually absorbed systemically remain unknown, as does to some extent the fate of systemically absorbed nafcillin.
7. Like other penicillins nafcillin is of low toxicity in laboratory animals by single administration. In laboratory rodents the minimum oral lethal dose was above 5000 mg/kg bw; the LD₅₀ following intramuscular administration was 2800 mg/kg bw, and the intraperitoneal LD₅₀ was 1200 mg/kg bw. The intravenous LD₅₀ was 1100 mg/kg bw, which is less than reported for benzylpenicillin (2200 mg/kg bw), but in the same order of magnitude as reported for oxacillin (1500 mg/kg bw). In dogs the LD₅₀ following intravenous administration was 600 mg/kg bw. For all routes signs of toxicity occurred near the LD₅₀ and included occasional mild convulsion followed by depression, loss of muscle tone and dyspnea.
8. As nafcillin belongs to a group of substances with a well known toxicological profile that, apart from their allergenic potential, does not include significant adverse effects in connection with repeated exposure and as there are no indications that nafcillin exhibits a different repeated dose toxicity profile than the other penicillins, no specific data for nafcillin concerning repeated-dose toxicity are considered necessary.
9. The bovine lactating udder showed acceptable tolerance to the currently marketed intramammary products containing nafcillin.
10. Reproductive studies in mice and rats employing doses up to 2000 and 4000 mg/kg bw/day, respectively, provided no evidence of foetal toxicity, including teratogenicity, and no negative effects were observed on postnatal development in the pups. Considering the class of compounds to which nafcillin belongs plus the fact that no teratogenic or other effects of reproductive toxicity of nafcillin were seen in the studies carried out, no additional reproductive toxicity studies are considered necessary.
10. Since nafcillin belongs to a class of compounds considered to be devoid of mutagenic and carcinogenic potential no specific studies on these endpoints for nafcillin are considered necessary.
11. A major determinant for the induction of allergic reactions is the opening of the β -lactam ring leading to formation of penicilloic acids. As the structure of nafcillin was designed to prevent this opening, the allergenic potential of nafcillin is most likely less than that of benzylpenicillin. A Polish study showed, that workers (n = 442) employed in the pharmaceutical industry revealed a higher prevalence of skin reaction following exposure to benzylpenicillin (6.2%) than after exposure to nafcillin (0.8%).

12. The effect of nafcillin on the growth of 74 anaerobic and 30 aerobic bacterial strains obtained from fresh human stool was evaluated after incubation in vitro. MICs were determined for up to ten isolates each of *Enterococci*, coliform bacteria, *Proteus ssp*, *Bacteroides ssp*, *Lactobacillus spp*, *Bifidobacterium spp*, *Prevotella spp*, *Eubacterium spp*, *Clostridium spp*, *Fusobacterium spp*, and anaerobic gram-positive cocci. The lowest MIC₅₀ values for the tested organisms ranged from 1.26 (anaerobic gram-positive cocci) to 128 µg/ml. The geometric mean for the 5 most sensitive species was 3.14 µg/ml.
13. The acidifying activity of three commercially available and widely used yoghurt cultures was significantly affected only at concentrations of nafcillin above 0.075 µg/ml. However, significant effects on cell morphology were observed at lower concentrations. At concentrations above 0.02 µg/ml *Lactobacillus bulgaricus* grew in long thin filaments. At concentrations above 0.05 µg/ml *Streptococcus thermophilus* were swollen and irregular. At concentrations in excess of 0.15 µg/ml only growth of *Lactobacillus bulgaricus* was observed. The effects of oxacillin and nafcillin on yoghurt cultures were qualitatively and quantitatively similar.
14. Nafcillin was originally developed for use in humans. Since the late 1960s the substance has been employed in the treatment of serious infections caused by penicillinase-producing staphylococci, e.g. endocarditis, septicemia, osteomyelitis and pneumonia. The recommended daily dose is 1 g administered intramuscularly; in the case of severe infections up to 6 to 18 g/day may be given in divided doses. The compound is highly bound to serum protein (about 90%). Although formulations for oral administration are available the oral route is not recommended due to irregular absorption. Data concerning adverse effects reported in connection with clinical use in humans have not been presented. Reports have been published associating the development of interstitial nephritis with clinical use of nafcillin. Cases have occurred mainly in patients exhibiting other signs of an allergic reaction concurrent with deteriorating renal function.
15. Based on the formula adopted by the CVMP and on the lowest MIC₅₀ a microbiological ADI was calculated as follows:

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

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

$$\text{ADI} = \frac{\frac{1.26 \times 1}{1} \times 150}{0.7 \times 60} = 4.4 \mu\text{g/kg bw i.e.} = 264 \mu\text{g/person}$$

The following assumptions were made:

- CF1 = 1 because the MIC₅₀ value for the most sensitive species was used
- CF2 = 1 because no data were available to correct for extrapolation from in vitro to the in vivo situation
- 150 ml was the volume of the daily faecal bolus
- 0.7 = the fraction of the oral dose available to the micro-organisms at the distal part of the gastrointestinal tract.

17. Depletion studies carried out in connection with treatment at drying off show that for animals with a dry period greater than or equal to 19 days the residues in all edible tissues were below 300 µg/kg at 24 to 72 hours after calving. The studies also show that with a dry period greater than or equal to 30 days, the residues in milk were below 30 µg/l at the 2nd milking after calving. Similarly, the depletion studies carried out in connection with treatment of lactating cows show that residues in all edible tissues were below 300 µg/kg at 72 hours after cessation of treatment and that residues in milk were below 30 µg/l from the 4th milking onwards, after cessation of treatment.
18. An validated analytical method for the determination of nafcillin in bovine tissues and milk was presented in the ISO 78/2 format and was based on HPLC succeeded by microbiological assay by agar diffusion as quantification of nafcillin. The limits of detection expressed in µg/kg were 0.5 for milk and 0.6 to 1.1 for edible tissues. The limits of quantification expressed in µg/kg were 5 for milk and 10 for edible tissues.

Conclusions and recommendation

Having considered that :

- a microbiological ADI of 4.4 µg/kg bw (i.e. 264 µg/person) was established for nafcillin,
- the parent compound is considered to be an appropriate marker residue for milk and all edible tissues,
- a validated routine analytic method was available,
- nafcillin is intended to be for intramammary administration only;

the Committee recommends the inclusion of nafcillin 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 tissues	Other provisions
Nafcillin	Nafcillin	Bovine	300 µg/kg 300 µg/kg 300 µg/kg 300 µg/kg	Muscle Fat Liver Kidney	For intramammary use only
			30 µg/kg	Milk	

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 of 264 µg/person.