



COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

PENETHAMATE (Extension to all mammalian species)

SUMMARY REPORT (4)

1. Penethamate is the diethylaminoethyl ester of benzylpenicillin. In formulations intended for veterinary use the compound is incorporated as the hydriodide. Penethamate hydriodide is used in intramammary products for treatment of mastitis in cows and as an injectable solution for treatment of bacterial infections in swine and cattle.

Currently, penethamate is included 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
Penethamate	Benzylpenicillin	Bovine	50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg 4 µg/kg	Muscle Fat Liver Kidney Milk	
		Porcine	50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg	Muscle Fat Liver Kidney	

Previously, provisional MRLs with the same values as the ones for bovine and porcine species had also been established for ovine species. The MRLs however expired as no validated analytical method was provided.

2. A request was submitted to the EMEA for the extension of the existing entry in Annex I of Council Regulation (EEC) No. 2377/90 to all mammalian species. The scientific justification for this extension was assessed taking into account the Note for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMA/CVMP/187-00-FINAL).
3. In the original assessment of penethamate, the following data were considered:
4. Penethamate is a prodrug from which benzylpenicillin and diethylaminoethanol are released by hydrolysis. Antimicrobial activity of the compound is exclusively related to benzylpenicillin.
5. Penethamate and procaine benzylpenicillin have similar profiles with respect to pharmacological properties. Penethamate possesses local anaesthetic activity, an effect also observed with other esters of diethylaminoethanol. In experimental animals rapid intravenous administration gives rise to anticholinergic (atropine-like) and cardiac depressing effects. These effects are not observed in connection with intramuscular injection.

6. Penethamate is a base with a pK_a of 8.4 at room temperature. At pH 7.2, 8% of the drug will be present as the uncharged molecule, while 92% will be in the cationic state.
7. Penethamate is rapidly converted into benzylpenicillin. At 37°C and pH 7.3 (physiological conditions) the half-life of penethamate in aqueous solution is 23 minutes. In tissue homogenate at 32°C half of the penethamate was hydrolysed within two hours and at 20 hours no penethamate remained.
8. Benzylpenicillin itself is of low toxicity. Consequently, the toxic effects of the penicillin esters will depend on the alcohol released by hydrolysis. Since procaine penicillin and penethamate hydroiodide both contain an esterified diethylaminoethanol group, which is released by hydrolysis, the two compounds have similar toxicological profiles.
9. In laboratory animals the LD_{50} of penethamate hydroiodide is 2000 mg/kg bw following oral or subcutaneous administration, 300 to 1650 mg/kg following intraperitoneal administration, and 30 to 90 mg/kg bw in connection with intravenous administration.

The oral LD_{50} of diethylaminoethanol in rats is 1300 to 5600 mg/kg bw. The LD_{50} following intraperitoneal administration is 1220 mg/kg bw. Like several other aliphatic amines diethylaminoethanol is an ocular and mucous membrane irritant. The small amounts of diethylaminoethanol released by hydrolysis of penethamate make any toxicological risk from this compound appear unlikely.

10. In repeated-dose toxicity studies subcutaneous administration of penethamate hydroiodide at a dose of 200 mg/kg bw daily for 7 weeks to rats did not affect growth rate or give rise to significant changes in haematological parameters. Nor were any treatment-related abnormalities detected at autopsy at the end of the study period. Similar results were seen in a study in rabbits treated by intramuscular injection with a daily dose of 25 or 50 mg/kg bw for 20 to 36 days.
11. No formal studies have been carried out regarding reproductive toxicity, mutagenic and carcinogenic potential, immunotoxicity, and antimicrobial properties of residues in animals treated with penethamate. Since penethamate is quickly hydrolysed to benzylpenicillin within the body, there is little reason to assume penethamate to act significantly different from benzylpenicillin. Benzylpenicillin belongs to a group of substances with a long history of safe use, which, apart from their allergenic potential, does not include significant adverse effects in connection with repeated exposure.
12. More than 400 persons have received penethamate hydroiodide in studies. From these, it appears that the toxicity of penethamate hydroiodide is low and comparable to that of procaine penicillin. Penethamate has been authorised for human use in several European countries and in the United States of America.
13. In considering penicillins as a group the CVMP noted that penicillins have a low toxicity; the therapeutic index is more than 100, and toxic effect have only been seen after extremely high doses. No teratogenic effects have been recorded.

In connection with therapeutic use of penicillins hypersensitivity reactions are by far the most commonly encountered side-effects. The amount of penicillin hapten necessary to sensitize a subject is several orders of magnitude higher than the quantity needed to trigger an allergic reaction in an already sensitized individual. Furthermore, it takes a much higher oral dose to induce an allergic reaction than if the drug is administered parenterally. Against this background it can be concluded that the small amounts of penicillins which may be present in food products of animal origin are not able to sensitize the consumer, but will, at the most, trigger a reaction in already sensitized subjects. Literature contains only very few references reporting allergic reactions traceable to penicillin residues in foods. At least 10 IU penicillin seems normally necessary to provoke an allergic reaction.

Penicillins are strong inhibitors of the bacteria used in production processes employed by the dairy industry. Concentrations as low as 0.01 IU penicillin per ml milk considerably inhibit starter cultures and delay acid production. In order to secure dairy products of acceptable quality, penicillin residues in milk must not exceed 0.005 IU.

In order adequately to protect the consumer and secure dairy production, the CVMP recommended maximum residue levels of 50 µg/kg for tissues and 4 µg/kg for milk (1 IU of benzylpenicillin 0.6 µg).

14. For the extension to include all mammalian species in the Annex I entry for penethamate the following information was taken into account:
15. As penethamate is rapidly converted into benzylpenicillin and the data available with regard to benzylpenicillin were considered relevant for the assessment of penethamate, no additional residue depletion studies were considered necessary.
16. Since the hydrolysis of penethamate to benzylpenicillin is rapid there is little reason to assume that penethamate will act significantly different from benzylpenicillin. Benzylpenicillin was retained as the marker residue for the establishment of MRL values for penethamate.
17. Benzylpenicillin has MRLs established for all food producing species. The established MRLs for benzylpenicillin and the ones established for penethamate in bovine and porcine species are identical, as were the provisional ones for ovine species. Therefore, it is reasonable to extrapolate the MRL values for penethamate in such a way that the same values would apply to all mammalian food-producing animals.
18. A validated HPLC-MS method for determination of penethamate (determined as benzylpenicillin) in porcine tissues, described in ISO 78/2 format is available. The limit of quantification is 25 µg/kg for porcine liver, kidney, muscle and fat. The limit of detection is 4.5 µg/kg in fat, 9.5 µg/kg in muscle, 6.3 µg/kg in liver and 8.3 µg/kg in kidney. A published RP-HPLC analytical method with UV detection is available that has been validated for bovine kidney, liver and muscle tissues and kidney, liver and muscle tissues from sheep. The limit of detection is 5 µg/kg for benzylpenicillin kidney, liver and muscle tissues.

A validated capillary gas chromatographic method with thermionic detection for quantification of benzylpenicillin in bovine tissue and milk is available. The limit of quantification is 3 µg/kg for milk and 10 µg/kg for edible tissues. The limit of detection is 0.9 µg/kg for milk and less than 2 µg/kg for edible tissues.

Furthermore, analytical methods routinely used for surveillance of benzylpenicillin residues in bovine and porcine muscle and kidney and ovine tissues, are also available. These methods should be acceptable for residue surveillance also in other mammalian species. The HPLC-MS method developed for porcine tissue would be the preferable method for use in other mammalian tissues, as no derivatisation of the analyte is necessary and because MS detection are relatively specific compared to UV detection and both MS and HPLC equipment are expected to be widespread and commonly used equipment in national control laboratories.

19. Considering the data above the Committee considered it acceptable to extrapolate the existing MRLs for bovine and porcine species to all mammalian food producing species.

Conclusions and recommendation

Having considered that:

- penethamate is a β -lactam antibiotic which has a long history of safe use in food-producing animals and is thus a well-known substance,
- like the other penicillins, penethamate is of very low oral toxicity,
- penethamate is rapidly and completely hydrolysed to benzylpenicillin for which MRLs have been established with benzylpenicillin itself as marker residue,
- due to the rapid hydrolysis of penethamate benzylpenicillin was chosen as the marker residue for penethamate,
- MRLs were previously established in bovine and porcine tissues; these MRLs are identical and are the same as those established for benzylpenicillin in all food producing species,
- analytical methods for the monitoring of residues in tissues and milk were available;

the Committee for Medicinal Products for Veterinary Use recommends the modification of the current entry for penethamate 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
Penethamate	Benzylpenicillin	All mammalian food-producing species	50 µg/kg 50 µg/kg 50 µg/kg 50 µg/kg 4 µg/kg	Muscle Fat Liver Kidney Milk	