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

EMEA/MRL/782/01-FINAL
March 2001

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TOSYLCHLORAMIDE SODIUM
(Extension to bovine)

SUMMARY REPORT (2)

1. Tosylchloramide sodium (N-chloro-p-toluenesulfonamide, sodium salt, chloramine T) is an organic chlorine compound with biocidal effect, with an available chlorine content of 28 – 30%. It is used in fish farming for prevention and control of bacterial gill disease as follows: 10 mg/l in water of flow-through basin for 1 hour for preventive purposes, which may be repeated every 15 to 30 days; 10 mg/l in water of flow-through basin for 1 hour for therapeutic purposes, which may be repeated up to 3 times within 1 week.

   Currently, tosylchloramide sodium is included in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance	Animal species	Other provisions
Tosylchloramide sodium	Fin fish	For water borne use only

   An application for the extension to use in bovine has now been received. Tosylchloramide sodium is intended for teat and udder disinfection to prevent udder disease in lactating cows. To obtain effective concentrations at least 50 to 100 mg active chlorine per litre solution are required. This is achieved if a 0.3% tosylchloramide solution is used. The teats of lactating cows are dipped in the solution after each milking throughout the lactation period and in nonlactating cows once daily. According to prescription 0.5 ml of the solution ready for application (3 mg per ml) should be used for each teat, i.e. 6 mg per animal.

2. Tosylchloramide sodium has a biocidal effect, which partly depends on the separation of chlorine from the molecule and partly on irreversible reactions to organic material of the tosylchloramide ion itself. It has effect against a wide range of bacteria, viruses, fungi and parasites. In a summary of published efficacy studies it was shown that the substance has effect against mastitis pathogens such as *Escherichia coli*, *Pseudomonas pyoceanea, Staphylococcus aureus* and *Streptococcus agalactiae* at concentrations of 0.5% in the solution. Bactericidal effect was tested *in vitro* with a suspension test without protein burden. The effect was observed at concentrations at 0.004% and 0.25%, and 5 minutes of action. In addition, the same test with protein burden resulted in bactericidal effects at concentrations between 0.015 and 0.25%, and 15 minutes of action. Water solutions of tosylchloramide have inhibiting effects on Gram negative bacteria, *Escherichia coli* and *Pseudomonas fluorescens,* at concentrations of 300 µg/ml and on Gram positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus,* at concentrations of 2000 µg/ml and 100 µg/ml, respectively.

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3. The percutaneous absorption of tosylchloramide sodium was investigated. Five lactating cows were treated twice daily during milking for 8 days. The teats were cleaned before milking with udder tissues dipped in a solution containing 0.5% tosylchloramide and thereafter dipped after milking into the same solution. The solutions containing 0.5% tosylchloramide were freshly prepared every day. Blood samples were taken from the Vena jugularis during the treatment, immediately before the last treatment and 30 minutes, 1, 2, 4, 8, 16 and 24 hours after the last treatment. The samples were tested with a High Pressure Liquid Chromatography (HPLC) method where tosylchloramide is hydrolysed into para-toluenesulfonamide before analysing of the samples. The detection limit of the method was 5 µg/kg of para-toluenesulfonamide and 8 µg/kg for tosylchloramide. No residues of para-toluenesulfonamide could be detected in any blood samples. It is concluded that the absorption of tosylchloramide in blood is negligible after percutaneous application to the teat.

4. A set of toxicity studies concerning tosylchloramide sodium, para-toluenesulfonamide, ortho-toluenesulfonamide and a mixture of para-toluenesulfonamide and ortho-toluenesulfonamide were previously presented in the application for fin fish. An ADI could not be established from these data. However, the highest dose tested without any effect after treatment with tosylchloramide sodium was 15 mg/kg bw in a 90 day study in rats.

5. The efficacy studies show that the substance might have effects on bacteria that are present in the human gut flora e.g. *Escherichia coli* . Tosylchloramide was tested for its *in vitro* activity against 6 bacterial strains of the human gut flora and the minimum inhibitory concentrations (MIC) were determined with the agar dilution test method. The MIC value was 1600 µg/ml for *Lactobacillus acidophilus*, 3200 µg/ml for *Escherichia coli* and *Clostridium* and 6400 µg/ml for *Enterococcus faecalis, Enterococcus faecium* and *Proteus mirabilis*. Considering the low residues (5 to 9µg/kg) that can be found in milk after treatment with tosylchloramide sodium it can be concluded that the residues of tosylchloramide sodium are devoid of activity on the bacteria in the human intestinal flora after intake of milk coming from treated udders.

6. Bacteria in 2 yoghurt starter cultures (Hansen Boll 3 and Bulgarian starter) and a starter H (mesophilic starter with Leuconostoc as a flavour producer) were tested in the pH test for sour activity. With an exposure to 300 mg/kg tosylchloramide sodium a small decrease of the sour activity of the 2 yoghurt starters as well as in starter H was observed. A new study was submitted investigating the effects of tosylchloramide on the growth of various starter cultures. The study was performed according to current guidelines. The substances tosylchloramide sodium and para-toluenesulfonamide, and the product containing 60% tosylchloramide sodium were tested at different concentrations on the mesophilic DL-starter Probat 505, the thermophile yoghurt culture V2 and single strains of *Lactococcus lactis* (8 strains), *Streptococcus thermophilus* (6 strains) and *Lactobacillus bulgaricus* (4 strains). The lowest NOEL was found for the product containing tosylchloramide at a concentration of 50 mg/kg, for tosylchloramide sodium at a concentration of 100 mg/kg and for para-toluensulfonamide at a concentration of 200 mg/kg. It was shown that the metabolite has weaker effect on the tested starter cultures compared to undiluted product and tosylchloramide sodium itself. Thus, it was shown that tosylchloramide sodium and its main metabolite para-toluensulfonamide have no effect on starter cultures at residue concentrations resulting from use as a teat dip.

7. The residues of tosylchloramide sodium in milk after teat dipping were investigated. Twenty bulk milk samples were taken from 5 farms with different number of cows (4 samples per farm during the same week; 12, 24, 28, 28 and 18 cows per farm) and different milk yields (180 to 610 kg per day). Furthermore, individual milk sampling was carried out on 1 farm from 5 cows (milk yields between 20 and 30 kg per cow and day). The milk samples were taken from the measuring can containing the individual milk of each of the 5 tested cows during the evening milking on 5 successive days. The teats were immediately dipped after milking. Tosylchloramide sodium was hydrolysed to the metabolite para-toluenesulfonamide before analysis with an HPLC method. The limit of detection for para-toluenesulfonamide was 5 µg/kg in milk. No residues of para-toluenesulfonamide were found in bulk milk, in the control sample or in 16 out of 25 individual milk samples. In the remaining 9 samples 5 to 9 µg/kg para-toluenesulfonamide was found.

Conclusions and recommendation

Having considered the criteria laid down by the Committee for the inclusion of substances in Annex II to Council Regulation (EEC) No 2377/90 and in particular that:

- the absorption of tosylchloramide sodium is negligible after topical application to the teat,
- residues of tosylchloramide sodium after teat dipping are far below the concentrations that show activity against microorganisms in the human gut flora and activity on the microorganisms used in dairy starter cultures,
- the amount of residues likely to be ingested by the consumer is very low and of no toxicological concern;

the Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for tosylchloramide sodium and recommends its inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Tosylchloramide sodium	Bovine	For topical use only