The European Agency for the Evaluation of Medicinal Products

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TOSYLCHLORAMIDE SODIUM

SUMMARY REPORT (1)

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

2. Tosylchloramide sodium is a biocidal agent, acting on the affected gills against a wide range of bacteria and fungi. The biocidal effect results from the separation of chlorine, which reacts with bacterial proteins and nucleic acids and from an irreversible binding of the tosylchloramide ion itself to organic material such as bacteria, viruses and fungi. Due to the mechanism of the biocidal action, is unlikely that any microbial resistance will develop.

   No studies have been presented that show other pharmacodynamic effects of tosylchloramide sodium.

3. No pharmacokinetic studies in mammals are available concerning either tosylchloramide sodium or para-toluenesulfonamide.

4. A set of toxicological studies is available concerning tosylchloramide sodium, para-toluenesulfonamide, the major metabolite in fish, another closely related substance, ortho-toluenesulfonamide, and defined mixtures of para- and ortho-toluenesulfonamide.

   The ortho-toluenesulfonamide is not formed either in water solutions of tosylchloramide sodium or as a metabolite in fish. Thus, the results of the studies with ortho-toluenesulfonamide are not relevant for the safety evaluation of tosylchloramide sodium.

5. The oral LD_{50} of tosylchloramide sodium is 935 and 1100 mg/kg bw in the rat and mouse, respectively. Gastric inflammation, apathy and gastric bleeding as well as intestinal haemorrhage were observed in animals which died.

   Para-toluenesulfonamide has an oral LD_{50} in the rat of 2330 mg/kg bw.

   LD_{50}-values for a mixture of ortho- and para-toluenesulfonamide (41% and 51%, respectively) in rats was approximately 2400 mg/kg bw.

   Tosylchloramide sodium is corrosive to skin and an extreme irritant for the ocular mucosa, wheras para-toluenesulfonamide has no significant irritation potential.

6. In a 28-day feeding study, rats were exposed to diets containing tosylchloramide sodium at doses of 0, 3000, 10 000 or 30 000 mg/kg feed, equivalent to approximately 150, 500 or 1500 mg/kg bw/day. Significantly reduced weight gain, slightly increased leukocyte count and pale discoloration of liver were present at doses equal to or higher than 10 000 mg/kg. Relative kidney and liver weight were increased in all treated groups. No significant treatment-related histological alterations were present. A NOEL was not identified.

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In a 90-day feeding study, Wistar rats (10/sex/group) were exposed to diets containing 0, 100, 300, 1000 or 3000 mg/kg feed of tosylchloramide sodium, equivalent to approximately 5, 15, 50 or 150 mg/kg bw/day. A slight reduction of weight gain and food efficiency was present in females at 3000 mg/kg feed. Relative kidney weight was significantly increased in both sexes at doses equal to or higher than 1000 mg/kg feed. In females at 1000 and 3000 mg/kg feed an increased severity and frequency of calcareous deposits in kidneys was observed. The NOEL was 300 mg/kg feed, equivalent to approximately 15 mg/kg bw.

In a 90-day feeding study, rats were exposed to diets containing 0, 300, 1000 or 3000 mg/kg feed of a mixture of para-toluenesulfonamide (68%) and ortho-toluenesulfonamide (32%), equivalent to approximately 15, 50 or 150 mg/kg bw/day. A slight reduction of weight gain and food consumption was present at 3000 mg/kg feed as the only treatment-related effect. As no haematological, biochemical urinalysis and histopathological parameters were studied in the 1000 and 3000 mg/kg feed groups, no NOEL can be derived from this study.

No treatment-related effects were observed in a 90-day feeding study on dogs exposed to doses up to 3000 mg/kg feed of a mixture of para-toluenesulfonamide (68%) and ortho-toluenesulfonamide (32%), equivalent to 75 mg/kg bw/day.

7. In a two-generation lifetime feeding study in Sprague-Dawley rats with the related substance ortho-toluenesulfonamide, weanlings (50/sex/group) were exposed to diets containing 0, 2.5, 25 or 250 mg/kg feed, or 250 mg/kg feed combined with 1% NH_4Cl in drinking water. The dietary levels were equivalent to approximately 0.125, 1.25, 12.5 and 12.5 mg/kg bw/day. After three months the F_0 animals were mated. From each group, 50 F_1 pups of each sex were randomly selected and kept on the the same dietary levels as the parental generation. F_0 animals were terminated after 142 weeks of test, while F_1 rats were terminated after 127 weeks.

   Systemic toxicity was observed only in F_0 and F_1 at the top dose level (either with and without NH_4Cl); signs included reduced weight gain and dose-related increases of several histological alterations such as reactive hyperplasia and haemosiderosis of the spleen, and centrilobular foci of basophilic cells and peliosis in liver. However, no histological alterations were consistently present in both sexes of both generations. The NOEL for systemic toxicity was 25 mg/kg feed, equivalent to 1.25 mg/kg bw/day.

   No impaired fertility or indication of teratogenic effects were seen. A significant reduction of average litter size and neonatal weight were observed at 250 mg/kg feed. No effects were seen at 25 mg/kg feed, equivalent to approximately 1.25 mg/kg bw.

8. A repeated dose toxicity and reproductive/developmental toxicity screening test on para-toluenesulfonamide was performed according to the relevant OECD guideline. Rats were treated by gavage with 0, 120, 300 or 750 mg/kg bw, males for 42 days before mating and females for 14 days before mating up to day 3 of lactation. Dams and litters were examined on post-natal day 4.

   Dose-related hypersalivation was evident in all treated groups. In the males of the highest dose group dark coloured livers were seen at gross pathology and thickening of the urinary bladder epithelium at histopathological examination. In addition, blood chemistry analyses demonstrated increased levels of blood urea nitrogen, serum aspartate aminotransferase and chloride in both sexes of the two highest groups and increased serum alanin aminotransferase levels in the high dose males. Reduced weight gain and food consumption were present in adults at the high and the medium dose level. No teratogenic effect or impaired fertility were observed. At 750 mg/kg bw a significant reduction of neonatal survival and body weight were present. No effect on litter size or survival were seen at 300 mg/kg bw. As no raw data and no complete report were provided for this study, no conclusion could be reached.

   In a GLP compliant embryotoxicity/teratogenicity study, pregnant rats were treated by gavage on gestational days 6 to 15 with 0, 50, 250 or 500 mg/kg bw of a mixture of para-toluenesulfonamide (68%) and ortho-toluenesulfonamide (32%). At 250 and 500 mg/kg bw maternal weight gain was significantly reduced during the treatment period. At the same dose levels, postimplantation loss showed a dose-related increase and foetal weight was reduced. No teratogenic effect was observed. The NOELs for maternal toxicity and embryotoxicity/fetotoxicity were 50 mg/kg bw.

9. The genotoxicity of tosylchloramide sodium was assayed in a set of test, including: a non-GLP *Salmonella*-microsomal test (*Salmonella typhimurium* strains TA98, TA100, TA1535, TA 1537 and TA 1538 with and without metabolic activation); a non-GLP DNA-repair test on *Escherichia coli*, with and without metabolic activation; a GLP compliant gene mutation assay in mouse lymphoma L5178Y cells with and without metabolic activation; a GLP compliant micronucleus assay in mice treated by gavage with 300, 600 and 1200 mg/kg bw/day for 2 days. All tests provided negative results.

   In a non-GLP *Salmonella*-microsomal test para-toluenesulfonamide was evaluated in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA 1537 and TA 1538 with and without metabolic activation. No mutagenicity was observed.

   It is concluded that tosylchloramide sodium and the metabolite para-toluenesulfonamide are devoid of genotoxic potential.

10. No carcinogenicity studies were performed on tosylchloramide sodium or para-toluenesulfonamide. Considering also the lack of genotoxicity shown by tosylchloramide sodium, no further testing for carcinogenicity is deemed necessary.

11. No specific studies on immunotoxicity are available.

    In repeated dose toxicity studies, neither tosylchloramide sodium nor para- or ortho-toluenesulfonamide induced significant alterations on haematological parameters and tissues involved in the immune response.

12. No specific studies on human gut microbes have been presented.

    The bacterial toxicity of tosylchloramide sodium has been determined using the inhibition of oxygen uptake of *Pseudomonas putida* in water: an EC_{10} of 10 mg/l. was determined. The type of study and the species used are not relevant to bacteria of the human intestinal flora.

    Chloramine acts on bacterial and fungal cells as a disinfectant, not as an antibiotic. Moreover, any resistance is unlikely to develop, due to the biocidal mechanism of action. Therefore, studies on antibacterial effects in order to determine a microbiological ADI are not needed.

    Since tosylchloramide sodium is intended for use only in aquaculture, no data are needed with regard to microorganisms used in industrial food processing.

13. No data are available concerning the human effects of tosylchloramide sodium or related compounds.

14. An ADI could not be established from the available data. Nevertheless the presented toxicity studies and the fact that very low residues of tosylchloramide sodium and para-toluenesulfonamide are found in fish during and immediately after the bath treatment demonstrate that the residues likely to be ingested are of no toxicological concern to the consumer.

15. Fingerlings and juvenile trouts were exposed to 20 mg/l (twice the therapeutic concentration) of ring UL-^{14}C tosylchloramide sodium (purity 93.7%, specific activity 1.2 µCi/µM) for up to 1 hour and then transferred to fresh water for recovery to assess tissue accumulation and distribution of resulting residues. The temperature of the well water was 11.6 to 12.2 °C.

    The estimated half-life of para-toluenesulfonamide equivalents in fingerlings was 27.3 hours whereas determined by HPLC the half-life of para-toluenesulfonamide residues in whole-body homogenates was 36.3 hours. The estimated half-life of residues in juvenile fish was 32.6 hours, based on radiometric data, while determined by HPLC the half-life for para-toluenesulfonamide residues in whole body samples was 40.3 hours.

    Elimination of total tosylchloramide sodium residues from fingerlings and juvenile whole-body homogenates, based on radiometric counts, was rapid but significantly faster from fingerlings ($t_{1/2}$ of 27.3 hours) than from juveniles ($t_{1/2}$ of 32.6 hours).

16. Fifteen adult rainbow trout were exposed to water solution of ^{14}C tosylchloramide sodium (purity 93.7%, specific activity 1.2 μCi/μM) at a concentration of 20 mg/l (twice the proposed treatment concentration) for a period of 1 hour and then transferred to fresh water. The temperature of the well water was 11 to 13°C. Samples of muscle fillet, residual carcass and gall bladder bile will be collected, frozen and stored for subsequent analyses. Residues of tosylchloramide sodium were not observed in any of the fish tissues analysed for this study. Tosylchloramide sodium was rapidly reduced to the primary metabolite para-toluenesulfonamide, but the levels were not quantified.

    In the above decribed study in fingerling and juvenile trouts tissue concentrations of tosylchloramide sodium residues were assessed by oxidation-liquid scintillation counting and by HPLC (limit of detection: 10 μg/kg para-toluenesulfonamide). Isolation and purification of a suspected metabolite of tosylchloramide sodium from whole body homogenates and fillet tissue was accomplished by gel permeation chromatography and HPLC.

    Tosylchloramide sodium was poorly absorbed from the bath by both fingerling and juvenile trout. No residues of tosylchloramide sodium, only of the primary metabolite para-toluenesulfonamide, were found in any of the fish tissues in this study therefore, all tissue residues determined either by radiometric or by HPLC methods were reported on the basis of equivalent concentrations of para-toluenesulfonamide. The para-toluenesulfonamide equivalents concentration in whole body homogenates after 1 hour, based on radiometric analysis, was 980 μg/kg, a value about 5% of that in the exposure water, in fingerlings. In juveniles, this value was 570 μg/kg or about 3% of the concentration in the exposure bath. The exposure of para-toluenesulfonamide in whole body homogenates, after 1 hour, based on HPLC analyses was 360 μg/kg in fingerlings and 170 μg/kg in juveniles.

    Differences in the concentration estimates of para-toluenesulfonamide equivalent residues between the radiometric and HPLC data and differences in the rates of clearance of the residues suggested the presence of a second unidentified tosylchloramide sodium metabolite in the tissues. However, 24 hours after termination of the treatment the radiometric data and HPLC analyses indicate that all activity was consistent with para-toluenesulfonamide. Therefore the occurence of a small amount of the unknown metabolite is not considered of relevance to the safety of the consumer.

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 animals are unlikely to be sent for slaughter during or immediately after treatment,
- tosylchloramide sodium is poorly absorbed and rapidly and extensively metabolised and excreted,
- the amount of residues likely to be ingested by the consumer is very low and of no toxicological concern;

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

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