



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TRICAINE MESILATE

SUMMARY REPORT

1. Tricaine mesilate (synonym: tricaine methanesulfonate, MS 222, *m*-aminobenzoic acid ethyl ester methanesulfonate), is normally a local anaesthetic, but when fish are immersed in its solution, the substance acts on the central nervous system as a general anaesthetic. An aqueous solution of the product prepared from its powder form is used as an immersion bath for temporary immobilisation, anaesthesia or sedation of *Salmonidae* and other fin fish. The employed concentrations of tricaine mesilate range from 15 to 200 mg/l (depending on type of fish and reason for sedation), with exposure times ranging from a few minutes for high concentrations and up to 48 hours for low concentrations. The recovery time varied between 1 and 30 minutes.

The maximum tolerable exposure time for *Salmonidae* at water temperatures of 10 to 17°C and concentrations of 80 to 135 mg/l is 4 to 12 minutes. At higher concentrations the exposure time limit is less. At concentrations of 50 to 60 mg/l the maximum tolerated exposure time is 30 minutes. Tricaine mesilate should not be used in water of a temperature of less than 10°C during first or second year smolt production.

2. Tricaine is structurally closely related to local anaesthetics. The structure of typical local anaesthetics contains a hydrophilic (tertiary or secondary amine group) and a hydrophobic (aromatic residue) domain, separated by an intermediate alkyl chain. Linkage to the aromatic group is of either the ester or amide type, and the nature of this bond determines certain of the pharmacological properties of these agents. Tricaine mesilate possesses an ethyl group attached by an ester linkage to aminobenzoic acid and the active component is therefore an isomer of benzocaine. It is distinct from benzocaine and procaine in having a *meta*-arrangement rather than a *para*-structure. No amide group is present.
3. An incomplete pharmacodynamic profile of tricaine mesilate is available. Tricaine mesilate acts on the central nervous system, cardiovascular system, neuromuscular junctions and ganglion synapse. Tricaine mesilate effectively crosses the blood-brain barrier. The free drug was detected in the brain within one minute following intravenous administration. Tricaine mesilate accumulates also in ventricular tissue and causes decreased cardiovascular function, decreasing blood flow through the gills, decreasing oxygen consumption in a dose-dependent manner. Its mechanism of action is the prevention of the generation and conduction of nerve impulses. Local anaesthetics block conduction by decreasing or preventing the large transient increase in the permeability of excitable membranes to Na⁺ that is produced by a slight depolarisation. This action of local anaesthetics is due to their direct interaction with voltage-sensitive Na⁺ channels. No further information is available.

None of the identified metabolites of tricaine mesilate is pharmacologically active.

4. Pharmacokinetic studies show that tricaine mesilate is absorbed through the gills when fish are immersed in solutions containing the anaesthetic agent. Tricaine mesilate enters the blood stream by rapid diffusion across the gills, in part owing to its very high lipid solubility. This solubility probably accounts for the rapid diffusion across gill membrane of fish in both directions. The time needed to reach the maximum plasma concentrations is less than 5 minutes. The elimination half-life of tricaine mesilate in salmonid muscle is 70 minutes for free living fish in fresh water. Doses between 60 and 150 mg/l produce concentrations in blood, liver and kidney ranging from 20 to 60 mg/kg, whereas muscle concentrations vary from 6 to 55 mg/kg, typical mean muscle values being 20 to 30 mg/kg. With doses of 30 to 40 mg tricaine mesilate/l initial muscle concentrations vary from 1 to 25 mg/kg.

Tricaine mesilate is rapidly metabolised in the liver, but to a lesser extent in kidney, blood, and muscle. Conjugation and hydrolysis are the main metabolic pathways. The acetyl-conjugates of ethyl *m*-aminobenzoate and *m*-aminobenzoic acid represent the principal products. Hydrolysis with the production of the free acid varies between fish species. This influences the extent to which renal excretion is important. The gill epithelium is the principal route of excretion of tricaine mesilate, acetyl-tricaine mesilate and the other non-polar metabolites in salmonids and other higher teleost fish. The polar products are rapidly excreted, principally through the kidneys. In rainbow trout, renal excretion accounts for 21% of an injected anaesthetic dose and acetyl-derivatives account for 77 to 96% of the urinary products. Similar metabolism occurs in elasmobranch fish, but hepatic metabolism, conjugation (acetylation) and excretion generally occur at a slower rate. The aromatic metabolites, together with any remaining parent compound, are excreted within 24 hours by salmonid species.

Studies in 11 fresh water species (channel catfish, rainbow, brook, lake and brown trout, northern pike, largemouth bass, walleye, coho salmon, carp, and bluegill) showed that they were able to metabolise tricaine mesilate with the appearance in the blood of an acetylated derivative. Among these species the highest extent of metabolism was 34%, whereas in 10 of the 11 species concentrations of the acetylated derivative were usually less than 20%. Evidence indicates that cleavage of the ester bond of tricaine mesilate also occurs, forming the non-polar acetyl-*m*-aminobenzoate derivative and the polar *m*-aminobenzoic acid. Thus residues must be measured in terms of both the parent drug and total aromatic amines (acetylated tricaine, *m*-aminobenzoate and acetyl *m*-aminobenzoic acid).

5. Tricaine mesilate is of low oral acute toxicity. Acute oral LD₅₀ values were 2400 mg/kg bw in mice, 5200 mg/kg bw in rats and 4000 mg/kg bw in dogs. Acute intravenous LD₅₀ values ranged from 70 mg/kg bw (dogs and rabbits) to 170 mg/kg bw (mice). In fish, the minimum LC₅₀ values was 31 mg/l for salmonid species weighing 2 to 146 g exposed for 96 hours, and 39.4 mg/l for a range of warm water fish species weighing 0.5 to 63 g also exposed for 96 hours.
6. The therapeutic index (LC₅₀/EC₅₀) for tricaine mesilate was 3, 2 and 2.5 for channel catfish at exposure times of 15, 30 and 60 minutes, respectively, and the margins of safety (LC₁/EC₉₉) for the same exposure times were 1.1, 1.2 and 1.3 respectively. LC₁/EC₉₉ values for rainbow trout for exposure times of 15 and 30 minutes were 1.4 and 1.3, respectively. Other data show a safety margin of 1.57 in young trout. A solution containing 82 mg/l tricaine mesilate produced 50% mortality in young trout after 15 minutes. The maximum tolerated concentration (LC₁) for trout was 63 mg/l and a solution of 40 mg/l produced anaesthesia in 99% of trout (EC₉₉) within 3 to 4 minutes.
7. No studies on short- and long-term repeated dose toxicity of tricaine mesilate were available.
8. No tolerance studies in target species were provided. However, a slight increase in tolerance with repeated treatment of fish was observed, owing presumably to an increase in the activity of enzymes that degrade the substance.
9. No multigeneration studies or teratogenicity studies in mammalian species were presented.

10. With regard to reproduction toxicity of tricaine mesilate only results on sperm motility and fertility in fish are available. Brook and rainbow trout sperm lose motility within 10 seconds of exposure to tricaine mesilate concentrations of 12.5 to 18.9 mg/l. Concentrations lower than 6.5 mg/l have no effect. Egg development is not endangered by exposure and anaesthesia of females to a range of normal doses. Egg fertility is not affected at concentrations below 50 mg/l. Spawning of northern pike, muskellunge and walleyes is not affected at concentrations up to 150 mg/l. No abnormalities in offspring of guppies occurred when fertilisation was preceded by frequent periods of anaesthesia in concentrations of 500 mg/l.
11. Tricaine mesilate was tested for mutagenicity in the rec assay (repair test) in *Bacillus subtilis* strains M45 (recsup⁺) and H17 (recsup⁺) and in the reverse mutation assay with five strains of *Salmonella typhimurium* (TA 100, TA 98, TA 1535, TA 1537 and TA 1538) in presence and absence of metabolic activation. No mutagenic effects of tricaine mesilate were observed in these studies.
12. No carcinogenicity studies were presented, however in view of the negative results of the mutagenicity studies with tricaine mesilate these were not considered necessary.
13. No data on the skin sensitising potential of tricaine mesilate were presented.
14. No observations in humans were available.
15. From the available data, neither a pharmacological nor a toxicological NOEL could be identified and thus an ADI cannot be established.
16. Data reported in published literature on 4 trout species and coho salmon, where tricaine mesilate was given in combination with quinaldine, show that initial muscle concentrations of tricaine mesilate ranged from 1.9 to 27.3 mg/kg at zero hour removal from the bath solution. Muscle values for aromatic amines attained background concentrations in all species within 24 hours following removal of the fish from the tricaine mesilate solution. Further literature data on fish of 4 trout species, treated with tricaine mesilate alone at concentrations ranging from 80 to 135 mg/l and water temperatures of 7, 12 and 17°C, show that residues of aromatic amines in muscle, liver, and kidney of these species declined to background concentrations within 24 hours following removal of the fish from the tricaine mesilate solution.

Rainbow trout were anaesthetised in solutions of tricaine mesilate at concentrations ranging from 18 mg/l to 135 mg/l, at water temperatures of 7, 12 and 17°C and an exposure duration ranging from 4 to 12 minutes. After a 24-hour withdrawal, the total aromatic amine residues in muscle ranged from 0.8 mg/kg to 1.4 mg/kg. No residue data were presented after 48 hours after removal from tricaine mesilate solution. Depletion of residues from muscle in trout species is temperature-dependent, being slowest at 7°C. From these data the maximum amount likely to be ingested in a portion of 300 g fish 24 hours after treatment amounts to 0.42 mg.

The data in salmonid species are substantiated by information in other fish species. Published data on channel catfish show that immediately after treatment muscle tissue contains 1.08 mg/kg acetylated aromatic amines, or 2.8% of the total aromatic amines. These acetylated derivatives accumulate to a mean maximum concentration of 6 mg/kg after 9 hours. Within this period free residues dissipate so that the relative amount of acetylated amines increases to 52.2%. The concentrations of both fractions then decline to background concentrations within 24 hours withdrawal. Investigations of residues in muscle tissue of northern pike following deep anaesthesia with 150 mg/l solution of tricaine mesilate show that after 24 hours at 8°C, 12°C and 17°C the residues of tricaine mesilate plus acetylated derivative ranged from 0.8 mg/kg to 2.4 mg/kg. The initial residues of tricaine mesilate in muscle vary according to water temperature from 17 mg/kg to 14.7 mg/kg.

17. A method, based upon HPLC with UV detection, for batch analysis of tricaine mesilate was described. However, it was not validated in accordance with Volume VI the Rules Governing Medicinal Products in the European Community in respect of the determination of residues of tricaine mesilate in biological matrixes.

Conclusions and recommendation

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

- tricaine mesilate is of low oral acute toxicity,
- the animals are unlikely to be taken for human consumption during or immediately after treatment,
- tricaine mesilate is extensively metabolised and rapidly excreted,
- 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 tricaine mesilate 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
Tricaine mesilate	Fin fish	For water borne use only