

## **MS222 (TRICAINE METHANE SULPHONATE)**

### **Introduction**

This bulletin is intended to provide a summary of the properties, characteristics and uses of MS 222 (synonyms: Tricaine mesilate, tricaine methanesulfonate, *m*-aminobenzoic acid ethyl ester methansulfonate).

MS 222 is the only fish anaesthetic agent licensed for fin fish intended for human consumption and ornamental fish.

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## **Chemical and physical properties**

MS 222 is closely related to local anaesthetic agents. It is an isomer of benzocaine with the amine group being in the *meta* position on the benzene ring rather than the *para* position. The presence of the methane sulphonate grouping allows MS222 to dissolve in water (unlike benzocaine which must first be dissolved in an organic solvent prior to placing it in water).

Molecular formula	C <sub>9</sub> H <sub>11</sub> O <sub>2</sub> N + CH <sub>3</sub> SO <sub>3</sub> H
Molecular weight	261.3
Appearance:	Fine, white, odourless crystalline powder
Melting Point:	147°C to 150°C
Flash Point:	Not applicable
Solubility:	Freely soluble in water (20°C) dissolving equally readily in sea water, spring water and tap water.
Free from	Chlorides, sulphates, alkaloids and heavy metals.
Storage	Store in a dry place, below 25°C and protect from light.
Stability	The dry powder is stable for up to 5 years. A stock solution (10g MS 222/litre water) can be made if desired. It is stable for at least a month when stored cool in a dark or opaque bottle.

## **Pharmacology**

MS 222 is rapidly absorbed through the gills. Its mode of action is by preventing the generation and conduction of nerve impulses. It has direct actions on the central nervous system, cardiovascular system, neuromuscular junctions and ganglion synapses.

MS 222 is rapidly metabolised primarily by the liver but also in the kidneys, blood and muscle. Conjugation and hydrolysis are the main metabolic pathways. The primary metabolites are the acetyl-conjugates of ethyl *m*-aminobenzoate (non polar) and *m*-benzoic acid (polar). When MS 222 dissolves in fresh water the sulphonate radical hydrolyses and can reduce the pH of the anaesthesia system to a limited degree.

MS 222 and its non-polar metabolites are excreted through the gills. Polar metabolites are excreted through the kidneys.

MS 222 like all fish anaesthetic agents has a dose dependent effect which varies with species as well as individual fish to fish variations.

Lower doses of MS 222 induce tranquillisation and sedation (useful for minor procedures) while higher doses lead to full general anaesthesia levels (used for example with surgical interventions).

## **Legal Status**

Legal category - PML

Marketing Authorisation number - Vm 1360/4000

MRL - The Committee for Veterinary Medicinal Products concluded that there is no need to establish an MRL for Tricaine mesilate and recommended 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

## Toxicity

- Mutagenicity -** no mutagenic effects were observed.  
**Carcinogenicity -** MS 222 is not mutagenic so carcinogenicity studies are not considered necessary by the EMEA (European Medicines Evaluation Agency)

## Anaesthesia

It is always advisable to monitor the effect on a few test fish to determine how anaesthesia proceeds in each situation. It is wise whenever possible to continue observation for 12 to 24 hours after anaesthesia to ensure full recovery of the fish.

Anaesthesia is an art as well as science. MS222 must be used carefully because the level of anaesthesia obtained will vary depending on water temperature, water hardness, salinity, oxygen concentration, length of time of immersion, the biomass and species of fish. Smaller fish are commonly more sensitive to anaesthesia than larger ones.

### Administration of MS 222

The water used to anaesthetise fish should be taken from the environment where the fish are living (e.g. aquarium, sea cage or tank).

MS 222 dissolves well in water and may be added directly to the anaesthetic bath.

A level 0.5ml scoop holds approx. 400mgm MS 222

Concentration of MS 222 required (mg/L or ppm)	No. of litres of water per 0.5ml scoop
10	40
25	16
50	8
100	4

The advantage of MS 222 is that it will dissolve directly in water. A stock solution is not therefore necessary.

### Main points when using MS222

- Weigh out the amount of MS222 you need for the anaesthetic procedure.

- Put it in a sealed container.
- When ready - add enough water to shake thoroughly to ensure the product dissolves.
- Add to the anaesthetic bath.
- Then add the fish - a few at a time at first until you are comfortable with the anaesthesia.

### **Acidity of anaesthetic solution**

The literature on fish anaesthesia frequently mentions the fact that addition of MS 222 to fresh water can lower the pH. A major reduction of pH would be stressful to fish. It is recommended that, prior to using MS 222 for the first time (especially in 'soft' water areas) the pH of the water is measured (using simple pH test strips) before and after the required concentration of MS222 is placed in solution. Be aware, that anaesthetising a large number of fish in the same container without adequate water changes or aeration will cause an increase of carbon dioxide in the water which will have a similar effect. pH changes of 0.5 to 1.0 pH point should not adversely affect most species of fish. However, as noted earlier, it is prudent to always test a small batch of fish with the anaesthetic solution prior to using it on large numbers of fish - especially if this is your first time.

As an example, the result of adding 50 to 100ppm of MS222 to Scottish Highland (soft, peaty) fresh water or English (hard) tap water are shown below:-

Scottish "Highland" water with MS222 @ 50ppm with MS222 @ 100ppm	pH 5 pH 4.5 pH 4 / 4.5	English tap water with MS222 @ 50ppm with MS222 @ 100ppm	pH 6.5 pH 6.5 pH 6.5
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These tests were performed using commercially available pH indicator papers.

This data shows that MS 222 has a minimal effect on the acidity of the water.

### **Induction of anaesthesia**

Before sedation or general anaesthesia, fish should be fasted for at least 12 to 24 hours.

It is good practice to provide aeration to the anaesthetic bath.

The fish should be carefully monitored either as the product is gradually introduced to the fish or the fish are introduced to the anaesthetic bath.

Doses of MS 222 required to afford full general anaesthesia cause loss of reflexes up to ten minutes after immersion, depending upon the concentration employed. Fish have increased gill cover movement, followed by a slowing down of the gill cover movements prior to losing their balance, eventually turning upside down and floating to the surface (although not all species do this). Every fish species can behave differently. Once the procedure has been completed, the fish should be removed from the anaesthetic bath and returned to a recovery tank as soon as possible. Recovery

will take place after one to 30 minutes. Fish should be closely observed during this period. Once they are fully recovered, they can be returned to their original container.

To minimise damage and loss when sedated for long periods (e.g. for transport), the level of sedation should allow the fish to maintain their equilibrium and swimming position.

A guide to the quantities of MS 222 required to achieve differing levels of anaesthesia in different species of fish is shown in table 1.

### **Remember:**

- Always test a small number of fish first to note the effects
- Use the lowest dose needed to effect the level of anaesthesia required
- Always monitor fish in the recovery tank until they can be safely returned to their original tank.

### **Uses**

MS 222 can be used as an aid in the following management procedures:-

Sedation	handling, transport
Anaesthesia	vaccination, minor surgery
Deep Anaesthesia	major surgery, euthanasia

For further details please contact us on 01425 651927  
or by email to [askanimalhealth.uk@alpharma.com](mailto:askanimalhealth.uk@alpharma.com)

### **References**

Brown, L (1993). Anaesthesia and Restraint. In: Textbook of fish medicine. M.Stoskopf. W.B.Saunders Company. Philadelphia 882 pages

Ross L.G. and Ross B.R. (1999) Anaesthetic and Sedative Techniques for Aquatic Animals Blackwell Science. Oxford. 159 pages

Bové, F.J. (date unknown) MS-222 Sandoz. The anaesthetic and tranquillizer for fish, frogs and other cold blooded organisms. Sandoz Technical Bulletins, Sandoz ltd., Switzerland

### **Dose rates and immersion times**

The following examples of dose rates and exposure times are based on laboratory and field experience.

**Table 1**

SPECIES	MS222 CONCENTRATION mg/litre, or ppm OF	PARTS MS 222 TO PARTS WATER	IMMERSION TIME MINUTES
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	<b>WATER</b>		
<b>Trout</b> (7C°-17° C)			
Sedation	10-30	1:100,000 to 1:33,333	Up to 480
Anaesthesia: light	30-80	1:33,333 to 1:12,500	Up to 30
Anaesthesia: deeper	80-180	1:12,500 to 5,556	Up to 10
<b>Salmon</b>			
Sedation	7-30	1:142,857 to 1:33,333	Up to 240
Anaesthesia: light	30-80	1:33,333 to 1:12,500	Up to 10
Anaesthesia: deeper	80-100	1:12,500 to 1:10,000	Up to 5
<b>Bass</b>			
Sedation	8-30	1:125,000 to 1:33,333	Up to 480
Anaesthesia: light	30-70	1:33,333 to 1:14,286	Up to 20
Anaesthesia: deeper	70-100	1:14,286 to 1:10,000	Up to 4
<b>Carp</b>			
Sedation	20-30	1:50,000 to 1:33,333	Up to 1440
Anaesthesia	30-200	1:33,333 to 1:5,000	Up to 8
<b>Fresh Water Tropical Fish</b> (general guide only)			
Sedation	30-50	1:33,333 to 1:20,000	Up to 1440

The information given in this table is for guidance only.