PHARMAQ

TRICAINE PHARMAQ 1000MG/G

powder for solution for fish treatment

A users guide for the inducement of anaesthesia through inhalation in fish

INTRODUCTION

Fish which are undergoing a procedure involving significant handling and/ or intrusion should be anaesthetised. This is to ensure handler safety, safeguard fish welfare and optimise the outcome of the procedure being undertaken. Such procedures include fish surgery for veterinary or research purposes (e.g. the introduction of PIT tags for population monitoring) and vaccination, which is unquestionably the most common procedure for which fish are sedated.

This manual has been compiled as a guide to the safe and effective use of "TRICAINE PHARMAQ 1000MG/G POWDER FOR SOLUTION FOR FISH TREATMENT", the product name for Tricaine methanesulphonate (mesylate, or TMS), which has been licensed by PHARMAQ in the UK, Ireland, Norway, Iceland, Italy, Spain, Greece and The Faroe islands; in most of these markets it is the only approved anaesthetic for use in fish which are intended for human consumption. Tricaine PHARMAQ (TPQ) has replaced the original presentation of this anaesthetic, which was called PHARMAQ MS 222. The composition of the replacement product is exactly the same as MS 222 (100% tricaine methanesulphonate), as is the legal category (POM-VPS in the UK), which means that TPQ requires a prescription, which can be provided by a Veterinarian, Pharmacist, or Suitably Qualified Person. In all other countries the product can be only be obtained through prescription.

ANAESTHESIA OF SALMONIDS WITH TRICAINE PHARMAQ 1000MG/G

Tricaine PHARMAQ (TPQ), a white odourless crystalline powder, is the ethyl ester of amino benzoic acid and operates by blocking action potentials exchanged between the brain and the extremities of treated fish thus eliminating sensory input. The extent to which this blocking action is achieved is dose dependent and it is this that dictates the depth of anaesthesia that is induced in fish under treatment. Whilst the state of anaesthesia is a matter of degree, attempts have been made to categorise levels of sedation in fish, the most notable of which is the so-called "Four Stages of Anaesthesia" described by WN McFarland in 1959 (Table 1).

Stage	Plane	Description	Physiological and behavioural indicators
1	1	Light sedation	Responsive to stimuli but motion reduced, ventilation decreased.
	2	Deep sedation	As above but only receptive to gross stimulation – some analgesia.
2	1	Light anaesthesia	Partial loss of equilibrium – good analgesia.
	2	Deeper anaesthesia	Total loss of muscle tone and equilibrium, ventilation almost absent.
3		Surgical anaesthesia	As above but with no reaction to major stimulation.
4		Medullary collapse	Ventilation ceases, cardiac arrest, eventual death. Overdose.

Table 1. "The four stages of Anaesthesia" After McFarland (1959) in Lindsay G Ross and Barbara Ross (2008)

The process of anaesthetisation should actually start some hours (between 12 and 24 depending upon species and water temperature) before the introduction of TPQ. This is because it is essential that the digestive tracts of fish under treatment are completely empty prior to the onset of anaesthesia, especially for the purpose of vaccination. It is also recommended that feed is withheld for up to 12 hours following recovery in order to allow time for the resumption of normal gut activity.

Figure 1. Salmon parr that have been anaesthetised to stage 2-2 in readiness for vaccination.

For the purpose of vaccination it is important that pre-vaccinates are quickly (<90 seconds) and safely brought into stage 2 (2) of anaesthesia. Fish in this state will exhibit some opercula activity but will not react to any stimulus. They can now be safely handled by an operator who can apply the vaccine confidently and accurately and with minimal impact on the welfare of the stock undergoing treatment. Following vaccination, fish should be returned to a well aerated environment that is free of anaesthetic and be monitored through recovery. As a guide, fish should not be exposed to TPQ for an interval which exceeds by greater than a factor of three, the time it took for them to achieve anaesthesia initially. Thus if 90 seconds have elapsed for the induction of anaesthesia, the fish must be removed from the anaesthetic solution within 4.5 minutes. Recovery, at least to stage 1(1) should be completed within 3 minutes of return to the ambient, well aerated, water supply.

To achieve all this, a 'bath' containing TPQ at a concentration of between 80 and 100 mg/Litre (see Table 2) of clean water should be made up on the day of use. The bath must be well aerated in order to provide adequate oxygen as well as reduce the build up of carbon dioxide. The advantage of TPQ over some other anaesthetics lies in its water-solubility which negates the requirement for spirit based solvents some of which may cause gill irritation and distress to fish. Whilst the best-practice recommendation is for dissolution of the anaesthetic on the day of use, stock solutions (usually 10 g/Litre) can be prepared, with buffer, in advance of use, provided that they are kept in a stoppered container which is stored in a cool dark place. Exposure of the solution to light will, over time, cause it to darken although this does not necessarily indicate a decrease in potency.

The volume of anaesthetic baths can vary between 80 to 1000 litres depending upon the scale of the operation being undertaken. Baths can be made up of freshwater e.g. for vaccination of salmon parr (FIGURE 2 a, and b) or seawater e.g. for sea lice counting (FIGURE 3).

Figure 2. a) automatic, and b) manual anaesthetisation of salmon parr.

In the former case it is important that the operator is familiar with the ionic buffering capacity of the local water. In poorly buffered or soft water, TPQ can cause a significant drop in the pH, which not only acts as a potential stressor to fish but can also result in hitherto benign metal ions suddenly becoming toxic, as well as the inducement of other undesirable physiological changes. It is important to remember that pH is measured on a log scale, so the difference between pH 7 and 6 represents a ten-fold increase in hydrogen ions.

To overcome this it may be necessary to buffer the water in the anaesthetic bath through the addition of an agent such as sodium bicarbonate (also available from PHARMAQ). The need for and extent of buffering will be best determined by monitoring pH, which should always be maintained at ambient. By and large, adequate buffering can be achieved by matching, weight for weight, the amount of TPQ with sodium bicarbonate. It is worth bearing in mind that the potency of TPQ increases as temperature rises and water hardness falls.

Figure 3. Anaesthetisation of large fish in seawater for lice counting. (Photo Sean Lydon Dawnfresh)

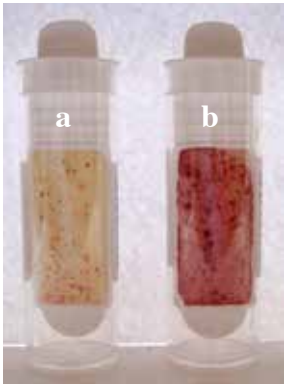

Figure 5. Dip slides from a 50 litre anaesthetic bath revealing bacterial contamination. A) prior to the introduction of fish. B) after 500 fish at 40g mean weight been sequentially passed through.

Apart from fixing the pH of the anaesthetic bath, many operators also include a low level of a disinfectant such as Halamid (Chloramine-T) to improve the bio-security of anaesthetisation by combating the build up of microorganisms, an inevitable consequence of exposing a large number of fish (between five and ten thousand) to a small volume of water. By way of example, a sample taken from a fresh anaesthetic bath (40 L, 6-8°C) was found to have a microbial load of 184 colony forming units (CFU) per ml. A sample taken from the same bath solution after 3000 50g fish had been sequentially passed through it revealed >1100 CFU ml^{-1}. Results from a similar trial are illustrated in FIGURE 5.

A further measure which can: a) mitigate the removal of mucus from the surface of anaesthetised fish (an effect of low pH) and b) bind unwanted metal ions, is the use of a water conditioner such as Vidalife. Vidalife can be regularly added to the vaccination table upon which anaesthetised fish are being placed and also to the anaesthetic bath itself. Vidalife not only helps to maintain the integrity of the protective mucus layer on the fish, it also reduces scale loss and physical damage.

Good hygiene and buffering of the anaesthetic bath will maximise the biomass of fish which can be treated before the contents of the batch must be discarded and replaced with a fresh solution. As a guide, the number of fish with an average weight of 40g that can be passed through an anaesthetic bath before it should be replaced is around 10 per litre. However operators should remain vigilant and be prepared to change the bath if anaesthesia induction and recovery times are observed to increase or decrease respectively or they notice a build-up of detritus in the bath.

OPERATOR SAFETY

Although not especially toxic, it is still important to remember that TPQ is a biologically active therapeutant the safety of which, in addition to the guidelines set out in this manual, must also be viewed from an operator, consumer and environmental perspective. It is advisable that operators minimise direct exposure to TPQ by wearing protective clothing including gloves, goggles and a mask which will guard against accidental or unintended contact. From the consumer's perspective, fish must not be presented for sale as food unless 70 degree days have elapsed since they recovered from exposure to TPQ (e.g. 7 days if water temperature is 10°C). TPQ can be eliminated from spent anaesthetic baths by filtration through a deactivated carbon filter to achieve < 1µg/Litre of TMS.

DOSE GUIDLINES

The following is a dose guide for the use of TPQ across a range of fish species commonly found both in aquaculture as well as in research. The doses are indicative only and operators should always test TPQ on a small number of fish initially, so that the interaction of the anaesthetic with the fish can be assessed within the context of the prevailing local conditions.

As well as being species specific, anaesthesia induction and recovery times are also influenced by temperature as well as fish size. Small size and higher temperatures tend to reduce the time taken for the onset and recovery from anaesthesia. In addition to this, because TMS is fat soluble, fattier fish will normally take longer to both succumb to, and recover from the anaesthetic.

TPQ should NOT be used for the anaesthetisation of the following tropical fish species:

The Ram cichlid *(Apistogramma ramirezi)*, The Bala Shark *(Balantocheilus melanopterus)*, Etroplus sp, MacCulloch's rainbow fish *(Elantoteaenia maccullochi)*, The Silver Moony *(Monodactylus argenteus),* and the Congo tetra *(phenacogrammus interruptus)*.

Species	Taxonomic name	Dose
Atlantic salmon	Salmo salar	100 $mg.l^{-1}$
Rainbow trout	Onchorynchus mykiss	80-100 $mg.l^{-1}$
Common carp	Cyprinus carpio	Adult <85 $mg.l^{-1}$ Juvenile >100 $mg.l^{-1}$
Tilapia	Oreochromis mossambicus	Adult 100-200 mg.l-1 Juvenile 60-70 mg.l-1
Cod	Gadus morhua	75 $mg.l^{-1}$
Gilthead bream	Sparus aurata	70 $mg.l^{-1}$
Sea bass	Dicentrarchus labrax	70 $mg.l^{-1}$
Atlantic halibut	Hippoglosus hippoglosus	200 $mg.l^{-1}$
Ballan wrasse	Labrus bergylta	70 $mg.l^{-1}$
Turbot	Scophthalmus maximus	80-100 $mg.l^{-1}$
Zebra Fish	Danio rerio	<100 $mg.l^{-1}$

Acknowledgements:

Dawnfresh; *"A procedure for Fish Anaesthesia"*. Richard Hopewell 2012

'Anaesthetic and Sedative Techniques for Aquatic Animals'. Lindsay G Ross and Barbara Ross 3rd ed. (2008)

A Review of Tricaine Methanesulfonate for Anesthesia of Fish. Carter. KM, Woodley CM and Brown RS in Reviews in Fish Biology and Fisheries (2011) 21 pp 51-59.

The Use of MS222 (Tricaine Methanesulphonate) as an Anaesthetic for Routine Blood Sampling in Three Species of Marine Teleosts. Bourne.P.K. Aquaculture, 36 (1984) 313-321

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