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Anesthetic Options for Fish (19-Aug-2001)

P.R. Bowser

Aquatic Animal Health Program, Dept of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA.

Introduction

The current most commonly utilized methods of anesthesia in fish will be summarized in this report. A wide variety of compounds have been utilized to anesthetize fish in fisheries research, fisheries management, aquaculture and fish health. Extensive lists of compounds used for anesthesia in fish are provided in [1-3]. For many of these compounds, little published information exists. In more practical terms, the vast majority of anesthetic procedures have been performed with a limited number of compounds. One survey of fishery workers [4] indicated the most commonly used anesthetic compounds were tricaine, quinaldine, and carbon dioxide.

The governmental regulatory environment must be considered when selecting the compound to be used for anesthesia of fish. In the United States, the Environmental Protection Agency (EPA) is charged with the regulation of pesticides while the Food and Drug Administration (FDA) regulates those compounds to be used on food animals. Currently, the number of "registered compounds" for which there is an EPA or FDA label for use as an anesthetic is limited to one chemical. Finquel[™] and Tricaine-S[™]; formulations of tricaine, are the only anesthetics with a label for use with fish granted by the FDA. Two other compounds, carbon dioxide and sodium bicarbonate, are listed by the FDA as Low Regulatory Priority compounds. The regulatory environment for chemicals used on fish, including those used for anesthetic purposes, is constantly changing. Individuals considering anesthetic procedures on fish should be cognizant of the current regulations regarding those compounds they may consider using. Individuals considering use of a compound for anesthesia of fish are referred to the web site of the Center for Veterinary Medicine of the U.S. Food and Drug Administration.

Routes of Administration

Although fish may be injected with anesthetics, the vast majority of anesthetic procedures are accomplished by a dip or bath treatment in a static bath or with flowing water. In either case the anesthetic must be soluble in water. In some cases, anesthetics are first dissolved in an organic solvent and then diluted in water. The fish is exposed to the aqueous solution of the compound at a predetermined concentration for a specific period of time. Concentrations are typically calculated in terms of parts per million (p.p.m.), which is equivalent to milligrams per liter of water (mg/L) or grams per cubic meter of water (g/m³). The degree of sedation in fish (Table 1) is a function of the concentration of the compound and the duration of exposure.

A common method of exposing fish to the anesthetic compound is to establish the desired concentration of anesthetic in container of water (bucket or tub) and placing the fish in the container. The fish are maintained in the container of anesthetic and removed when they reach the desired level of anesthesia. Once the manipulations of the sedated fish are completed, they are typically placed in a recovery container of fresh water with aeration. This procedure is applicable when the desired procedures can be completed on sedated fish in a relatively short period of time (e.g. less than 1 minute). In cases where fish must be maintained under sedation for extended periods, alternate anesthetic delivery methods are required. This may consist of a method of alternatively delivering water containing anesthetic and water without anesthetic. A schematic diagram for such an anesthetic (e.g. MS222) delivery system is shown in (Fig. 1). The system provides for the ability to irrigate the gills with aqueous MS222 or non-treated water, depending on the observed level of sedation of the fish. In each case the treated or non-treated water is directed back to a separate pump from which it can be reused.

Table 1. Stages of Anesthesia (modified from [3])		
Stage	Descriptor	Behavioral Response of Fish
0	Normal	Reactive to external stimuli; opercular rate and muscle tone normal
1	Light sedation	Slight loss of reactivity to external stimuli; opercular rate slightly decreased; equilibrium normal
2	Deep sedation	Total loss of reactivity to all but strong external stimuli; slight decrease in opercular rate; equilibrium normal
3	Partial loss of equilibrium	Partial loss of muscle tone; swimming erratic; increased opercular rate; reactivity only to strong tactile and vibration stimuli
4	Total loss of equilibrium	Total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes
5	Loss of reflex reactivity	Total loss of reactivity; opercular movements slow and irregular; heart rate very slow; loss of all reflexes
6	Medullary collapse (stage of asphyxia)	Opercular movements cease; cardiac arrest usually follows quickly

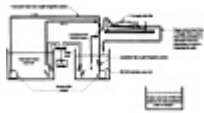


Figure 1. Flow diagram for delivery of water containing MS222 anesthetic or water containing no anesthetic in support of anesthesia in fish (diagram adapted from Wooster GA, Hsu H-M and Bowser PR 1993 [9]; used with permission). - [To print diagram click here](#) - To view this image in full size go to the

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Commonly Used Compounds

Tricaine - Chemical names of tricaine include: 3-aminobenzoic acid ethyl ester methanesulfonate, ethyl m-aminobenzoate methadesulfonate, methadesulfonate salt of alkyl aminobenzoate, and methandsulfonate salt of ethyl meta-aminobenzoate. Common and proprietary names include tricaine methanesulfonate, MS-222, Tricaine-S^m and Meta-caine. Finquel[™] refers to the specific formulation registered by Fort Dodge Laboratories, Fort Dodge, Iowa, and sold by Argent Chemical Laboratories, Redmond, Washington. Tricaine-S^m refers to the specific formulation registered by Western Chemical, Inc, Ferndale, Washington. The FDA has granted a label for the use of Finquel[™] and Tricaine-S^m on fish. These compounds should not be used within 21 days of harvesting fish for food.

The compound is readily soluble in water and is also lipid soluble and readily moves across the gills. The compound is dissolved in water to achieve a concentration ranging from 15 to 330 mg/L. Dose depends on the degree of anesthetization desired, the species and size of fish, water temperature and water hardness. The compound is more potent in warm water with low hardness. Solutions of tricaine are acidic and should be buffered to pH 7.0 - 7.5 with NaHCO₃ or Tris buffer. It is recommended that preliminary test solutions be evaluated with small numbers of fish to determine the specific dose to be used. Recovery from anesthesia depends upon the concentration of tricaine used and exposure time. The compound is readily excreted across the gills during recovery. The tissue concentration of the compound decreases to near zero within 24 hours.

Quinaldine and Quinaldine Sulfate - The chemical name of quinaldine is 2-methylquinoline while quinaldine sulfate is also known as quinate.

Quinaldine is slightly soluble in water, but is soluble in acetone and ethanol. Quinaldine sulfate is readily soluble in water. In solution, quinaldine sulfate is acidic and should be buffered with sodium bicarbonate (0.45 g NaHCO₃/1 g quinaldine sulfate). Although it has been used successfully by fisheries workers, a number of adverse effects have been reported. The compounds are irritants to the gills and corneal damage in fish has been reported. The solvents used to dissolve quinaldine have been known to irritate fishery workers where ventilation of the work area is inadequate.

These compounds are used at a dose of 15 - 60 mg/L, depending on the same conditions as for tricaine. Quinaldine and quinaldine sulfate are more potent at higher pH and are ineffective below pH 5.0. These compounds are more potent in hard water. Following use, tissue concentrations of these compounds decrease to undetectable concentrations within 24 hours.

Carbon Dioxide - Carbon dioxide gas is soluble in water and as such, has weakly acid properties. Typically the gas is bubbled in the water. It is difficult to control the concentration of carbon dioxide by this method and concurrent consideration must be

given to maintaining adequate oxygen concentrations in the same water. Carbon dioxide has been used primarily to sedate fish during transport or to allow handling of large numbers of fish.

In addition to using pressurized gas to introduce carbon dioxide into the water, sodium bicarbonate (NaHCO_3) has also been used as a source of carbon dioxide. When NaHCO_3 is dissolved in water, it slowly releases carbon dioxide gas. The gas is released more rapidly under conditions of low pH. One recommendation suggests adding NaHCO_3 at 640 mg/L at pH 6.5 as the most efficient means for producing carbon dioxide. However, it may be difficult to maintain the pH of the water.

Both carbon dioxide and sodium bicarbonate are listed as Low Regulatory Priority compounds by the FDA. The specific listings are as follows [5]: carbon dioxide gas is listed for anesthetic purposes in cold, cool and warm water fish. Sodium bicarbonate at a concentration of 142 to 642 p.p.m. (= mg/L) for 5 minutes as a means of introducing carbon dioxide into the water to anesthetize fish.

AQUI-S - The active ingredient for AQUI-Ssm is isoeugenol (2-methoxy-4-propenylphenol). AQUI-Ssm is manufactured by AQUI-S New Zealand Ltd., and is dispersed directly into seawater or fresh water. No buffers or solvents are required. It is used at 5 - 10 mL per 1000 litres of water for light sedation or 17 - 20 mL per 1000 litres for heavy anaesthesia, depending upon the species, age and level of exhaustion in the fish as well as associated environmental factors. It is currently approved for use in Chile, the Faroe Islands, New Zealand and Australia, with no withdrawal period. AQUI-Ssm does not yet have full FDA approval but may be used in the US through the Investigational New Animal Drug (INAD) program with the US Fish and Wildlife Service. It was placed under an expedited review status by the FDA in 1996.

Eugenol - Eugenol (4-allyl-2-methoxy-phenol), the active compound in clove oil. Eugenol has only recently been considered as a compound for anesthesia in fish [6-8]. Clove oil originates from the flowers, flower stalks and leaves of the clove tree *Eugenia aromatica*. It has been used for many years as a food flavoring and in the dental profession as a local anesthetic. The active ingredient, eugenol, makes up 90 - 95% of clove oil. Clove oil has been used at concentrations of 25 - 100 mg/L, depending on fish species and degree of anesthesia. The US FDA has determined that eugenol is not Generally Recognised as Safe (GRAS) when used as a fish anaesthetic and that a New Animal Drug Application (NADA) is required for this compound.

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