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RESEARCH PAPER

A review of tricaine methanesulfonate for anesthesia of fish

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Abstract Tricaine methanesulfonate (TMS) is an anesthetic that is approved for provisional use in some jurisdictions such as the United States, Canada, and the United Kingdom (UK). Many hatcheries and research studies use TMS to immobilize fish for marking or transport and to suppress sensory systems during invasive procedures. Improper TMS use can decrease fish viability, distort physiological data, or result in mortalities. Because animals may be anesthetized by junior staff or students who may have little experience in fish anesthesia, training in the proper use of TMS may decrease variability in recovery, experimental results and increase fish survival. This document acts as a primer on the use of TMS for anesthetizing juvenile salmonids, with an emphasis on its use in surgical applications. Within, we briefly describe many aspects of TMS including the legal uses for TMS, and what is currently known about the proper storage and preparation of the anesthetic. We outline methods and precautions for administration and changes in fish behavior during progressively deeper anesthesia and discuss the physiological effects of TMS and its potential for compromising fish health. Despite the challenges of working with TMS, it is currently one of the few legal options available in the USA and in other countries until other anesthetics are approved and is an important tool for the intracoelomic implantation of electronic tags in fish.

Keywords Anesthesia · TMS · MS222 · FDA · Salmonids

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Introduction

Anesthetics are used to sedate fish during transport or handling, and immobilize fish for surgical procedures by depressing their central and peripheral nervous systems (Summerfelt and Smith 1990). This nervous system depression reduces voluntary movement and reduces sensory perception during the procedure (Spath and Schweickert 1977; Hensel et al. 1975; Arnolds et al. 2002; Sneddon 2002). Although the capability of fish to feel pain is a highly controversial topic (for counterarguments, see Rose (2002), Sneddon et al. (2003), and Braithwaite and Boulcott (2007)), considerable evidence suggests that using anesthetics increases the wellbeing of fish. Prolonged and/or cumulative stressors associated with fish husbandry

and research, such as repeated handling, blood sampling, or surgery, can lead to several physiological changes that can ultimately leave fish susceptible to disease or predation (Wedemeyer et al. 1990; Barton 2002). The use of anesthetic agents has been shown to reduce physiological indicators of stress resulting from blood sampling (Iwama and Ackerman 1994; Wagner et al. 2003) and to reduce mortality when exposed to severe and repeated stressors (Strange and Schreck 1978). It is now considered standard practice to use anesthetic agents for humane handling of fish during stressful or invasive procedures (Olfert et al. 1993; AFS 2004). For this reason, it is essential for handlers to understand its proper use.

Tricaine methanesulfonate (TMS), known also as MS-222, ethyl 3-aminobenzoate methanesulfonic acid, tricaine mesilate, Aqualife TMS^{TM} (Syndel, Qualicum Beach, BC Canada) metacaine, methanesulfonate, Finquel^{TM} (Argent Chemical laboratories, Redmond, WA, USA), or Tricaine-S^{TM} (Western Chemical, Inc., Ferndale, WA, USA), is classified as an ester-type synthetic local anesthetic and is commonly used in the fisheries industry (Sato et al. 2000). The main purpose of this paper is to inform fisheries researchers and practitioners on the use of TMS for anesthetizing fish, with particular emphasis on its use in surgical procedures. Within, we discuss important aspects and proper use of TMS as it relates to research with juvenile salmonids, mechanisms of action, and intended and unintended physiological side effects in fish. Although special focus is made on juvenile salmonids due to the large amount of research that is conducted on them, this review should also be useful for others working on other sizes or species of fish.

TMS belongs to the local anesthetic family of drugs which has been widely studied but not entirely understood. The limited use of TMS in human and veterinary applications has resulted in relatively little research into properties and physiological effects specific to TMS when compared to anesthetics used in mammals. Therefore, we also highlight areas of ambiguity in the literature regarding the use or effects of TMS.

Designated uses for tricaine methanesulfonate

Guidelines and regulations regarding the use of TMS vary between manufactures and between countries. We encourage our readers to consult local and regional agencies for rules and regulations on TMS. In the United States, TMS is the only legal anesthetic for use on a limited number of food fish (FDA 2006). The limitations on TMS use with food fish include a 21-day withdrawal period before harvesting, and use is restricted to the families Ictaluridae (catfishes), Salmonidae (salmon, trout, char, whitefish, and grayling), Esocidae (pike and pickerel), and Percidae (perch, walleye, ruffe, and darters) (FDA 2006). For other species, the drug should be limited to hatchery or laboratory applications in which fish will not be released into the wild or consumed. According to the U.S. Food and Drug Administration (FDA) and manufacturers, water temperatures during the 21-day withdrawal period should exceed 10°C (50°F; FDA 2006; Argent 2008; Western 2008a). However, a Freedom of Information Act factsheet (FDA 1997) stated that water should not exceed 10°C (50°F) during the 21-day period. The reason for this discrepancy is not clear and possibly a typographical error. The authors were not successful at confirming this suspicion.

In Canada, TMS is the only approved drug for use in food fish and is sold under the name Aqualife TMS^{TM}. The usage of TMS in food fish is limited to the family Salmonidae and is available only by veterinarian prescription. Treated fish cannot be slaughtered 5 days after the last exposure and must be held in water warmer than 10°C (Health Canada 2010).

In the United Kingdom (UK), TMS is available for use in fin fish intended for human consumption and ornamental fish. The company PHARMAQ holds the only marketing authority to manufacture TMS in the UK, as granted by the Veterinary Medicines Directorate (VMD 2010a). According to the VMD summary of product characteristics sheet, TMS has a 70 degree day withdrawal period (or 7 days in water at mean temperatures of 10°C or higher) in the UK before harvesting (VMD 2010b). This also states that TMS should not be used in the tropical fish species *Apistogramma ramirezi, Balantocheilus melanopterus, Etroplussurrantensi spp, Elanoteaenia maccullochi, Monodactylus argenteus, Phenacogrammus interruptus* and *Salopagus argus*.

Storage and preparation

TMS is a white, odorless crystalline powder with a high solubility in water (1 g/0.8 mL of TMS at 20°C;

11%; Merck and Company 1989; Argent 2008; Sigma 2008). Storage areas for the powder form should be dark, dry, well-ventilated, and cool (Merck and Company 1989; Argent 2008; Sigma 2008). TMS in powder form easily can become airborne and could become hazardous to the handler. Although there is evidence that TMS is not mutagenic (Yoshimura et al. 1981), users should be aware that TMS is retinotoxic and an irritant to mucous membranes, including the upper respiratory tract (Berstein et al. 1997; Argent 2008; Sigma 2008). Prior to handling or using, one should read manufacturer-specific material safety data sheets (MSDSs) and avoid contact with the powder or solution by using accepted personal protective equipment. This can include gloves, eye protection, and general skin protection when dealing with all forms of the chemical; and masks and/or fume hoods when dealing with the powdered form (Argent 2008; Sigma 2008). Additionally, hand washing after handling TMS, whether in powder or aqueous form, is recommended.

TMS is generally prepared as a concentrated stock solution of TMS and water. Some manufacturers agree that the TMS solution is photosensitive (its color changes with light exposure), although the color change does not necessarily indicate significant activity decrease (Bové 1962; Argent 2008; Western 2008a) and thus color should not be used to gauge degradation. In contrast, Bell (1987) stated that TMS solutions can be toxic to fishes in seawater with sunlight exposure, although no mechanism for this acquired toxicity was noted.

Due to the difficulty of handling powdered TMS in situations in which proper personal protective equipment may not be readily available or practical to use (e.g., field settings), some researchers pre-mix and store TMS stock solutions in dark bottles. Although this practice is widely used, there is some question as to how long TMS solutions can stand without degradation. Based on Bové (1962) and fact sheets on Argent Laboratories Finquel™ (2008) and Western Chemical Tricaine-S™ (2008a, 2008b), no significant degradation of TMS stock solution occurred after 3 d of standing. However, a 5% activity decrease was observed within 10 d. In contrast, the Sigma–Aldrich MSDS (2008) stated TMS solutions degrade quickly and therefore recommended solutions be made immediately prior to use. Further diverging from Bové (1962), ALPHARMA (2001) reported that stock solutions were stable for 1 month when stored in dark or opaque bottles in cool environments. Ross and Ross (2008) concur these solutions are stable, but indicate that solutions should be stored for only 3 months in cooled dark, sealed containers. Neither ALPHARMA (2001) nor Ross and Ross (2008) provided a TMS activity degradation curve at a specific temperature. The authors' investigation into the research on the storage of stock TMS solutions is inconclusive, so these discrepancies warrant further examination. We recommend that until additional research is conducted, TMS solutions be prepared immediately prior to use to ensure maximal and predictable TMS potency.

Mechanisms of anesthesia

Although classified as a local anesthetic, TMS acts systemically when absorbed through the gills and skin of fish (e.g., scaleless fish with well-vascularised skin) in an anesthetic bath (McFarland 1959; Hunn and Allen 1974; Ferreira et al. 1984). Once in the gills, TMS enters the bloodstream and is distributed throughout the body (Hunn and Allen 1974; Summerfelt and Smith 1990). Once inside the body, TMS concentrations are metabolized rapidly by acetylation reactions and excreted (Burka et al. 1997). The major excretion route for TMS and its non-polar metabolites is through the gills (Wayson et al. 1976). Unmetabolized TMS and its more polar metabolites are excreted via the kidneys (Hunn and Allen 1974; Wayson et al. 1976; Burka et al. 1997). The estimated plasma half-life of TMS is 1.5–4 h (Hunn and Allen 1974). After 8 and 24 h, TMS is undetectable in whole blood and urine, respectively (Hunn and Allen 1974; Ohr 1976; Wayson et al. 1976; Burka et al. 1997). Local TMS injections are ineffective because the drug is eliminated too quickly to induce anesthesia (Allen and Hunn 1986; Burka et al. 1997). Therefore, for the vast majority of procedures involving fish, TMS is administered by immersion in an anesthetic bath and, when appropriate, followed by continuous irrigation of the gills with anesthetic solution. Fish immersion or gill irrigation in TMS provides continual uptake of the anesthetic during exposure. For this reason, induced fish should be monitored to prevent overdosing or deeper stages of anesthesia.

The major mode of action for TMS is nervous system suppression whereby the entrance of sodium (Na^+) into the nerve is inhibited, thus limiting nerve membrane excitability (Carmichael 1985; Butterworth and Strichartz 1990; Burka et al. 1997). Nerve inhibition is facilitated by the lipid solubility of TMS, which allows it to move easily into the cell membrane to bind with sodium channels (Hunn and Allen 1974; Butterworth and Strichartz 1990). In mammals, the loss of nerve function starts as a loss of the senses of pain, temperature, touch, and the loss of equilibrium and proprioception, followed by loss of skeletal muscle tone (Rang et al. 2003). Although the loss of nerve function is not as well documented in fish, predictable behavioral changes during anesthesia induction are documented and used to gauge the level of anesthesia being experienced by the fish (McFarland 1959; Summerfelt and Smith 1990; Ross and Ross 2008) (See "Induction of Anesthesia" below).

The potency of anesthetic agents similar to TMS, such as 2-phenoxyethanol, benzocaine, tetracaine, and lidocaine, can increase dramatically with cooler temperatures (Sehdev et al. 1963; Butterworth and Strichartz 1990). Although pharmacokinetics have not been directly studied in fish, changes in pharmacokinetics at cooler temperatures and slowed diffusion rates decrease the clearance rate from the nerve site (Ohr 1976; Butterworth and Strichartz 1990). Thus, at 10°C, a lower dose may more readily block nerve function than at 22°C.

Administration

Induction of anesthesia

Differential exposure time and dosage of TMS can induce stages of anesthesia in fish corresponding to differing states of narcosis or level of sedation with changes in neural functioning that initiate in the peripheral neural system. As neural function decreases, fish exhibit predictable changes in behavior that can be used to gauge the current level of anesthesia (McFarland 1959). Summerfelt and Smith (1990) derived a 6 Stage scale where at Stage 1, light sedation, there is a slight loss of reactivity to external stimuli and slightly decreased opercular rates; Stage 2, deep sedation, where there is a total loss of reactivity to external stimuli with the exception of strong pressure and there is a slight decrease in opercular rate; Stage 3, partial loss of equilibrium, where there is a partial loss of muscle tone, and hyperactive behavior such as erratic swimming and increased opercular rate and reaction only to strong tactile or vibrational stimuli; Stage 4, total loss of equilibrium, where there is a total loss of muscle tone and equilibrium, slow and regular opercular movements, and a loss of spinal reflexes; Stage 5, loss of reflex reactivity, where opercular movements are slow and irregular, and there is a total loss of reflexes and reactivity; and Stage 6, asphyxia, where opercular movements cease and cardiac arrest follows shortly after.

The rate of decline for neural function, as well as the level to which it declines, varies primarily with the anesthetic dosage due to the rapid diffusion of TMS across the gill (Hunn and Allen 1974). For minor handling procedures (e.g., measurements, blood samples) or transport, lower dosages (15–30 mg of TMS/L to water for Salmonidae) result in tranquilization and light sedation (Stages 1–2), which can be held for long periods (Schoettger and Julin 1967). Major procedures require higher doses (60–100 mg/L of TMS to water) to quickly (within 4 min) induce deep anesthesia levels (Stages 4–5; Schoettger and Julian 1967; Hunn and Allen 1974; Summerfelt and Smith 1990).

For invasive procedures, such as intracoelomic transmitter implantation, the fish must be in a deep level of anesthesia (≥Stage 4) to be rendered completely immobile. Several authors have suggested that an ideal anesthetic should induce Stage 4 anesthesia quickly (in under 3 min), while allowing for quick recovery (less than 5 min; Marking and Meyer 1985; Bell 1987; Iwama and Ackerman 1994). Because TMS moves across the gills rapidly and is metabolized readily, blood concentrations will diminish if bath concentrations are not high enough to overcome the speed at which the active form of the drug leaves the body (Hunn and Allen 1974). TMS moves quickly into the blood to be dispersed throughout the body and can peak in 1–3 min (Hunn and Allen 1974). Higher doses will induce and maintain Stage 4 anesthesia quickly. However, the risk for adverse side effects would increase if fish were not able to be processed in a timely manner (Marking and Meyer 1985; Bell 1987; Iwama and Ackerman 1994).

Exact dosages for salmonids vary with anesthesia stage targeted, species, body size, health, age and life stage, and water quality (Summerfelt and Smith 1990; Ross and Ross 2008). In addition, the tolerance of salmonids for specific dosages varies between stocks and/or sexes (Marking 1967; Schoettger and Julian 1967; Houston and Corlett 1976; Burka et al. 1997). The optimal TMS dosage to induce anesthesia varies between 60 mg/L and 100 mg/L of TMS to water (Iwama and Ackerman 1994). Induction and recovery times are inversely correlated with body weight, especially for salmon (Burka et al. 1997; Houston and Corlett 1976). Water quality parameters, such as temperature, pH, salinity, and hardness, can affect metabolic rate, acid–base regulation, osmoregulation and ion regulation (Schoettger and Julin 1967; Heisler 1988; Iwama et al. 1989; Perry and Gilmour 2006). These factors can also affect the pharmacodynamics of TMS (Marking 1967; Ohr 1976). Before a full study and/or fish-handling events are initiated, or if environmental conditions have changed, it is recommended that sample fish be tested by anesthetizing at the desired TMS dosage (Schoettger and Julian 1967; Iwama and Ackerman 1994; Argent 2008; Western Chemical 2008a). When possible, fish should be monitored for 24–48 h after anesthetic administration and the associated procedure to ensure full recovery.

Anesthetic baths (whether induction, surgery table, or recovery) should be changed when the temperature varies from ambient water temperature by more than 2°C, when the bath becomes noticeably frothy or cloudy, or when reduced water quality is suspected due to excreta, blood or other bodily fluids, (Summerfelt and Smith 1990; Portz et al. 2006). Anesthetic baths also should be changed when induction or recovery times are noticed to increase or decrease, respectively. Changes in induction and recovery times occur due to the decay of TMS within the induction bath as it becomes metabolized or diluted with the physical movement of fish into and out of the induction bath (Schoettger and Julin 1967; Burka et al. 1997; Summerfelt and Smith 1990).

TMS has side effects, such as changes to the cardiovascular and endocrine systems as well as to osmoregulation and ion regulation. During deeper levels of anesthesia when opercular movements are slowed and respiration is depressed, severe hypoxia (inadequate oxygen in blood or tissues) and respiratory and metabolic acidosis can develop (Iwama et al. 1989). Reduced blood flow through the gills and the concomitant increased anatomical and physiological dead spaces significantly slow the exchange of gas (oxygen and carbon dioxide) across the gills. Declined gas exchange results in a consequent reduction of blood oxygen tension, an increase in blood carbon dioxide tension, and a concomitant decrease in blood pH (Iwama et al. 1989; Iwama and Ackerman 1994; Hill and Forster 2004). If allowed to continue, the condition results in hypoxemia, a potentially lethal decrease in arterial oxygen tension. Depressed respiration combined with the change in blood oxygen and carbon dioxide levels can cause hypotension and changes to heart rate and cardiac output (Randall 1962; Houston et al. 1971; Hill and Forster 2004). Therefore, it is important to aerate both induction and recovery baths as well as the anesthetic solution being used for gill irrigation during any surgical procedures. Aeration will increase passive gas exchange at the gills (Summerfelt and Smith 1990; Ross and Ross 2008).

In addition to elevating catecholamine levels, TMS exposure increases the level of circulating cortisol (Iwama et al. 1989; Molinero and Gonzalez 1995; Mommsen et al. 1999). Cortisol, a principal corticosteroid that plays a role in intermediary metabolism, ion regulation and osmoregulation, and immune function, has been shown to cause swelling of erythrocytes (red blood cells) (Sovio et al. 1977; Iwama et al. 1989; Mommsen et al. 1999). This swelling can subsequently block gill lamellae and lead to reduced oxygen uptake. As induction time increases, cortisol concentrations increase along with associated hematological components such as glucose, lactate, sodium, and potassium concentrations in freshwater fish (Hattingh 1977; Sovio et al. 1977; Strange and Schreck 1978; Wedemeyer et al. 1990; Sladky et al. 2001). Yet, lethal doses of TMS (i.e., 200 mg/L of TMS to water) do not elicit cortisol or hyperglycemic responses compared to lower immobilizing doses (Strange and Scheck 1978; Wedemeyer et al. 1990). In addition, water quality and biological factors, such as species, length and weight, sex, time of year, condition, disease, and stress, can alter and/or amplify physiological responses (e.g., cortisol production) to anesthetics and the handling or surgical procedure.

Each side effect mentioned above may not be life threatening in its intensity or duration; however, the amalgamation or amplification of these effects can hinder the recovery and health of the fish during exposure and recovery. The possibility of these potentially detrimental side effects makes it important to consider how TMS is administered to achieve a proper level of anesthesia for surgical procedures and how the fish is handled in the timeframe surrounding anesthesia. Gentle netting and handling techniques should be employed whenever possible before, during, and after anesthesia. Handling has been shown to be an important factor in the stress response during surgical procedures (Hill and Forster 2004), and multiple stressors can lead to mortality in and of itself (Strange and Schreck 1978). The handler should be aware of the number of fish in the anesthetic bath and the length of time each fish has been in the bath by following a conscientious anesthesia protocol. This observation will reduce avoidable differential effects of anesthesia on the fish caused by varying times in the bath and the decay of the anesthetic due to its absorption or interaction with byproducts released by the fish.

To limit hypoxemia and acute stress responses, induction and handling times should be minimized. It is recommended that stages required for surgery, like Stage 4, should be achieved in less than 3 min (Wedemeyer et al. 1990; Wedemeyer 1997; Mommsen et al. 1999; Wagner et al. 2003). Focused research into physiological differences between longer and shorter induction times is somewhat scarce. However, many of the negative physiological effects occur in deeper stages of anesthesia and as fish approach and reach medullary collapse (Stage 3 and higher; Schoettger and Julin 1967; Iwama et al. 1989; Burka et al. 1997). Therefore, decreasing time spent in these stages by increasing the dosage of TMS is warranted. When large numbers of fish need to be processed, higher dosages of anesthetic could improve fish induction time in the bath, thus reducing numbers of fish in the anesthetic bath at any one time. This method would not only reduce the time each fish is in the anesthetic solution but also allow for more flexibility when managing anesthetized fish in case of processing delays. Higher doses require diligence on the part of the researcher. Fish will transition to deeper stages of anesthesia more quickly and overdose can occur.

Maintenance of anesthesia during surgery

Once fish are placed on the surgery table, an anesthetic maintenance dose (a lower dose than the induction anesthetic bath) is necessary to maintain Stage 4 anesthesia for a short (<10 min) surgical procedure. A well-oxygenated maintenance dose can be delivered via a flexible hose that is placed in the mouth to irrigate the fish's gills. The flow rate should be adequate to ensure sufficient gas exchange across the gills. Published guidelines, although vague, can be used to estimate a sufficient maintenance dose to maintain a fish at the appropriate level of anesthesia (Schoettger and Julin 1967; Ross and Ross 2008). Therefore, a maintenance water system that incorporates two water sources, one containing anesthetic and one with fresh water, that mixes the water sources just before reaching the gills is preferred for anesthesia maintenance, fish stress minimization, and surgeon ease. In the field, this method can be easily set up using two buckets (one containing TMS water and one containing fresh water) and a hose from each that converges at or before the fish's mouth. It is preferable to have controls on the maintenance water system to either increase or decrease contributions of each water source. This method allows the surgeon to control the level of anesthesia during the surgical procedure. After the surgical procedure is completed, the fish should be transferred immediately to a recovery bath containing fresh, aerated water and monitored to ensure recovery.

Buffering the anesthetic bath

In general, salmonids should be maintained in waters with a pH range of 6.5–9.0 to avoid experiencing detrimental changes in physiological functions (Piper et al. 2001). Dissolving TMS in freshwater can reduce the anesthetic bath pH due to the hydrolysis of the sulfonate radical (Ohr 1976). If the water in the bath does not have adequate buffering capacity, the subsequent pH drop from the TMS additions could alter the water pH below the ideal physiological range of a given species. Fish held in acidic environments (pH $\leq$ 5) have difficulty maintaining many physiological functions (Iwama et al. 1989; Burka et al. 1997). In low pH conditions, disturbances in ionic and osmotic balance can lead to haemoconcentration (Iwama et al. 1989; Burka et al.

1997), increased blood pressure (Milligan and Wood 1982), and suppressed metabolic rate (Packer 1979; Pelster and Randall 1998).

The potency of TMS decreases with decreasing pH (Ohr 1976). In acidic environments with a pH of 5.5 or less, the anesthetic portion of tricaine is positively charged, which inhibits its diffusion across the gill surface (Ohr 1976; Ross and Ross 2008). Therefore, induction time increases inversely with pH at these low values, increasing the time in the anesthetic bath. Reduced pH after the addition of TMS can be counteracted by adding buffers, such as sodium bicarbonate ($NaHCO_3$), tris (tris [hydroxymethyl] aminomethane; $[HOCH_2]_3CNH_2$), or sodium hydroxide (NaOH), to the water bath. The need for additional buffers to maintain an acceptable pH (6.5–9) will depend on the dose of TMS used and the alkalinity of the local water source (Summerfelt and Smith 1990). Saltwater and freshwater with higher alkalinity contain enough buffers to maintain an acceptable pH (Piper et al. 2001). However, waters without sufficient alkalinity can experience wide shifts in pH if the addition of TMS overcomes the buffering capacity. Therefore, it is important to check the pH of the anesthetic bath before its use at each new location or when a change in local water chemistry is suspected (Summerfelt and Smith 1990; Iwama and Ackerman 1994).

Future Research

The long-term effects of TMS exposure on salmonids and other fish species after surgical implantation of transmitters have not been thoroughly elucidated. Therefore, more research is needed to determine these effects. In addition, research exploring ideal storage protocols for stock TMS solutions and the physiological effects of various induction and recovery times needs to be conducted to increase the safety and efficacy of this anesthetic.

There is a need for additional laboratory research on other anesthetics because some aspects of TMS are undesirable. The required withdrawal period for potential food fish, the need for personal protective equipment (sometimes difficult in a field setting), and the ease of accidental overdose and sublethal negative physiological effects make TMS less than ideal as an anesthetic for fish. However, wide scale use of other anesthetics is difficult as TMS is the only approved anesthetic for use in some food fish species in the US, Canada and the UK (FDA 2006; Health Canada 2010; VMD 2010a). In the future, additional anesthetics may be legalized for use, so we emphasize that it is necessary to monitor progress and developments in the testing and regulation of other fish anesthetics.

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References

AFS (American Fisheries Society) (2004) Guidelines for the use of fishes in research. American Fisheries Society Press, Bethesda, MD

Allen JL, Hunn JB (1986) Fate and distribution of some drugs used in aquaculture. Vet Human Toxicol 28:21–24

ALPHARMA (Animal Health Limited) (2001) MS222 (Tricaine methane sulphonate). Technical Bulletin. Available via www.europharma.no/fiskehelse/datablad/bedovelse/MS222.pdf. Accessed 10 April 2009

Argent (Argent Laboratories) (2008) Anesthetics and tranquilizers – Finquel (MS-222 or tricaine). Available via

Arnolds DE, Zottoli SJ, Adams CE, Dineen SM, Fevrier S, Guo Y, Pascal AJ (2002) Physiological effects of tricaine on the supramedullary/dorsal neurons of the cunner, *Tautogolabrus adspersus*. Biol Bull 203:188–189

Barton BA (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr Comp Biol 42:517–525

Bell GR (1987) An outline of anesthetic and anesthesia for salmonids, a guide for fish culturists in British Columbia. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1534

Berstein PS, Digre KB, Creel DJ (1997) Retinal toxicity associated with occupational exposure to the fish anesthetic MS-222. Amer J Ophthalmol 124:834–843

Bové FJ (1962) MS-222 Sandoz. The anesthetic of choice for fish and other cold-blooded organisms. Sandoz News 3

Braithwaite VA, Boulcott P (2007) Pain perception and fear in fish. Dis Aquat Org 75:131–138

Burka JF, Hammel KL, Horsberg TE, Johnson GR, Rainnie DJ, Spears DJ (1997) Drugs in salmonid aquaculture–a review. J Vet Pharm Therap 20:333–349

Butterworth JF, Strichartz GR (1990) Molecular mechanisms of local anesthesia: a review. Anesthesiology 72:711–734

Carmichael FJ (1985) General anaesthetics and local anaesthetics. In: Kalant H, Roschlau WHE, Sellers EM (eds) Principles of medical pharmacology, 4th edn. Department

of Pharmacology, University of Toronto, Toronto, Ontario, pp 265–289

FDA (U.S. Food and Drug Administration) (1997) Freedom of Information Summary for NADA 200-226. Available via http://www.fda.gov/cvm/FOI/1635.htm. Accessed 07 April 2009

FDA (U.S. Food and Drug Administration) (2006) Database of approved animal drug products. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine. VMRCVM Drug Information Laboratory. Available via http://www.accessdata.fda.gov/scripts/AnimalDrugsAtFDA/. Accessed 10 December 2009

Ferreira JT, Schoonbee HG, Smit GL (1984) The uptake of the anaesthetic benzocaine hydrochloride by the gills and skin of three freshwater fish species. J Fish Biol 25:35–41

Hattingh J (1977) The effect of tricaine methanesulphonate (MS-222) on the microhaematocrit of fish blood. J Fish Biol 10:453–455

Health Canada (2010) List of veterinary drugs that are authorized for sale by health Canada for use in food-producing aquatic animals. Available via http://www.hc-sc.gc.ca/dhp-mps/vet/legislation/pol/aquaculture_anim-eng.php. Accessed 13, October 2010

Heisler N (1988) Acid-base regulation. In: Shuttleworth TJ (ed) Physiology of elasmobranch fishes. Springer, Berlin, pp 215–252

Hensel H, Bromm B, Nier K (1975) Effect of ethyl m-aminobenzoate (MS-222) on ampullae of lorenzini and lateral-line organs. Experientia 31:958–960

Hill JV, Forster ME (2004) Cardiovascular responses of Chinook salmon (*Oncorhynchus tshawytscha*) during rapid anaesthetic induction and recovery. Comp Biochem Physiol 137:167–177

Houston AH, Corlett JT (1976) Specimen weight and MS-222. J Fish Res Board Can 33:1403–1407

Houston AH, Madden JA, Woods RJ, Miles HM (1971) Some physiological effects of handling and tricaine methanesulphonate anesthetization upon the brook trout, *Salvelinus fontanalis*. J Fish Res Bd Can 28:625–633

Hunn JB, Allen JL (1974) Movement of drugs across the gills of fishes. Ann Rev Pharmacol 14:47–55

Iwama GK, Ackerman PA (1994) Biochemistry and molecular biology of fishes, vol 3 – Analytical Techniques. In: Hochachka P, Mommsen T (eds) Anaesthetics. Elsevier Science, Amsterdam, p 15

Iwama GK, McGeer JC, Pawluk MP (1989) The effects of five fish anaesthetics on acid–base balance, hematocrit, cortisol, and adrenaline in rainbow trout. Can J Zool 67:2065–2073

Marking LL (1967) Toxicity of MS-222 to selected fishes. In: Investigations in fish control. Resource Publication 18, U.S. Department of the Interior. Bureau of Sport Fisheries and Wildlife, Washington, DC, pp 3–10

Marking LL, Meyer FP (1985) Are better anesthetics needed in fisheries? Fisheries 10:2–5

McFarland WN (1959) A study of the effects of anesthetics on the behavior and physiology of fishes. Publication of the Institute of Marine Science, University of Texas at Austin 6:23–55

Merck and Company (1989) The Merck index, 11th edn. Merck and Company, Inc., Rahway, NJ

Milligan CL, Wood CM (1982) Disturbances in haematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. J Exp Biol 99:397–415

Molinero A, Gonzalez J (1995) Comparative effects of MS-222 and 2-phenoxyethanol on gilthead sea bream (*Sparus aurata* L.) during confinement. Comp Biochem Physiol 111:405–414

Mommsen TP, Vijayan MM, Moon TW (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev Fish Biol Fish 9:211–268

Ohr EA (1976) Tricane methanesulphonate–l, pH and its effects on anaesthetic potency. Comp Biochem Physiol 54:13–17

Olfert DE, Cross BM, McWilliam AA (1993) Guide to the care and use of experimental animals, 2nd edn, vols 1 and 2, vol 1 and 2, 2nd edn. Canadian Council on Animal Care, Ottawa

Packer RK (1979) Acid-base balance and gas exchange in brook trout (*Salvelinus fontainalis*) exposed to acidic environments. J Exp Biol 79:127–134

Pelster B, Randall D (1998) The physiology of the root effect. In: Perry SF, Tufts B (eds) Fish physiology, vol 17. Academic Press, New York, pp 113–139

Perry SF, Gilmour KM (2006) Acid-base balance and CO2 excretion in fish: unanswered questions and emerging models. Resp Physiol Neuro 154:199–215

Piper RG, McElwain IB, Orme LE, McCraren JP, Fowler LG, Leonard JR (2001) Chapter 1: Hatchery requirements. In: Wedemeyer GA (ed) Fish hatchery management. American Fisheries Society, Bethesda, MD, pp 11–12

Portz DE, Woodley CM, Cech CC Jr (2006) Stress-associated impacts of short-term holding on fishes. Rev Fish Biol Fish 16:125–170

Randall DJ (1962) Effect of an anaesthetic on the heart and respiration of teleost fish. Nature 195:506–508

Rang HP, Dale MM, Ritter JM, Moore PK (2003) Chapter 43: local anesthetics. In: Rang HP, Dale MM, Ritter JM, Moore PK (eds) Pharmacology, 5th edn. Churchill Livingstone, Edinburgh, Scotland

Rose JD (2002) The neurobehavioral nature of fishes and the question of awareness and pain. Rev Fish Sci 10:1–38

Ross LG, Ross BR (2008) Anaesthetic and sedative techniques for aquatic animals. Blackwell Science, Oxford, United Kingdom

Sato T, Kitayama S, Mitsuhata C, Ikeda T, Morita K, Dohi T (2000) Selective inhibition of monoamine neurotransmitter transport by synthetic local anesthetics. Naunyn-Schmiedeberg's Arch Pharmacol 361:214–220

Schoettger RA, Julin AM (1967) Efficacy of MS-222 as an anaesthetic on four salmonids. In: Investigations in fish control, Resource Publication 19. U.S. Department of the Interior, Bureau of Sport Fisheries and Wildlife, Washington, DC, pp 3–15

Sehdev HS, McBride JR, Fagerlund UHM (1963) 2-phenoxyethanol as a general anesthetic for sockeye salmon. J Fish Res Bd Can 20:1435–1440

Sigma (2008) Material safety data sheet: ethyl 3-aminobenzoate methanesulfonate, E10521. Sigma-Aldrich Corporation. Available via http://www.sigmaaldrich.com/site-level/msds.html. Accessed 10 April 2009

Sladky KK, Swanson CR, Stoskopf MK, Loomis MR, Lewbart GA (2001) Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (*Piaractus brachypomis*). Amer J Vet Res 62:337–342

Sneddon LU (2002) Anatomical and electrophysiological analysis of the trigeminal nerve in a teleost fish, *Oncorhynchus mykiss*. Neurosci Lett 319:167–171

Sneddon LU, Braithwaite VA, Gentle MJ (2003) Do fish have nociceptors? Evidence for the evolution of a vertebrate sensory system. Proc Royal Soc Lond B 270:1115–1121

Sovio A, Nyholm K, Huhti M (1977) Effects of anesthesia with MS222, neutralized MS222 and benzocaine on the blood constituents of rainbow trout, *Salmo gairdneri*. J Fish Biol 10:91–101

Spath M, Schweickert W (1977) The effect of metacaine (MS-222) on the activity of the efferent and afferent nerves in the teleost lateral-line system. Naunyn-Schmiedeberg's Arch Pharmacol 297:9–16

Strange RJ, Schreck CB (1978) Anesthetic and handling stress on survival and cortisol concentration in yearling Chinook salmon (*Oncorhynchus tshawytscha*). J Fish Res Bd Can 35:345–349

Summerfelt RC, Smith LS (1990) Methods for fish biology. In: Schreck CB, Moyle PB (eds) Anesthesia, surgery and related techniques. American Fisheries Society, Bethesda, MD, pp 213–272

VMD (Veterinary Medicines Directorate) (2010a) Product Information Database. Available via http://www.vmd.gov.uk/ProductInformationDatabase/Search.aspx. Accessed 13 October 2010

VMD (Veterinary Medicines Directorate) (2010b) Summary of Product characteristics for MS222 SPC-88144. Available via http://www.vmd.gov.uk/ProductInformationDatabase/SPC_Documents/SPC_88144.doc. Accessed 13October 2010

Wagner GN, Singer TD, McKinley RS (2003) The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquacult Res 34:1139–1146

Wayson KA, Downes H, Lynn RK, Gerber N (1976) Studies on the comparative pharmacology and selective toxicity of tricaine methanesulfonate: metabolism as a basis of the selective toxicity in poikilotherms. J Pharmacol Exp Therap 198:695–708

Wedemeyer GA (1997) Effects of rearing conditions on the health and physiological quality of fish in intensive culture. In: Iwama GK, Pickering AD, Sumpter JP, Schreck CB (eds) Fish stress and health in aquaculture. Cambridge University Press, Cambridge, pp 35–72

Wedemeyer GA, Barton BA, McLeay D (1990) Stress and acclimation. In: Schreck CB, Moyle PB (eds) Methods for fish biology. American Fisheries Society, Bethesda, MD, pp 451–489

Western (Western Chemical, Inc.) (2008a) Fact sheet: Tricaine-S. Available via http://www.wchemical.com/Assets/File/tricaineS_instructions.pdf. Accessed 07 April 2009

Western (Western Chemical, Inc.) (2008b) Material safety data sheet: Tricaine-S. Available via http://www.wchemical.com/Assets/File/tricaineS_MSDS.pdf. Accessed 07 April 2009

Yoshimura H, Nakamura M, Koeda T (1981) Mutagenicity screening of anesthetics for fishes. Mutation Res 90: 119–124