ANESTHETICS[1]

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The use of anesthetics facilitates work with fish at the research level and is required for invasive studies such as surgical preparations for physiological investigations, where the fish must be held immobile for extended periods of time. Sedation with the use of anesthetics is also used for the manipulation of animals during procedures such as transport, grading or vaccination. Although the use of anesthetics is primarily for the purpose of holding fish immobile while the animal is being handled for sampling, anesthetics are also used to lower the level of stress associated with such procedures. Overdose of anesthetics is also used routinely as an effective and humane means of euthanizing fish.

Anesthesia is generally defined as a state caused by an applied external agent resulting in a loss of sensation through depression of the nervous system. Anesthetics may be local or general, depending on their application.

The method of administration for each anesthesia is fairly well defined, but the appropriate time or circumstance for using it is less clear. The choice of anesthetic depends on many factors. For example, if the maintenance of gill ventilation during an experimental procedure is desirable, then ketamine hydrochloride would be one possible anesthetic (Graham & Iwama, 1990), but because it is best administered by injection, an initial anaesthetization with another suitable anesthetic such as buffered TMS (MS-222) or metomidate may be required. If transport stress is a concern, it may be minimized through a light sedation brought about by low concentrations of an anesthetic such as TMS (buffered with sodium bicarbonate if necessary).

The nature of the application as well as any local regulations and legislation may dictate the choice of anesthetic. Currently, only TMS and metomidate are registered for veterinary use with fish in Canada, though researchers have the luxury of obtaining many compounds not available to the public. Lengthy withdrawal times are mandatory for chemical anesthesia of food fish prior to harvest, and this has led to an interest in less persistent and more natural anesthetics such as clove oil.

While effective and lethal doses for the major chemical anesthetics used for fish are well established, there has been a trend towards the prohibition of their use in fisheries and aquaculture-related sciences. The few available anesthetics registered for use with fish in Canada, and the trend towards not using chemical agents, has stimulated renewed interest in anesthetic research and the search for non-chemical means of anaesthetizing fish. Research into the optimization of anesthesia through the use of electricity and CO_2 are necessary, as are investigations into developing the use of agents such as clove oil into viable alternatives to chemical anesthesia.

To be of use to a researcher, an anesthetic should induce anesthesia in less than 3 minutes and recovery should occur within 5 minutes of placement of the fish in clean water (Marking

[1] Trade and company names mentioned in this appendix are for information purposes only and do not imply endorsement by the authors.

& Meyer, 1985; Bell, 1987). The anesthetic chosen should not have toxic side effects for either the fish or the handler. It should be biodegradable and have properties which allow the body to clear it from the tissues following exposure. It should have no persisting physiological, immunological or behavioral effects which could reduce the likelihood of survival of the fish or interfere with later measurements. Cost effectiveness and availability of the anesthetic should be considered, as should characteristics such as foaming, which could reduce gas transfer into and out of the water.

Because the efficacy of most anesthetics are affected by species, body size, the density of fish in the bath, as well as water quality (e.g., hardness, temperature, or salinity), it is imperative that preliminary tests be performed with small numbers of the fish to determine the optimal dosage and exposure time. Due care should be taken to control the level of anesthesia desired, through the application of the appropriate concentration, and to maintain constant observation of the fish as they go through the various stages of anesthesia (see Table 1).

Table 1 Stages of anesthesia and recovery

Stages of Anesthesia	Description
I	Loss of equilibrium
II	Loss of gross body movements but with continued opercular movements
III	As in Stage II with cessation of opercular movements
Stages of Recovery	
I	Body immobilized but opercular movements just starting
II	Regular opercular movements and gross body movements beginning
III	Equilibrium regained and preanesthetic appearance

From Iwama *et al*., 1989

All anesthetics should be handled with care, and appropriate Material Safety Data Sheets (MSDS) should be consulted to ensure the safety of users. Cautionary notes have been included within the body of this text on each anesthetic. In the first section, some characteristics of the major anesthetics that are in use for fishes are outlined, as well as essential parameters for the application of those anesthetics, including optimum and lethal dosages, and induction and recovery times. Possible physiological effects are also noted. Table 2 outlines the dose ranges for recommended anesthetics. For additional information, readers are referred to Iwama & Ackerman (1994).

Physiology of Anesthesia

Many descriptions of the stages of anesthesia exist for fish, but for the scope of this review, the stages displayed in Table 1 should be sufficient for the investigator to ascertain the level of anesthesia experienced by the fish. For more detailed distinctions between the stages, readers are referred to McFarland (1959), Bell (1987), or Summerfelt & Smith (1990).

Many of the anesthetics used on fish are similar to those used in mammalian research or even on humans. However, some of these are considered topical anesthetics in mammals, whereas they are applied in a general manner to fish. As such, a progressive depression of both the central and peripheral nervous system activities occurs (Summerfelt & Smith, 1990). Immobility of the fish is achieved by Stage III for most anesthetics; however, some anesthetics (e.g., 2-phenoxyethanol, metomidate, quinaldine sulfate) may not completely block involuntary muscle movements and muscle twitching may still occur. Such side effects may make the anesthetic unsuitable for use if blood sampling or surgery is required.

Anesthesia as a Potential Stressor

Stage III anesthesia generally involves a cessation of breathing which, in turn, reduces gas transfer leading to hypoxia and respiratory acidosis due to the reduction of blood oxygen (O_2) tension and a concomitant rise in blood CO_2. As a result of the lack of respiration, increases in blood concentrations of adrenaline and cortisol have been demonstrated in fish anaesthetized with buffered TMS, 2-Phenoxyethanol, Benzocaine, Metomidate, and CO_2 (Iwama *et al.*, 1989; Molinero & Gonzalez, 1995). In most cases, prolonged maintenance of Stage III anesthesia without gill irrigation will result in death.

Potential Hazards to Humans

Many of the anesthetics in use have the potential to cause harm to humans if they are misused. For example, lack of proper ventilation when anaesthetizing fish with CO_2 could prove deadly to the user. Some of the chemicals such as urethane have been shown to have toxic qualities, and impacts of misuse could have far reaching health repercussions if mishandled. While some specific hazards have been noted in the paragraphs relating to each anesthetic, it is imperative that any anesthetic be investigated for possible human hazard prior to its use and that the appropriate precautions taken if a more innocuous choice is not available. In all instances, MSDS sheets should be consulted to ensure proper handling.

Chemical Anesthesia

TMS

TMS (MS-222), [3-aminobenzoic acidethyl ester methanesulfonate] is the most widely used fish anesthetic, and it is extremely effective for rapid induction of deep anesthesia. TMS is commonly used in research laboratories and has been registered by Health Canada for veterinary use with fish. It is a white crystalline powder that is easily dissolved in water, with a solubility of 1.25 g/mL water, at 20 ^{o}C.

Precautions:

TMS is generally safe to handle, but contact with eyes and mucous membranes should be avoided (Merck and Company, 1989), as irritation can result. Exposure of a stock solution to sunlight can make it toxic to fish in seawater (Bell, 1987). Because it is an acid, care should be exercised to buffer soft waters with an equal weight of sodium bicarbonate if necessary.

Dosages:

Dose is related to species, size and density of the fish, as well as water temperature and hardness, but in general, anesthetic doses are usually between 25 to 100 mg/L and

excessively long exposures at 50 mg/L or more should be avoided as mortalities may be induced (Marking, 1967). Aeration should always be used. Induction and recovery times have been shown to be inversely correlated with body weight, with these effects being more pronounced in small fish (Houston & Corlett, 1976). A lethal dose of 400 - 500 mg/L is generally used for euthanasia of salmonids.

Notes:

- TMS may have a haemodilution effect on the blood (Macavoy, 1997).
- The initial absorption of the anesthetic by the fish has an excitatory effect, which is reduced by buffering, but such an effect may have impacts on physiological measurements. TMS has been shown to produce a stress response in sea bream (*Sparus auratus*) at doses as low as 25 mg/L with significant effects on cortisol, glucose and lactate levels following exposure (Molinero & Gonzalez, 1995).
- TMS is also known as MS-222, [TM18]Finquel, Tricaine, tricaine methanesulfonate and Metacaine.

Benzocaine

Benzocaine [p-aminobenzoic acid ethyl ester] has two forms: a crystalline salt with a water solubility of 0.4 g/L, or a freebase form which must be dissolved in ethyl alcohol first at 0.2 g/mL (Merck and Company, 1989).

Precautions:

Benzocaine hydrochloride is generally harmless to humans and is commonly used as a local anesthetic in cough drops, sprays, sunburn creams, and haemorrhoid preparations (McErlean & Kennedy, 1968). However, the powder is a respiratory irritant and reasonable care should be exercised. It is also used as a topical and local anesthetic for veterinary purposes (Merck and Company, 1989).

Dosages:

The efficacy of benzocaine has been shown to be affected by the size of the fish, where the smallest fish require the lowest dose, as well as by the temperature of the water (Gilderhus, 1989). Reported doses range from 25 – 100 mg/L (Ferriera *et al.*, 1979; Yesaki, 1988; Gilderhus, 1989; Gilderhus, 1990; Gilderhus, 1991) with doses for salmonids falling in the range between 25 – 45 mg/L (Gilderhus, 1989). Induction time is generally in less than 4 minutes and when fish are placed in clean water, recovery is usually within 10 minutes. Lethal doses are dependant on the water temperature, and the safety margins (difference between lethal and effective doses) are widest in cooler temperatures (Gilderhus, 1989).

Notes:

- Fish may retain some locomotory functions throughout all stages of anesthesia, making this an unsuitable anesthetic for use in procedures involving surgery.
- Benzocaine is also known as [TM1]Anesthesin, [TM14]Anesthone, [TM2]Americaine, ethyl aminobenzoate, Orthesin and Parathesin.

Lidocaine

Lidocaine [2-(diethylamino)-N-(2,6-dimethylphenyl) acetimide], in freebase form, is insoluble in water, but freely soluble in acetone or alcohol. It is generally used in the hydrochloride salt form which is freely soluble in water (Merck and Company, 1989). It is a cardiac depressant which is used by veterinarians topically or injected as a nerve block (Merck and Company, 1989).

Dosages:

Lidocaine has been used in combination with sodium bicarbonate to anaesthetize carp (*Cyprinus carpio*), tilapia (*Oreochromis/Tilapia mossambica*) and catfish (*Ictalurus punctatus*) (Carrasco *et al.*, 1984). The addition of sodium bicarbonate, at 1 g/L, has been demonstrated to enhance the anesthetic effects of lidocaine. Without the addition of bicarbonate, there are huge variations in required doses. For example, tilapia required in excess of 800% more lidocaine than carp when it was administered in the absence of sodium bicarbonate. Carrasco *et al.* (1984) showed a reasonable safety margin between anesthetic and lethal doses.

Notes:

- Lidocaine is also known as [TM3]Xylocaine.

Metomidate and Etomidate

Metomidate [1-(1-phenylethyl)-1H-imidazole-5-carboxylic acid methyl ester] is a water-soluble powder which has the properties of a hypnotic, or sleep-inducing, drug. Etomidate [1-(1-phenylethyl)-1H-imidazole-5-carboxylic acid ethyl ester] is a colourless, odourless crystalline analogue of metomidate and propoxate (Merck and Company, 1989). It has been used on humans as a hypnotic drug, but it is very expensive and difficult to obtain (Bell, 1987).

Precautions:

A side effect of anesthesia with metomidate is muscle twitching which can make blood sampling difficult (Gilderhus & Marking, 1987). This effect has not been reported for etomidate. Metomidate has been demonstrated to be ineffective for use on larval fishes causing high mortalities (Massee *et al.*, 1995).

Dosages:

Metomidate is effective in both fresh and saltwater, and has been reported to be more potent in adult salmon adapted to sea water (Olsen *et al.*, 1995). Efficient dosages range from 1 – 10 mg/L (Olsen *et al.*, 1995) and very large safety margins have been reported with cod (*Gadus morhua*), Atlantic halibut (*Hippoglossus hippoglossus*) and Atlantic salmon (*Salmo salar*) with no mortalities (Mattson & Riple, 1989; Malmstroem *et al.*, 1993; Olsen *et al.*, 1995).

Both drugs are fast acting with induction times of less than 3 minutes and lengthy recovery times (up to 40 minutes) (Amend *et al.*, 1982; Limsuwan *et al.*, 1983b; Plumb *et al.*, 1983; Gilderhus & Marking, 1987; Mattson & Riple, 1989).

Notes:

- The efficacy of etomidate is pH dependent and it has proven to be more effective in alkaline waters (Amend *et al.*, 1982). Temperature influences the toxicity of etomidate, with higher temperatures rendering the drug less toxic (Limsuwan *et al.*, 1983b).
- Metomidate does not cause hyperactivity in the fish, but concentrations above 3 mg/L have been shown to block the cortisol response, and result in increases in blood lactate levels and haematocrit (Olsen *et al.*, 1995).
- Metomidate and etomidate are also known as Marinil, Methomidate or [TM19]Methoxynol, and [TM10]Hypnomidate or [TM4]Amidate, respectively. Both are relatively fast acting drugs.

Propoxate

Propoxate [propyl-DL-1-(phenylethyl) imidazole-5-carboxylate hydrochloride] is a crystalline powder which resembles metomidate and etomidate structurally, and is freely soluble in both fresh water and salt water. It is stable in solution for long periods and is 100 times more soluble than TMS (Thienpont & Niemegeers, 1965).

Precautions:

Caution should be exercised at higher doses as respiratory arrest occurs after 15 minutes at 64 mg/L, and after 1 hour at 16 mg/L (Thienpont & Niemegeers, 1965).

Dosages:

Propoxate is 10 times more potent than TMS. Effective concentrations range from 0.5 mg/L to 10 mg/L (Summerfelt & Smith, 1990). A level of 0.25 mg/L is safe for anesthesia of lengths up to 16 hours. Ross & Ross (1984) recommend a dose of between 1 and 4 mg/L to anaesthetize fish resulting in induction times ranging from 30 seconds for higher doses.

Ketamine hydrochloride

Ketamine hydrochloride [2-(0-chlorophenyl)-2-(methyl-amino) cyclohexanone hydrochloride] is a white crystalline powder, which has a water solubility of 200 g/L at 20 ^{o}C (Merck and Company, 1989). It has been widely used as an anesthetic both in human and veterinary medicine, and is safe for the handler (Merck and Company, 1989).

Dosages:

Ketamine has a wide safety margin between lethal and effective doses. It is an injectible drug, which is generally dissolved in saline and administered intravascularly (i.v.) at a dose of 30 mg/kg for salmonids which results in anesthesia in under 3 minutes, with a recovery time of 1 – 2 hours (Graham & Iwama, 1990). Intramuscular (i.m.) injections can result in variability with respect to the depth and length of anesthesia (Graham & Iwama, 1990). Because the drug is an injectible, it is not appropriate for large groups of fish. However, intramuscular injections with a dart gun to specific individual fish in a tank or stream may be a successful application. Graham & Iwama (1990) used double the i.v. dose for such i.m. administration.

Notes:

- Fish may struggle in the early stages of anesthesia, which would indicate some degree of stress, but the drug does not block ventilatory rhythm (Williams *et al.*, 1988; Graham &

Iwama, 1990). It may, therefore, be appropriate or desirable for long-term anesthesia, where it is not possible to maintain constant irrigation of the gills with water.

- Ketamine is also known as [TM6]Ketaject, [TM14]Ketalar, Ketanest, [TM6]Ketaset, [TM6]Ketavet, Ketalean and [TM14]Vetalar.

Quinaldine sulfate

Quinaldine sulfate [2-methylquinoline sulfate] is a light yellow crystalline powder which has a water solubility of 1.041g/L (Merck and Company, 1989). It is one of the most widely used anesthetics by marine biologists to collect tidepool and coral reef fishes (e.g., Munday & Wilson, 1997).

Precautions:

Extended exposure of fish to quinaldine sulfate has been shown to be toxic (Amend *et al.*, 1982), and is therefore only useful as a short-term anesthetic.

Dosages:

The effective dosage varies widely with species, size, and temperature (Schoettger & Julin, 1968). Larger fish are more heavily sedated at a given dose and the recovery is longer at higher temperatures (Schoettger & Julin, 1968). Quinaldine sulfate is effective at water pH levels above 6.

Notes:

- Gilderhus & Marking (1987) reported that quinaldine sulphate did not completely block involuntary muscular movement; therefore, it may not be appropriate for applications such as surgery or marking fish.
- Quinaldine sulfate is also known by the trade name [TM11]Quinate.

Propanidid

Propanidid (4-[2-(diethylamino)-2-oxoethoxy]-3-methoxybenzeneacetic acid propyl ester) is a pale yellow liquid which is insoluble in water, but soluble in alcohol (Merck and Company, 1989).

Dosages:

Induction and recovery times for propanidid have been shown to be in the range of 2 - 4 minutes and 5 -10 minutes, respectively, at 1.5 - 3.0 mL/L or for intraperitoneal injections of 2.0 mg/kg in salmonids ranging from 2 to 2500 g (Siwicki, 1984).

Notes:

- Propanidid causes little or no change to the blood chemistry (red cell numbers, haematocrit, haemoglobin content, and serum concentrations of total bilirubin, total protein, urea, glucose, chloride, iron and magnesium) of the exposed fish either during anesthesia or for a period of 24 hours post anesthesia. However, a significant mixed respiratory and metabolic acidosis, lasting for approximately 1 hour after recovery from anesthesia has been observed. The anesthetic also caused no change in water CO_2 or pH (Siwicki, 1984).
- Propanidid is also known as [TM5]Epontol or [TM8]Sombrevin.

Clove oil and derivatives

Clove oil has recently been suggested as an alternative fish anesthetic. Clove oil is a pale yellow liquid derived from the leaves, buds and stem of the clove tree (*Eugenia* sp.). Its active ingredients are eugenol (4-allyl-2-methoxyphenol) and iso-eugenol (4-propenyl-2-methoxyphenol), which can comprise 90-95% of clove oil by weight.

Precautions:

Clove oil has been used for many years as a food additive and a topical analgesic in dentistry, and is recognized as a GRAS (Generally Recognized As Safe) substance by the US FDA for use in humans. ^{TM25}AQUI-S is a pharmaceutical derivative that contains 50% active ingredient and is registered for use with food fish in New Zealand and Australia with a nil withdrawal period. However, neither anesthetic is approved for use with fish in North America. Both substances are safe to handle, but as with all chemical anesthetics, contact with eyes and mucous membranes should be avoided.

Dosages:

Clove oil is most effective as an anesthetic at concentrations of 40-60 mg/L for salmonids, and should be dissolved in ethanol (e.g., 1:9) before mixing into the water. Clove oil has a slightly faster induction time and a longer recovery time than similar concentrations of TMS (Anderson *et al.*, 1997; Keene *et al.*, 1998). ^{TM25}AQUI-S can be dissolved directly into fresh or salt water, and has been shown to be effective at 20 mg/L for anaesthetizing juvenile chinook salmon (AQUI-S New Zealand Ltd., 2004). Both compounds have a wide margin of safety between effective and lethal doses, and fish do not show signs of distress when being anaesthetized.

2-Phenoxyethanol

2-Phenoxyethanol (2-PE) [1-hydroxy-2-phenoxyethane] is a colourless, oily, aromatic liquid with a burning taste, and has a solubility in water of 27 g/L at 20 ^{o}C (Merck and Company, 1989). It is often used as a topical anesthetic (Merck and Company, 1989).

Precautions:

2-Phenoxyethanol is a mild toxin and may cause some irritation to the skin, therefore any contact with the eyes should be avoided (Bell, 1987). Based on human toxicology data, it may also cause liver and kidney damage (Summerfelt & Smith, 1990).

Dosages:

The efficacy of 2-PE varies with the size of the fish and with the temperature of the water (Sehdev *et al.*, 1963). While the effective dosage for salmonids is in the range of 200 – 300 μL/L, the lethal dose is as low as 500 μL/L, which leaves little margin for safety.

Notes:

- 2-phenoxyethanol does not block the stress response of fish and low doses have been shown to cause changes in plasma levels of cortisol, glucose, and lactate, with glucose and lactate levels being affected for over 24 hours post exposure (Molinero & Gonzalez, 1995). Data from our laboratory has shown that it does not block the involuntary muscle reflexes (unpublished observations T.Y. Yesaki and G.K. Iwama) which could interfere with blood sampling and surgical procedures. Fredricks *et al.* (1993) have shown that

cardiovascular activity is significantly reduced when fish are exposed to 2-PE, and that though heart rate and EKG patterns during recovery returned to normal rapidly, dorsal and ventral aortic pressures rose to above baseline values for an extended period of time. There is also potential for habituation as described by Weyl *et al.* (1996) who reported an increased tolerance to 2-PE upon repeated anaesthetization.

- While 2-PE has been widely used, the narrow margin between inductive and lethal doses, its potential for toxic side effects, and significant impacts on the cardiovascular system and physiological stress response, make it a less than ideal anesthetic for use with fish.
- 2-phenoxyethanol is also know by the names phenyl cellosolve, phenoxethol, phenoxetol, ethylene glycol monophenyl ether, and beta-hydroxyethyl phenyl ether.

Methylpentynol

Methylpentynol [3-methyl-1-pentyn-3-ol] is a liquid with a noxious odour and a burning taste, and has a solubility in water of 128 g/L at 25 ^{o}C (Merck and Company, 1989). It is a hypnotic sedative which, like 2-phenoxyethanol, varies in effectiveness with size and species of fish, as well as with water temperature. Other water quality parameters such as pH do not seem to have significant effects on the efficacy of anaesthetization.

Methylpentynol is not recommended for use with fish.

Notes:

- Methylpentynol is also known by a large number of alternate names: Meparfynol, [TM9]Allotropal, [TM21]Anti-stress, Apridol, Atemorin, [TM13]Atempol, Dalgol, Dorison, Dormalest, Dormidin, Dormigen, Dormiphen, [TM20]Dormison, Dormosan, Formison, Hesofen, Hexofen, Imnudorm, Oblivon, Pentadorm, Perlopal, Riposon, Seral, and [TM7]Somnesin.

Chlorobutanol

Chlorobutanol [1,1,1-trichloro-2-methyl-2-propanol] is a crystalline powder with a camphor odour. It has a high solubility in alcohol (1g/1mL [Merck and Company, 1989]), although it can also be dissolved in water (McFarland & Klontz, 1969). Stock solutions can be prepared well in advance of use and stored for long periods of time at 4^{o}C. In humans, chlorobutanol is used as a dental analgesic (Merck and Company, 1989). Chlorobutanol has limited use in aquaculture as it is toxic to small fish, and the fish response to this anesthetic is highly variable (McFarland & Klontz, 1969; Mattson & Riple, 1989).

Chlorobutanol is not recommended for use with fish.

Notes:

- Chlorobutanol is also known as Chloretone, [TM15]Coliquifilm, Methaform or Sedaform.

Halothane

Halothane [2-bromo-2-chloro-1,1,1-trifluoro-ethane] is a non-flammable, highly volatile liquid with a sweetish smell. It is used as an inhalant anesthetic in humans (Merck and

Company, 1989), but it is very light sensitive and has been shown to become toxic within 10 minutes of exposure to the effective concentration (Gilderhus & Marking, 1987).

Halothane is not recommended for use with fish.

Notes:

- Halothane is also known as [TM4]Fluothane or [TM17]Rhodialothon.

Urethane

Urethane [carbamic acid ethyl ester] is a crystalline powder with a water solubility of 2 g/mL (Merck and Company, 1989). Until it was shown to be a carcinogen for humans, it was a popular fish anesthetic as it has a wide margin of safety between lethal and effective dosages, and there seemed to be no ill effects to the fish with repeated exposures (McFarland & Klontz, 1969).

Urethane is not recommended for use with fish.

Notes:

- Urethane is also known as urethan or ethyl urethan.

Diethyl ether

Diethyl ether [1,1' -oxybesethane] is a very volatile, highly flammable liquid which, when exposed to light and air, will form explosive peroxides. It has a sweet pungent odour and a burning taste. It is slightly soluble in water, with saturation occurring at 8.43%, weight/weight (w/w), at 15 ^{o}C (Merck and Company, 1989). It is a skin irritant to humans and inhalation can lead to narcosis and unconsciousness, with death occurring due to respiratory paralysis (Merck and Company, 1989). Although reports of its use with fishes date in the 1940s and 1950s, the irritation to users has discouraged its common use (McFarland & Klontz, 1969).

Diethyl ether is not recommended for use with fish.

Notes:

- Diethyl ether is also known as ethyl ether, ethoxyethane, ethyl oxide, sulfuric ether and anesthetic ether.

Chloral hydrate

Chloral hydrate [2,2,2-trichloro-1,1-ethanediol] is an aromatic, acrid smelling powder with a bitter taste. Its solubility in water is temperature dependent: 2.4 g/mL at 0 ^{o}C; 5 g/mL at 10 ^{o}C; 8.3 g/mL at 25 ^{o}C; and 14.3 g/mL at 40 ^{o}C (Merck and Company, 1989). Chloral hydrate can irritate the skin, and is a potentially addictive drug which has sedative, narcotic, hypnotic, as well as depressant qualities (Merck and Company, 1989). Anesthesia is not deep, however, and chloral hydrate is more useful where sedation rather than deep anesthesia is required (McFarland & Klontz, 1969), such as in transport or various research applications.

Chloral hydrate is not recommended for use with fish.

Notes:

- Chloral hydrate is also known as [TM23]Escre, [TM22]Noctec, [TM12]Somnos, [TM24]Lorinal and [TM16]Chloraldurant.

Non-chemical Anesthesia

Electroanesthesia

Electrofishing is a common method for capturing juvenile and adult fish in fisheries management (Cowx & Lamarque, 1990; Reynolds, 1996). Electroanesthesia has primarily been used to immobilize adult fish for tagging or hatchery broodstock. Three types of electric current have been used to immobilize fish: alternating current (AC), direct current (DC), and pulsating forms of AC and DC. Direct current can cause anodotaxis (movement to the anode pole), electronarcosis (stunning) and electrotetany (tetanic muscle contractions), whereas alternating current causes only electronarcosis and tetany. The purpose of electroanesthesia is to induce electronarcosis, and avoid severe muscle tetany which can result in spinal injuries. The response of the fish to electricity depends on the intensity of the electric field and the duration of the shock. Others factors such as water conductivity, temperature, fish size and species can also affect the efficacy of electroanesthesia.

When used appropriately, there appear to be few long term deleterious effects on fish; however, there are acute physiological perturbations and some evidence of increased susceptibility to predation after recovery from electrofishing (Schreck *et al.*, 1976).

It has been found that electroshocking induces immediate elevation in plasma corticoid and lactate concentrations in rainbow trout, with persistent increases in plasma glucose and corticoids for at least 6 hours following capture, and cardiovascular changes including rythmn changes. These responses were attributed to trauma, oxygen debt, and general adaptation syndrome, hence the use of electroanesthesia should be regarded as an invasive and stressful procedure. This procedure in nature has notable impact on other taxa; electrofishing induces up to 10 fold increase in the number of macroinvertebrates drifting after shock (Bisson, 1976).

Precautions:

Great care must be taken when using electricity in water, and proper protective equipment (e.g., neoprene rubber gloves) should be worn when handling fish. Only properly trained individuals should operate electroanesthesia units, operators should never work alone, and first aid should be readily available in case of an accident.

Application:

Alternating current was widely used in the past (e.g., Madden & Houston, 1976; Ross & Ross, 1984); however, it is now known to be the most damaging wave form to fish (Lamarque, 1990; Walker *et al.*, 1994) and most electroanesthesia is now carried out with DC or pulsed DC. A popular design is a 12-V DC shocking basket that was developed for handling adult salmonids at sea (Gunstrom & Bethers, 1985; Orsi & Short, 1987), and that has recently been used with adult fish in freshwater (Sterritt *et al.*, 1994; Jennings & Looney, 1998). Pulsed DC is the most common waveform used for electrofishing at present, and a setting of 60 V and 50 Hz has been used successfully for electroanesthesia (Walker *et al.*, 1994; Redman *et al.*, 1998). Low voltages should be used for electroanesthesia of adult

fish, as higher voltages (>100 V) can cause physical injury and reduced survival (Tipping & Gilhuly, 1996).

Notes:

- The main advantages of electroanesthesia over chemical anesthetics include faster induction and recovery times, and the immediate release of treated fish without the need for a withdrawal period. Drawbacks include the need for specialized equipment, and the potential of physical injury to the operator and fish.

Hypothermia

Hypothermia is accomplished by lowering the ambient temperature of the fish with ice or cold water.

Precautions:

The only potential danger to the handler is the risk of exposure to high concentrations of CO_2 from the use of dry ice as the coolant. The use of dry ice could result in hypercapnic (high CO_2) and acidic conditions in the water, if it is placed in the water. Fish acclimated to higher temperatures may experience stress as a result of cold shock.

Application:

Hypothermic anesthesia is more effective for fish acclimated to waters above 10 ^{o}C, as sedative effects are not induced if acclimation temperatures are lower than this. In the latter case, an additional chemical anesthetic may be necessary to induce deep anesthesia (Mittal & Whitear, 1978). Generally, hypothermic anesthesia has been induced in a variety of fishes by inducing a temperature change of about 10 to 25 ^{o}C, or to near 0 ^{o}C, by immersing them in crushed ice or ice water (see Summerfelt & Smith, 1990). Hypothermia results in a slow, light anesthesia, which is characterized by an absence of motion, reduced power of exertion and diminished nerve sensitivity (Bell, 1987). This is useful for transport, but it is not deep enough for any type of lengthy surgery. While this is not a common method of anesthesia today, it presents an alternative method when chemical anesthetics are not available or desirable.

Carbon dioxide

Carbon dioxide (CO_2) is a colourless, odourless, non-flammable gas with a water solubility of 1.71 L/L water at 0 ^{o}C and 760 mmHg (Bell, 1987).

Precautions:

CO_2 is safe to use, but a level of 10% or more in the air will cause anesthesia or even death to the operator; therefore, ample ventilation is necessary (Bell, 1987). The hydration of CO_2 will acidify water, and therefore, should be buffered to reduce this potential stress to the fish.

Application:

The exposure of fish to hypercapnia in the water (1 – 5% in air) is a commonly practiced method to induce respiratory acidosis in fish, as it produces a consistent and reproducible decrease in blood pH upon exposure to the fish. Studies by Iwama *et al.* (unpublished) have shown that buffering the water to minimize pH change with high CO_2 and adding 8.5 g/L

NaCl significantly reduced the irritation as well as blood haematology and chemistry changes in 40 g steelhead trout (*Oncorhynchus mykiss*) exposed to a mixture of 50% CO_2 in air.

Sodium bicarbonate (Booke, 1978) and carbonic acid (Post, 1979) are both listed in Table 2 under this heading since they are essentially the same treatment. The acidification of bicarbonate or carbonic acid evolves CO_2, which is similar in producing a hypercapnic condition in the water. One difference is the unknown contribution of the added acid to the pH change of the water.

The recent interest in fisheries and aquaculture practitioners in using CO_2 anesthesia is based on its gaseous nature, and the fact that it leaves no residues in the tissues. This popularity raises the need for research into the best method for administration since it is a potent method for disturbing the acid-base and ionic balance of all fishes (Iwama *et al.*, 1989). Although such disturbances might be lessened in sea water, due to the higher bicarbonate buffering, caution must be exercised by monitoring the acidification of the anesthetic bath.

Table 2 List of selected anesthetics and estimates for optimum doses, as well as induction and recovery times, for various fishes.

Anesthetic	Dose	Induction time	Recovery time	Test Fish	References
TMS	25 – 100 mg/L	< 3 min	< 10 min	Salmonids, Carp, Minnows	(Bell & Blackburn, 1984; Gilderhus & Marking, 1987; McFarland & Klontz, 1969; Schoettger & Julin, 1967; Sylvester & Holland, 1982; Yesaki, 1988)
	250 – 480 mg/L	< 5 min	< 10 min	Atlantic halibut	(Malmstroem *et al.*, 1993)
	150 mg/L	< 3 min	< 10 min	Striped bass	(Lemm, 1993)
	75 mg/L	Rapid	3.7 – 7.1min	Cod	(Mattson & Riple, 1989)
	80 – 100 mg/L	2.6 – 6.8 min	2.5 – 1.2min	Tilapia	(Ferriera *et al.*, 1979; Ross & Ross, 1984)
Benzocaine hydrochloride	40 mg/L			Cod	(Ross & Ross, 1984)
	25 – 50 mg/L	3 min	4.3 – 6.32 min	Salmonids	(Yesaki, 1988)
	55 – 85 mg/L	3 min	< 10 min	Bass	(Gilderhus, 1989; Gilderhus, 1990; Gilderhus & Marking, 1987; Gilderhus, 1991; Gilderhus, 1991)
	50 – 100 mg/L	1.2 – 3.9 1.6 – 6.5min	3.1 – 2.2 2.9 – 2.2min	Carp Tilapia	(Ferriera *et al.*, 1979)
Lidocaine plus	350 mg/L	53 sec	13 min	Carp	(Carrasco *et al.*, 1984)

1g/L $NaHCO_3$ *	250 mg/L	88 sec	12.6 min	Catfish	(Carrasco *et al.*, 1984)
	350 mg/L	89 sec	10.2 min	Tilapia	(Carrasco *et al.*, 1984)
Metomidate	5 – 20 mg/L	Rapid	8.2 – 19.2 min	Cod	(Mattson & Riple, 1989)
	7.5 – 10 mg/L	< 3 min	< 10 min	Striped Bass	(Lemm, 1993)
	10 – 60 mg/L	< 5 min	< 20 min	Atlantic halibut	(Malmstroem *et al.*, 1993)
	5 mg/L	2.7 min	18 min	Rainbow trout	(Gilderhus & Marking, 1987)
Etomidate	1 – 7 mg/L	~ 3 min	< 20 min	Salmonids	(Bell, 1987; Gilderhus & Marking, 1987)
	2 – 7 mg/L	90 sec	40 min	Tropicals	(Amend *et al.*, 1982)
	1.35 – 2.2 mg/L	3 – 4 min	5 – 20 min	Catfish	(Limsuwan *et al.*, 1983b)
	0.5 – 2.3 mg/L	5 – 18 min	~30 min	Golden shiners	(Lisumwan *et al.*, 1983a)
	1.0 mg/L	5 min		Striped Bass	(Plumb *et al.*, 1983)
Propoxate	1 – 4 mg/L	< 10 min			(Ross & Ross, 1984)
Ketamine hydrochloride	30 mg/kg	10 – 300 sec	1 – 2 hrs	Salmonids	(Graham & Iwama, 1990)
Quinaldine sulfate	15 – 40 mg/L	2 – 4 min	1 – 20 min	Salmonids	(Bell, 1987; Gilderhus & Marking, 1987; McFarland & Klontz, 1969)
	30 – 70 mg/L	2 min	1 – 24 min	Catfish	(Schoettger & Julin, 1968)
	10 – 30 mg/L	2 min	2 – 60 min	Bluegill	(Schoettger & Julin, 1968)
	25 – 55 mg/L	< 3 min	< 10 min	Striped Bass	(Lemm, 1993)
	15 – 70 mg/L	2 min	1 – 60 min	Largemouth Bass	(Schoettger & Julin, 1968)
Propanidid	1.5 – 3 mL/L	1 – 4 min	4 – 10 min	Salmonids	(Siwicki, 1984)
Clove oil & AQUI-S	40 mg/L	2.5 – 4 min	3 min	Rainbow trout (FW, 11°C)	(Anderson *et al.*, 1997)
	40 – 60 mg/L	3 – 4 min	12 – 14 min	Rainbow trout (FW, 9°C)	(Keene *et al.*, 1998)

	100 mg/L	1 – 2 min	0.5 – 2.5min	Rabbitfish (SW, 28°C)	(Soto & Burhanuddin, 1995)
	100 mg/L (for field use)	11 sec	2 min	Damselfish (SW, 29°C)	(Munday & Wilson, 1997)
	20 mg/L (AQUI-S)	5 min	5 – 10 min	Chinook salmon (FW)	(AQUI-S New Zealand Ltd., 2004)
2-Phenoxyethanol	200 – 500 mL/L	3 min	2 – 10 min	Salmonids	(Barton & Helfrich, 1981; Bell & Blackburn, 1984; Sehdev *et al.*, 1963; Yesaki, 1988)
	100 – 500 mL/L	3 min	< 4 min	Various species	(Mattson & Riple, 1989; McFarland & Klontz, 1969)
Hypothermia	Instant drop of 6°C			Tilapia	(Ross & Ross, 1984)
	Immersion in ice water			Various species	(McFarland, 1959; McFarland, 1959)
Carbon Dioxide	200 – 1500 mg/L (50%CO_2 : 50%O_2)	< 3 min	8.14 min	Salmonids	(Barton *et al.*, 1986; Bell, 1987; Bell & Blackburn, 1984; Britton, 1983; Gilderhus & Marking, 1987; Iwama *et al.*, 1989; Turvey & Genoe, 1984)
	290 – 460 mL/min	20 min	30 min	Carp	(Itzawa & Takeda, 1982)
	1 – 1.78 L/min @50%CO_2	30 min	20 – 30 min	Carp	(Mitsuda *et al.*, 1988; Mitsuda *et al.*, 1980; Mitsuda *et al.*, 1982)
	100 – 250 mmHg CO_2	~ 30 min	< 40 min	Carp	(Yoshikawa *et al.*, 1988; Yoshikawa *et al.*, 1991)
Sodium bicarbonate	pH 6.5 + 642mg/L $NaHCO_3$	5 min	10 min	Trout/Carp	(Booke, 1978)
	900 mg/L	5 min	12.1 min	Adult salmon	(Gilderhus & Marking, 1987)
Carbonic acid	150 – 600 mg/L H_2CO_3				(Post, 1979)
Electro-anesthesia	12 V DC	Rapid	Immediate	Chinook salmon (17 ‰)	(Gunstrom & Bethers, 1985)

	12 V DC 150 mA	Rapid	Immediate	Chinook salmon (SW)	(Orsi & Short, 1987)
	12 V DC 1-3 A	Rapid	30 sec	Coho salmon (FW)	(Sterritt *et al.*, 1994)
	12 V DC 30 mA	8 sec	9 sec	Striped bass (5 ‰, 21°C)	(Jennings & Looney, 1998)
	60 V pulsed DC 50 Hz, 7.3 ms pulse width pulsed for 10 – 15 sec	Rapid	2 – 3 min	Brown trout (FW, 12°C) Northern pike (FW, 11°C)	(Redman *et al.*, 1998) (Walker *et al.*, 1994)

*These values are the lethal concentrations of Lidocaine without the addition of sodium bicarbonate.

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