An Outline of Anesthetics and Anesthesia for Salmonids, a Guide for Fish Culturists in British Columbia

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February 1987

Canadian Technical Report of Fisheries and Aquatic Sciences No. 1534

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Oceans et Océans

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AN OUTLINE OF ANESTHETICS AND ANESTHESIA FOR SALMONIDS, A GUIDE FOR FISH CULTURISTS IN BRITISH COLUMBIA

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Cat. No. Fs97-6/1534E ISSN 0706-6457

Correct citation for this publication:

Bell, G. R. 1987. An outline of anesthetics and anesthesia for salmonids, a guide for fish culturists in British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1534: 16 p.

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ABSTRACT

Bell, G. R. 1987. An outline of anesthetics and anesthesia for salmonids, a guide for fish culturists in British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1534: 16 p.

Greatly increased activity in salmonid enhancement and farming in British Columbia indicated that an update of anesthetics and methods of anesthesia was necessary. This report discusses the properties, characteristics, and uses of certain physical and chemical anesthetics, emphasizing those of special interest to fish farmers. Common general anesthetics and promising newer ones are discussed. Although this report is primarily directed to salmonid culturists on Canada's Pacific coast, much of the information is more widely applicable.

RESUME

Bell, G. R. 1987. An outline of anesthetics and anesthesia for salmonids, a guide for fish culturists in British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1534: 16 p.

L'importante augmentation des activités de mise en valeur et d'élevage des salmonidés en Colombie-Britannique exige que l'on procède à une mise à jour des anesthésiques et des méthodes d'anesthésie utilisés. L'auteur traite des propriétés, caractéristiques et utilisations de certains anesthésiques physiques et chimiques en mettant l'accent sur ceux qui présentent un intérêt particulier pour les pisciculteurs. Les anesthésiques généraux courants et certains nouveaux anesthésiques prometteurs sont présentés. Ce rapports adresse surtout aux éleveurs de salmonidés de la côte canadienne du Pacifique, mais une grande partie de son contenu est d'application beaucoup plus générale.

PREFACE

With the much increased activity in fish culture, particularly salmonid farming, in B.C. there is need for a critical update on the status and application of anesthetics and anesthesia. This report attempts to fill this need and deals with common general anesthetics and methods of anesthesia as well as with promising newer ones that have special application or that may become accepted for use with market fish. The report is not intended to be a review of anesthesia but does include key references.

It is hoped that the information provided will be of practical value to both experienced and novice fish culturists whether producing fish for enhancement of wild stocks or for market consumption.

INTRODUCTION

Many manipulations of fish such as transportation, tagging, measuring,and injecting require some degree of sedation or immobilization of the subject to facilitate handling and reduce the stress associated with these procedures. This stress may kill fish immediately or it may kill over a period of time by rendering them more susceptible to poor environmental conditions or to opportunistic pathogens.

General anesthetics, the topic of this report, are used to sedate or immobilize fish rapidly and reversibly. Anesthesia may be induced by physical means such as hypothermia or electroshock, but more commonly by the use of chemicals dissolved in the water. Anesthetics administered in the water ("inhalants") pass over the gills during respiration where the chemicals cross the delicate respiratory membranes to enter the circulatory system. The chemicals then induce a state of sedation or general anesthesia by depressing the central nervous system while permitting the continuation of vital respiratory and cardiac functions. Local anesthetics, by definition affecting only the site of application, are not discussed here.

A guide to the use of general anesthetics has already been published (Bell 1967), and many aspects of fish anesthesia have been described in a useful booklet by Ross and Ross (1984). However, the former publication requires updating and the latter requires amplification, particularly to meet the needs of salmonid culturists.

The ideal anesthetic should be inexpensive, non-toxic and non-irritating to fish and man, rapid acting (without causing hyperactivity), water soluble (at least to effective levels), stable, easily administered, rapidly reversible, readily cleared from the body, biodegradeable, and non-foaming. The qualities of the ideal anesthetic are outlined in Table 1.

There are some general precautions to be taken in the administration of anesthetics, as there are in the administration of any chemicals in the water. First of all, make sure that the fish are not fed for 48 hours before and after anesthesia and that the anesthetic water (aerated) is at or near the same temperature as the holding water. Thoroughly mix the anesthetic in the water to avoid "hot spots." Test the anesthetic at the recommended dosage on a few fish under the conditions to be used and observe for aberrant behavior and/or mortalities over the next 24 hours. Preferably, test several dosages within the recommended or estimated range and choose that which is safest and most suits your requirements. If the fish show no ill effects, proceed with the larger group. The reasons for certain of the above precautions are that fish often regurgitate under conditions of anesthesia and that feeding and digestion add further stress. Also, abrupt temperature changes, up or down, can cause mortalities or debilitation. Finally, unless one has had previous experience, dosages should be considered as only approximate because the effect of the anesthetic can vary with the species used, its size, developmental stage, previous history (rearing conditions), and state of health, in addition to the temperature and type of water (fresh, salt, brackish, hard or soft, acid or alkaline).

There are a number of stages of anesthesia that are summarized in Table 2, along with relevant comments. So-called planes of anesthesia within these stages are not given because they are irrelevant to this report. Ideally, Stage I should be evident in 30-45 seconds, Stage II in 45-90 seconds, and Stage III in 120-240 seconds. More rapid induction suggests that the dose (concentration) of anesthetic is too high and that it may be toxic, whereas a slower induction period suggests that the dose is too low and that the fish may not reach the deeper "planes" (levels) of Stage III.

There are about 15 anesthetic chemicals but only 5 or 6 are commonly used, and only 2 or 3 find favor in any region. The choice of anesthetic is often highly subjective, based on casual experience, and word-of-mouth or historic usage. The most widely used anesthetics for salmonids in North America are probably MS222 (TMS)and 2-phenoxyethanol (2-PE). Use of these and other chemicals is not specifically regulated in Canada. However, in the USA, whose practice we usually recommend, only MS222 is registered for use with food fishes (Schnick et al. 1986). Further, even this anesthetic must not be used less than 21 days before the treated fish are slaughtered, to allow ample time for clearance of the chemical from the body (Walker and Schoettger 1967). At present the only safe and prudent way to anesthetize food fish (e.g. mature Pacific salmon) under the 21-day limit is to use electroanesthesia, hypothermia, or carbon dioxide.

A guide to the properties, characteristics, and uses of certain anesthetics is given below. Common anesthetics and anesthesia procedures will be discussed first, followed by discussion of less common ones that have noteworthy features or specialized uses.

A. ANESTHETICS COMMONLY USED IN B.C.

TMS (MS222)

Common or Trade Names[1]: TMS, Tricaine, Metacaine, Finquel. MS222 (Sandoz Pharmaceuticals) is the most familiar trade name but this awkward sounding, obsolete epithet will be used as seldom as possible herein.

Chemical Names: tricaine methanesulfonate, methane sulfonic acid salt of meta-amino ethyl benzoate, ethyl m-aminobenzoate.

Use: general anesthesia for handling, tagging, fin clipping. TMS is particularly useful for severe surgical intrusions. Wedemeyer et al. (1985) found that 10 ppm MS222 in transportation waters significantly reduced immediate and delayed mortalities of smolts of some salmonid species at loading densities of 225-450 g/3.8 L (0.5-1.0 lb/U.S. gal).

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[1]Mention of trade names or suppliers is for information only and does not imply endorsement by DFO.

Characteristics and Properties: white powder, stable, non-flammable, very soluble in water (125 g/100 mL, 20°C); in sunlight, solutions unstable and can become toxic to fish in sea water.

Dosage Range: 50-100 ppm (1 ppm = 1 mg/litre) for anesthesia (Bell and Blackburn 1984, [see Appendix], Schoettger and Julin 1967).

Precautions: dosages much above 100 ppm where Stage III anesthesia (Table 2) can occur in <1 min can be lethal. There is a limited margin of safety between the effective dose and the lethal dose (Marking 1967). TMS is an acid and at 75 ppm can cause soft (coastal) waters to drop to about pH 4. For other than brief (i.e., $\leq$ 5 min) exposure it is advisable to neutralize the anesthetic by adding, for example, 5-6 mL of a saturated solution of sodium bicarbonate (ca. 10% $NaHCO_3$) to 1 L of 100 ppm TMS. Avoid contact of TMS with milt, or sperm motility may be lost.

Lewis et al. (1985) found that channel catfish exposed for 10 min to a solution of only 0.5 ppm MS222 temporarily lost the cilia on their olfactory sensory epithelia. The authors felt that chemosensory discrimination could thus have been impaired for at least 11 days after exposure. The finding, if applicable to Pacific salmon, could contraindicate use of MS222 on migrating fish. Recently, however, Dr. T. Quinn (pers. comm.) obtained evidence suggesting that MS222 does not affect the migratory orientation of adult chinook salmon (Oncorhynchus tshawytscha).

Hazards and Toxicity to Man: generally safe to handle but avoid contact with eyes and mucous membranes. Toxicity low as evidenced by the fact that 5-10 g TMS/kg body weight fed to rats are required to kill 50% of the test animals, and by its acceptance as an anesthetic for humans and food fish.

Combination Anesthesia: Gilderhus et al. (1973b) found that combinations of quinaldine sulfate (q.v.) and MS222, while retaining the properties of both anesthetics, considerably reduced the dosage of each anesthetic required to give rapid, deep anesthesia.

2-Phenoxyethanol

Common Names: 2-PE, 2-phenoxy

Chemical Names: 2-phenoxyethanol, phenyl cellosolve, β-hydroxyethyl phenyl ether

Use: general anesthesia for handling, tagging, fin clipping; wherever trauma is not likely to be severe.

Characteristics and Properties: colorless, oily, aromatic liquid, slightly heavier than water, moderately soluble in water (about 2.6 g/100 mL at 25°C), low flammability; mild antibacterial activity led to use as a topical antiseptic.

Dose Range: 0.25-0.5 mL/litre (275-555 ppm, or mg/L) (Bell and Blackburn 1983). Effective dose decreases with decrease in temperature (Sehdev et al. 1963). Cost, about one third that of MS222.

Margin of Safety: the lethal dose is 3-5 times the anesthetic dose, indicating a good margin of safety.

Precautions: mix thoroughly; 2-PE dissolves slowly in cold water. Dispersion could be aided by dissolving 2-PE in 5 volumes of ethyl alcohol or a small volume of hot water.

Hazards and Toxicity to Man: avoid contact with eyes. Feeding tests with rats indicate moderate toxicity (1.26 g 2-PE/kg body wt. killed 50% of test group; i.e., LD_{50} = 1.26 g/kg). Some verbal reports of skin irritation following prolonged exposure.

Carbon Dioxide

Chemical Names: carbonic acid gas, carbonic anhydride, CO_2

Characteristics and Properties: colorless and odorless gas that dissolves readily in water (171 mL/100 mL at 0°C and 1 atmosphere) to produce an acid solution. Commercially available in cylinders as a compressed gas, or as a solid ("dry ice"). Can also be produced by adding acid to carbonates but this method is usually impractical.

Dosage Range: 200-500 mg/L: optimum about 250 mg/L should anesthetize in about 4 min. Levels can be determined titrimetrically.

Dosage System (cylinder gas): a practical system developed at Robertson Creek Hatchery (Britton 1984, [see Appendix]) is to bubble CO_2 at 150 psi (lb/in^2) from a cylinder through an aeration device placed on the bottom of the tank. The more finely and widely the gas is dispensed, the sooner the concentrations will reach anesthetic levels. Commercially available porous tubing can be efficacious if used as described by Turvey and Genoe (1984) [see Appendix]. When the CO_2 concentration reaches 250-300 ppm (or the desired level as measured by titration), decrease the pressure to 85-100 psi and bubble in O_2 through a separate system.

Precautions: there are two undesirable changes in water quality associated with pure CO_2 dosage. One is that the oxygen concentration decreases (the water is "sparged" or degassed of O_2), the other is that there is usually a marked drop in pH which on prolonged exposure may cause problems for the fish. The first effect is countered by oxygenation (O_2 should be at least 7 mg/L) and the second by adding sufficient $NaHCO_3$ (practical grade) to bring the pH from around 5 to 6.5-7. Thorough mixing is, as always, very important.

Hazards and Toxicity to Man: make sure that the valve stem of the CO_2 cylinder cannot be broken and that the cylinder is securely fastened down. Do not carbonate in a closed space because 10% or more CO_2 in the atmosphere will induce anesthesia or death of the operator!

Perspective: carbon dioxide meets many of the criteria of an ideal anesthetic, especially in not leaving a residue toxic to the consumer. However, in the experience of several people anesthetizing large numbers of adult salmon, induction is much too slow and is accompanied by marked, often injurious, hyperactivity. Possibly this hyperactivity may be reduced by a preliminary exposure of the fish to about 100 ppm CO_2 or to mild electronarcosis (see Ross and Ross 1984) before exposing them to the anesthetizing levels of 200-500 ppm. Also, hyperactivity might be reduced if the fish are not exposed to anesthetizing dosages while in an agitated condition. van Mechelen (1985) and Lim and McLean (MS in preparation: see References) should be consulted for details. Unfortunately, van Mechelen's publication is difficult to obtain.

Whatever the actual or perceived limitations of CO_2 are as an anesthetic, it definitely warrants more experimentation and field testing under a variety of conditions.

B. ANESTHETICS LESS COMMONLY USED IN B.C.

1. Chemical methods of anesthesia

Although the following anesthetics are rarely used in B.C., many are used elsewhere. These anesthetics are included here principally to familiarize the reader with some agents having special properties and to stimulate a useful expansion of our repertoire of anesthetics. However, it must be re-emphasized that only TMS is cleared (in the U.S.A.) for limited use on food (farmed) fish. From the standpoint of human consumption all anesthetics listed below could probably be safely used if fish are destined for release because there should be ample time for their clearance from the fish.

Amyl alcohol

Tertiary amyl alcohol has found some favor for anesthetizing Atlantic salmon in the Maritime provinces and in Europe. Induction and recovery periods may be unacceptably long, however. Its advantages are low cost, availability, and good solubility. Suggested dosage range is 2-5 mL/litre. Relatively small volumes are toxic to man when ingested, and it can be an irritant.

Benzocaine (ethyl-4-aminobenzoate)

Benzocaine (chemically related to TMS) is generally used as the hydrochloride salt because the salt is much more soluble in water. However, the free base which has the advantage of being neutral can be taken up in ethyl alcohol or acetone for ease of dispersion in water (Dawson and Gilderhus 1979). If employing the base, make a concentrated stock solution using minimum solvent and test at a dosage range similar to that for TMS. Add the

stock solution with vigorous mixing. Benzocaine is considered essentially harmless to humans under these conditions of use.

As inhalant anesthetics benzocaine and its hydrochloride salt have some advantages over TMS in cost and efficacy.

Etomidate (Hypnomidate)

Etomidate is an analogue of two other potent anesthetics, Metomidate (Hypnodil) and Propoxate and was first evaluated by Amend et al. (1982). Their data, using certain aquarium fish, showed that the minimum effective dose was 2-4 ppm and the maximum safe dose 7-20 ppm, depending on the species used. Bell and Blackburn (unpublished), using chinook smolts, found that 5 and 10 ppm Metomidate safely induced Stage III anesthesia in about 120 and 45 s, respectively. Recovery times were slow, however, compared to TMS.

At present, the etomidate and its analogues are relatively expensive and difficult to obtain but their potency, good "margin of safety" (ratio of the lethal dose to the effective dose), neutrality in solution, and other properties make them prime candidates for further evaluation. It will doubtless be years, if ever, before these or other compounds are cleared for use on food fish but they may find special application in the field or in enhancement facilities.

Quinaldine sulfate

Quinaldine sulfate is readily soluble in water and was found to anesthetize coho salmon and rainbow trout in less than 4 minutes at 25 ppm. However, anesthesia was only to Stage II and the fish responded to tactile stimuli (Gilderhus et al. 1973a). The authors also found that the pH of the anesthetic bath must be kept above 6 for the anesthetic to be effective. Toxicity may be evident at concentrations above 40 ppm.

As previously discussed, the efficacy of quinaldine sulfate can be improved by mixing with TMS (Gilderhus et al. 1973b).

The parent compound, quinaldine, has so many disadvantages that its use as an anesthetic should be discontinued. For example, its solubility in water is very low, it is unstable, and it is highly irritating to mucous membranes.

2. Physical methods of anesthesia

Hypothermia

Although hypothermia is usually impractical, it might suit special needs. Cooling fish to near freezing can induce early levels (planes) of Stage III anesthesia (Table 2). Complex, lengthy or surgically intrusive manipulations of the fish are not usually possible but simple, quick procedures may be undertaken. Hypothermia is useful for transporting fish.

Be sure, however, that wet ice used for chilling the transport water is not made from chlorinated water, or that it is added in sealed bags. If dry ice is used to accomplish the chilling, the CO_2 evolved should not come in contact with the water.

The main advantage of hypothermia is that no chemicals are added to the fish or the water. Its disadvantages are the slow and light anesthesia obtained, the high cost per unit volume of water treated, and the difficulty for those working in chilled water. In addition, if it is not possible to acclimatize the fish to temperature changes of more than a few degrees, stress related mortalities may occur.

Perspective: hypothermia will likely continue to have limited use as in fish transportation.

Electroanesthesia (Electronarcosis)

Recent advances in equipment design and safety suggest that electroanesthesia may find wide application in public and private fish culture operations (Ross and Ross 1984 and W. McLean, pers. comm.). Direct current, pulsed direct current, and alternating current at various voltages may be used depending upon the conductivity of the water and the size and species of fish. Responses of the fish vary from brief initial stimulation followed by quiet rigor to violent spasms often causing vertebral disjunction and hemorrhaging. However, large power outputs need not be required and one system for repeatedly anesthetizing adult salmon (salinity ca. 18°/$_{oo}$) used a single 12 volt car battery. Operators manipulating the fish with bare hands under the applied voltage (unstated but probably around 0.5-1 v.cm^{-1}) felt only a slight "tingling" (Gunstrom and Bethers 1985). Ross and Ross (1984) describe other apparatus and methods worth considering.

The main advantages of electroanesthesia are that no chemicals are used, induction of anesthesia and recovery are rapid, and operating costs are low. The main disadvantages are the electrical hazard, the short recovery time and need for sustained power for continuous anesthesia.

Perspective: electroanesthesia holds perhaps the greatest promise for use in many fish culture operations, and electrical engineers should be actively encouraged to produce safe, effective, and convenient apparatus. Experimentation with and operation of most electroanesthesia apparatus should be left to the professionals!

3. Anesthesia by injection

It is possible to anesthetize fish by injecting them with certain general anesthetics (Oswald 1978). The dosage is based on body weight. The captive fish may be lightly sedated with an inhalant anesthetic or physically restrained to facilitate determining the weight and subsequently to aid the injection. Routes of injection would most likely be intramuscular or intraperitoneal rather than intravenous. Ross and Ross (1984) recommend Alphaxalone (Saffan) and Propanidid (Epontol, Sombrevin), the latter being a

potentially useful inhalant anesthetic for chinook smolts (Bell and Blackburn, unpublished).

Two applications of injectable anesthetics are noteworthy. First, injection is indicated if it is necessary to immobilize fish for more than a few minutes out of water and there is no means of irrigating the gills with oxygenated anesthetic for stabilization (e.g. field work). Oxygenation of the blood can then be maintained by simply irrigating the gills with ambient water. Second, if it is desirable to capture an individual fish, for example, an adult chinook in a stream or large container, it is now possible to anesthetize the individual from a remote position in or out of the water. A Victoria firm has developed a laser sighted gun for firing an anesthetic-containing projectile to immobilize selected fish.

ACKNOWLEDGEMENTS

I am very grateful to Drs. Trevor Evelyn and Leo Margolis for helpful criticisms of the manuscript and to Bill McLean of DFO's Quinsam Hatchery for useful discussion of electro and carbon dioxide anesthesia.

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Table 1. Qualities of an ideal anesthetic.

Factor	Comments
Inexpensive	Cost per effective dose is the basic consideration.
Non-toxic, non-irritant	Low toxicity to fish and humans is desirable. For fish there should be a wide margin of safety between the effective and toxic doses. Substance should not irritate sensitive tissue such as the gill and mucous membranes. Further, it should be non-toxic to gametes with which it might come in contact.
Rapid acting	Anesthetic should immobilize quickly to minimize struggling, without causing stressful hyperactivity.
Solubility	Ready water solubility aids dispersion, administration, and subsequent removal. A solubility limit below the toxic dose would be a useful property.
Stability	A substance should be stable on storage and in solution, and should not react adversely with sea water, light, heat or combinations of these.
Rapid recovery	It is usually advantageous for fish to recover rapidly. They should move in the water column to escape predators or to prevent suffocation should they lie heaped in the bottom of a container.
Body clearance	It is desirable for the fish and consumer that the anesthetic be excreted as soon as possible. Only MS222 is registered for use on food fish in the USA whose practice is usually followed in Canada. Further, this anesthetic must not be used less than 21 days before slaughter. Carbon dioxide anesthesia could be used at any time less than the 21 day limit.
Biodegradeable	Substance should be decomposable by natural agencies to simple, non-toxic compounds.
Non-foaming	Substance should not cause foaming, especially in transportation tanks where foam interferes with observation and exhaust of gases.

Table 2. Stages of fish anesthesia, their description and significance.

Stage		Behavior and Comments
I.	Losing equilibrium	After some agitation and head snapping, fish begin to lose equilibrium but still actively evade capture.
II.	Loss of equilibrium	Swimming on sides or upside down but still evade capture.
III.	Immobilization	Swimming ceased but regular opercular movements (respiration) continuing. Fish lying on their sides andcan be handled and removed from water. Recovery in anesthetic-free water routine. A desirable level of anesthesia.
IV.	Immobilization and cessation of opercular movement	A dangerous level of anesthesia that should be avoided. However, fish can frequently be revived by gently flushing gills with a hose or by pulling fish backwards through the water. Once regular, shallow opercular movements begin, recovery should follow unaided.

Appendix

NI memo

For internal, informal, rapid exchange of information between data producers & users.

DATE February 1, 1983

MEMO NO. 49

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DISTRIBUTION

T. Bird - 5
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A. Lill - 3
D. Narver - 17
K. Sandercock - 30
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West Van. Lab. - 6
Pac. Biol. Stn. - 30

For further details contact:

Ed Britton,
Robertson Creek
Hatchery
(724-6521)

SUBJECT - Anesthetizing Adult Salmon

Item - Constant carbon dioxide (CO_2) and oxygen (O_2) aeration to anesthetize adult salmon.

An improved method of anesthetizing brood stock salmon in high concentrations of dissolved CO_2 gas has been developed at Robertson Creek Hatchery. Previously, problems associated with this technique included low dissolved O_2 levels and rapid reduction in dissolved CO_2 levels as the anesthetic was used (later groups were poorly anesthetized). This necessitated frequent recharging of the tank with fresh water and CO_2.

The following procedure can be used to anesthetize up to 1,000 adult salmon (50 salmon x 20 loads) after a single charge (with maintenance level charging).

The anesthetic tank measures 2 m x 2 m x 0.84 m deep, holds 3.36 m^3 water, and is charged with CO_2 by forcing the gas through a large carborundum stone in the bottom of the tank. Charge time is 30 minutes at 150 psi. A CO_2 concentration of 350-450 mg/l is reached. This reduces the dissolved O_2 from 10 mg/l to 7-8 mg/l. Partial restoration of O_2 is achieved by aeration with O_2 at 10 psi during the last 5 minutes of the CO_2 charge so that the dissolved O_2 concentration at the beginning of the sort is 8-9 mg/l. The pH is adjusted to > 6 with sodium bicarbonate (2-3 kg). During the sort, the anesthetic tank is continuously charged at maintenance levels of 85-100 psi CO_2 and 10 psi O_2.

In the initial experiment conducted on October 9, 1982, 405 coho and chinook were anesthetized in 12 loads. Initial gas concentrations were: CO_2 - 363 mg/l; O_2 - 7.9 mg/l. Concentrations after loading were: CO_2 - 285 mg/l; O_2 - 8.4 mg/l. Exposure was 4.0 minutes from first to last load. Dissolved gas levels are typically 220 mg/l CO_2 and 9.5 mg/l O_2 after these large loads. Since response of salmon to levels below 200 mg/l CO_2 is less than optimal and the water is very murky after 1,000 adults, this is probably near the maximum for a single charge of the tank.

EMPHASIS:
(1) Identification of priority problems or potentials, or
(2) Announcement of new data or insights into solutions to current problems prior to final analysis or formal reporting.
Information items are preliminary and not final. Recipients are invited to participate. Send your items (please be brief) to one of the above contacts.

INFO memo

For internal, informal, rapid exchange of information between data producers & users.

DATE February 1, 1983

MEMO NO. 51

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D. Narver - 17
K. Sandercock - 30
F.E.A. Wood - 72
West Van. Lab. - 6
Pac. Biol. Stn. - 30

For further details contact:

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SUBJECT - Fish Culture Anesthesia.

ITEM - Introducing carbon dioxide (CO_2) anesthesia via Micro-Por tubing.

To reduce the initial charge time of an anesthetic tank (2,000 l), CO_2 was introduced using Micro-Por tubing rather than the standard 165 cm carbon stone. Oxygen was introduced into the tank in the same manner, but with a separate line.

The tubing was fastened to an aluminum frame in a circular fashion to cover as much of the bottom area as possible. The CO_2 was introduced via 11.3 meters of tubing and the oxygen through 7.9 meters of tubing. Stopcocks were fastened to the end of each line for drainage purposes. This eliminated freeze-up problems when carbon stones were left in the tank while the gas was turned off.

At a flow of 55 cfh and using a 165 cm carbon stone, it took 25-30 minutes to obtain a concentration of 300-330 mg/l in a 2,000 l tank. When the same flow was used with Micro-Por tubing, the time was reduced to 13-17 minutes to obtain the same concentration. The decrease in time is primarily due to the added surface area of the tubing. Once the desired concentration was reached, this level was maintained by trickling CO_2 in at 10 cfh. (See Info Memo #49: maintenance level O_2 at 5 cfh.) Cost of the Micro-Por tubing vs carbon stones is:

Micro-Por tubing (30.5 m - 100 foot roll)	$131.25	single roll
	$111.57	- 4 or more
11.3 m of tubing	$ 48.56	
Carbon stone	$ 58.55	
	$ 52.05	- 2 or more

* Prices in U.S. funds as of Dec. 82

The Micro-Por tubing is relatively easy to work with but should be protected from damage (i.e. thrashing fish) by putting it under screens lying on the bottom of the anesthetic tank.

EMPHASIS:
(1) Identification of priority problems or potentials, or
(2) Announcement of new data or insights into solutions to current problems prior to final analysis or formal reporting.
Information items are preliminary and not final. Recipients are invited to participate. Send your items (please be brief) to one of the above contacts.

INFO memo

For internal, informal, rapid exchange of information between data producers & users.

DATE August 4, 1983

MEMO NO. 70

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Vancouver, B.C. 666-3855

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DISTRIBUTION

T. Bird - 5
D. Deans - 40
A. Lill - 3
D. Narver - 17
K. Sandercock - 30
F.E.A. Wood - 72
Pac. Biol. Stn. - 45

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SUBJECT - Anesthetizing chinook smolts

Item - Use of MS-222 (TMS) and 2-phenoxyethanol (2 PE)

Bouck and Johnson (Trans. Amer. Fish. Soc. 108: 63, 1979) found TMS at 100 or 75 ppm caused 100% and 20% mortality, respectively, of coho smolts that had been transferred immediately to sea water (SW) following 6 and 8 min treatment, respectively. 100 ppm caused 12% mortality when fish were returned to fresh water (FW) for 4 days before being introduced to SW. No mortalities occurred over 10 days in the FW to SW or direct to SW groups when 50 ppm TMS or 2.5 ppm quinaldine was used.

Because certain hatchery managers were concerned about Bouck and Johnson's results as they might apply to their juvenile tagging programs, we tested TMS and 2 PE on chinook smolts using essentially the same protocols and procedures as these authors. In addition, we estimated adaptation to SW by measuring plasma sodium concentrations (Clarke and Blackburn, Fish. Mar. Serv. Tech. Rep. 705, 1977). In view of space limitations the results and recommendations are summarized below.

There were no mortalities over a 15-day observation period when fish were treated for 4 min with TMS at 75 or 100 ppm and returned to FW (14°C), or switched to SW (11°C, 29‰) 4 days post treatment but there was a 10% loss in FW using 2 PE at 0.5 mL/L (no losses using 0.25 mL 2 PE/L). There was about a 5-10% loss with either anesthetic when fish were immediately transferred to SW post treatment.

Based on efficacy, mortalities, and adaptation to SW we recommend the use of 75 ppm TMS for anesthetizing smolts to be returned to FW, or after a recovery period of at least 4 days, to be introduced to SW. If 2 PE is used, dosage should be $\leq$ 0.5 mL/L. Immediate introduction of anesthetized smolts to SW should be avoided if possible. Fish should not be fed for 72 h prior to anesthetization; exposure to anesthetic should be only of sufficient time to immobilize, and stressors such as handling, low pO_2, high temperatures and crowding should be minimized before, after and during treatment.

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