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AQUI-S, A New Anesthetic for Use in Fish Propagation

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Abstract: AQUI-S is a new fish anesthetic approved for use in several countries and until recently was being developed in the USA as a sedative for immediate release of fish after sedation. Many hatcheries and research studies use AQUI-S to immobilize fish for rested harvesting and to suppress sensory systems during invasive procedures. AQUI-S still has the potential to be approved as an anesthetic with a short withdrawal time. Within, we briefly describe many aspects of AQUI-S including the legal uses for it, anesthesia mechanism, harvesting and what is currently known about, behavioral, pathologic effects of the anesthetic. We outline methods and precautions for administration and changes in fish behavior and discuss the physiological effects of anesthetic, its potential for compromising fish health and effectiveness of water quality parameters.

Key words: AQUI-S ・Anesthesia ・ Rested Harvest ・ Withdrawal ・ Fish

INTRODUCTION

Anesthesia is a biological state with the partial or complete loss of sensation or loss of voluntary neuromotor control induced by chemical or nonchemical means [1,2]. Anesthesia abolishes pain in fish and induces a calming effect followed by loss of equilibrium, mobility and consciousness [1]. Anesthetics in fish farms are used to minimize motility during handling and transport. This may reduce susceptibility to pathogens and infection [3,4]. Anesthetics are also used in fish during artificial spawning, weighing, tagging, grading, blood sampling, surgery and surgical procedures [4, 5].

The main purpose of this study is to inform fisheries researchers and practitioners on the use of AQUI-S for anesthetizing fish. This review should also be useful for others working on other sizes or species of fish.

When choosing an anesthetic, a number of considerations are important, such as efficacy, cost, availability and ease of use, as well as toxicity to fish, humans and the environment [6,7] and the choice may also depend on the nature of the experiment and species of fish [1,8]. Anesthesia in fish may be produced by different agents, mainly tricaine methanesulphonate (MS-222), quinaldine sulphate, benzocaine and phenoxyethanol, which are hazardous.

The useful features of clove oil (a clove oil-based) prompted the development of a new anesthetic compound for fish, named AQUI-S, at the Seafood Research Laboratory in Australia, Chile, New Zealand, Korea, Costa Rica and Honduras. The active ingredient of the product is isoeugenol [9], which, although very similar to eugenol, is not present in natural clove oil. In addition to the principal active ingredient, 50% isoeugenol (2-methoxy-4-propenylphenol, about 12% cis-isomer and 88% trans-isomer), the product is also reported to contain 50% polysorbate 80 (sorbitan mono-9-octadecanoate poly (oxy-1, 2-ethanediyl, derivatives) which acts as an emulsifier. Polysorbate is widely used in this way in many proprietary pharmaceuticals [10]. AQUI-S is a clear viscous yellow, dispersible liquid anesthetic, which is dispersible in freshwater or seawater (It does not require the use of hazardous solvents) and used for finfish, crustacea and shellfish to form a stock solution, husbandry and transport applications.

Designated Uses for AQUI-S: AQUI-S affords a more effective and controlled anesthesia than the mixture of compounds found in clove oil [10]. Clove oil contains active ingredients chemically similar to iso-eugenol, the active ingredient in AQUI-S™, but clove oil is approximately 100% active ingredient and AQUI-S™ is 50% active ingredient. However, it is less effective than

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Synonyms
(of the principal active ingredient)
Isoeugenol
Propenylguaiacol
4-Propenylguaiacol
2-Methoxy-4-propenylphenol phenol
4-Hydroxy-3-methoxypropenylbenzene
1-Hydroxy-2-methoxy-4-propenylbenzene
4-Hydroxy-3-methoxy-1-propenylbenzene

IUPAC name
2-Methoxy-4-prop-1-enyl-phenol
Molecular formula
$C_{10}H_{12}O_2$

Structure

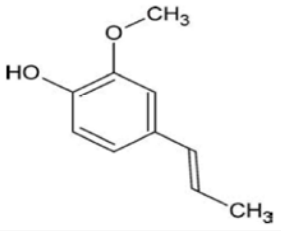

Fig. 1: AQUI-S [11]

AQUI-S and can produce adverse reactions and flavor problems in harvested fish. Neither clove oil nor eugenol has approval in any of the above countries as a veterinary medicine. Therefore, clove oil and eugenol are not usually acceptable for harvesting applications [12]. Although clove oil is effective and inexpensive to use, it is a crude product, does not have a sponsor and therefore stands no chance of gaining FDA-approval. A. U. S. Department of Health and Human Services guidance describes FDA's current position on clove oil that it is not legal to use. The USA Fish and Wildlife Service's (FWS) Aquatic Animal Drug Approval Partnership (AADAP) program has assumed primary responsibility for generating data to support such an approval for the use of AQUI-S on all fish species. Therefore, AQUI-S™ appears to be a safe and effective anesthetic for rested-harvest [13].

Presently, tricaine methanesulphonate (MS-222) is the only fish anesthetic approved by the U.S. Food and Drug Administration (FDA) for use in the United States [14]. Use of this anesthetic is constrained in the United States by a 21-day withdrawal period, i.e. fish cannot be made available for human consumption for 21 days after exposure to the anesthetic. This constraint limits anesthetic applications in USA, public aquaculture and fishery management programs and fostered the search and development of an alternative anesthetic that would allow for immediate release of exposed fish.

Other common anesthetics such as quinaldine sulphate, benzocaine, 2-phenoxyethanol and metomidate, are not registered for use in Australia, New Zealand, Canada, USA or Europe. Their potential residual toxicity to humans prevents their use during harvesting. Commonly used non-toxic fish handling, alternatives such as induced hypothermia or CO_2 cause relatively shallow sedation of the animals yet considerable trauma [12]. Isoeugenol and polysorbate 80 were in the FDA's GRAS category (i.e. for food use) and required no withdrawal period (i.e. time between last treatment and potential consumption or release). For this reason, AQUI-S is often described as a ''food grade'' anesthetic [10, 15, 16].

The procedures requiring zero withdrawal time include: artificial spawning with carcasses allowed as fish meal for feeds or with live fish released for sport, commercial, or subsistence fisheries, humane rested harvest of cultured fish to improve product quality and extend shelf life, sorting brood fish, marking, tagging and immediate release or harvest of both cultured and captured fish, facilitating vaccine administration to market-sized fish, grading fish by size for immediate harvest or release and transport of market-size fish for immediate harvest or release.

Attributes of a drug that must be characterized before the drug is approved include characterizing the depletion of a drug's total residues after exposure and characterizing the depletion of a marker residue (a marker residue is the parent compound, a metabolite, or combination of residues that persist for the longest time in the target tissue, e.g. edible fillet tissue). The marker residue is selected on the basis of data generated during the total residue depletion study. Characterizing marker residue depletion allows the FDA to establish withdrawal times for exposed fish ensuring total residue concentrations reach safe levels before fish are made available for human consumption [17].

However, AQUI-S has recently been placed in the (Investigative New Animal Drug) INAD category. AQUI-S was a candidate for USA approval as a sedative that would allow for the immediate release of treated fish. Because of a recent ruling by the U.S. National Toxicology Program classifying isoeugenol (the active ingredient in AQUI-S) as a carcinogen in male mice, AQUI-S will no longer be pursued in the USA as an immediate-release sedative.

Rested-Harvest: AQUI-S is now commercially available and targeted specifically at 'rested' harvesting for aquaculture as well as transportation for aquaculture and of ornamentals. It is the only aquatic anesthetic that can be used for harvesting fish in those countries. AQUI-S has a gentle action and fish do not usually show any adverse reaction to its presence. It is particularly useful in harvesting of fish, with slow, stress-free induction in less than 10 minutes resulting in improved product quality and consistency [11]. AQUI-S was developed to enable the ''rested harvest'' (is defined as application of an anesthetic immediately before harvest to reduce fish activity associated with a normal harvest) of aquatic species and is also promoted as being beneficial during transportation of aquatic species. Rested or low stress harvesting of fish has been reported to result in significant quality benefits by a number of researchers [15,18,19]. Reduced gaping, delayed onset of rigor, reduced blood spotting, improved color and appearance, reduced bruising, improved muscle texture and improved consistency all result from a rested harvesting process [12]. Simple act of dip netting a fish will deplete the aerobic resources of the muscle, accelerating the process of post-harvest autolytic spoilage. Therefore, most existing methods of harvesting farmed fish will result in stress, leading to a reduction in product quality and consistency. True rested harvesting can only be achieved by careful, stress-free marshalling of the animals followed by sedation using an anesthetic which does not produce any adverse reaction [12, 15]. Measurements of the surface pH of a freshly cut muscle provide a reliable method of estimating the lactate levels and corresponding rested state of the animal in the farm situation. Low pH values of less than approximately 7 indicate that significant exercise has occurred. Lactate levels are correspondingly high. Values of pH 7.6 and above indicate a well rested animal. Most commercial applications using AQUI-S achieve pH values above 7.2 which is considered to be the minimum pH necessary to consistently obtain the benefits of rested harvesting. Gaping can also be affected by poor handling of the fillets. Gaping score is a subjective evaluation of the number and extent of separation of muscle blocks. Rested harvesting results in a delay in the onset of rigor mortis, less intense rigor contractions and, consequently, stronger, more elastic tissue after the rigor contractions have finished. Fish that are harvested in a rested state show improved color and appearance, reduced gaping, a delay in the onset and severity of rigor and improved firmness. Improved quality, consistency and yield translate into greater demand for the fish product and profits for the fish farmer [12].

AQUI-S is effective at low concentrations of 10 to 20 mg/l corresponding to active ingredient concentrations of 5 to 10 mg/l. Fish do not usually show any adverse response to the presence of AQUI-S at 20 mg/l and therefore they do not demonstrate struggling or avoidance reaction as with some other anesthetics. In commercial most other anesthetics require at least three times and up to twenty times this concentration of active ingredient to be effective. For all fish species tested, increasing concentrations of AQUI-S resulted in significantly faster times to handle able. Fish have a fast recovery from AQUI-S. Sedative effects and changes to behavior can be detected at lower concentrations. AQUI-S has a wide margin of safety when used at low concentrations. This means that fish can remain in the treatment water for relatively long periods of time without fear of mortalities. Also, it is effective at lower temperatures than other anesthetics such as benzocaine and MS-222, allows a faster recovery [9, 20]. There is a substantial difference between coldwater species and cool- or warm-water species with respect to times to handle able. The concentrations of 40-60 mg/l rapidly anesthetize coldwater species to the handle able stage in <3-5 min. whereas 60-80 mg/l is necessary to anesthetize cool and warm-water species in a comparable amount of time.

AQUI-S has been studied on the following species: chinook salmon *Oncorhynchus tshawytscha*, Yellowstone cutthroat trout *Onchorynchus clarki bouvieri*, bull trout *Salvelinus confluentus*, lake trout *Salvelinus namaycush*, mountain whitefish *Prosopium williamsoni*, channel catfish *Ictalurus punctatus*, largemouth bass *Micropterus salmoides*, hybrid striped bass *Morone chrysops* × *M. saxatilis*, Tilapia *Oreochromis mossambicus* and carp goldfish hybrids *Cyprinus carpio* × *Carassius auratus*. *Anguilla australis* [12], *Cyprinus carpio* [21], *Oncorhynchus tschawytscha* [22, 23], *Pagrud auratus* [23], salmonids [12] and *Stizostedeon vitreum vitreum* [9, 11].

In New Zealand, AQUI-S is being extensively used for the transportation and harvesting of farmed salmonids, such as Chinook salmon *Oncorhynchus tshawytscha* [24]. Also, AQUI-S has been shown to be effective at 20 mg/L for anaesthetizing juvenile chinook salmon [12]. Fingerlings and adult rainbow trout (RBT) *Oncorhynchus mykiss* became handleable and recovered in about

Table 1: Stage of anaesthesia [1]

Stage	Notable behavior
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
Stage 6	asphyxia, where opercular movements cease and cardiac arrest follows shortly after

5 minutes when exposed to concentrations of 40-60 mg/l AQUI-S. At AQUI-S concentrations of 20-60 mg/l, subadult pallid sturgeon *Scaphirhynchus albus* and shovelnose sturgeon *Scaphirhynchus platorynchus* took substantially longer to become handleable and recover from handleable than such times observed in studies with salmonids. However, based on preliminary safety data and discussions with FDA, it appears that 60 mg/l AQUI-S is not safe for salmonids. Therefore, it is anticipated that a concentration of 40-50 mg/l AQUI-S will be the highest concentration approved for use by FDA for salmonids. In addition, preliminary safety data have indicated that 80 mg/L AQUI-S may be a safe concentration for use on cool- and warm-water fish. Use to anesthetize fish to the handleable stage at concentrations ranging from 20-50 mg/l for coldwater species and at concentrations ranging from 40 - 80 mg/L for cool- and warm-water species.

Induction of Anesthesia: Summerfelt and Smith [1] derived a 6 Stage scale (Table 1).

Stage 3 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. In most cases, prolonged maintenance of Stage 3 anesthesia without gill irrigation will result in death.

The movement of anesthetic across the gills is presumably regulated by factors such as branchial ventilation, perfusion and effective exchange area. Furthermore, opercular rates at high temperature were greater than low temperature. As the gills in fish are the main route of entry and the excretion of anesthetics, increasing gill ventilation and cardiac rates at high temperature would increase the gill permeability of anesthetic and result in increasing the efficacy of anesthetic.

Cortisol is the principal glucocorticoid secreted by the interrenal tissue (steroidogenic cells) located in the head-kidney of teleost fish. This hormone is released by the activation of the hypothalamus-pituitary-interrenal axis (HPI axis). Small [25] documented that anesthetics reduce or block the activation of HPI axis, so blood chemicals would not be altered at sampling process. Isoeugenol was shown to diminish 60% blood cortisol in channel catfish exposed to confinement, whereas metomidate showed greater effectiveness in blocking cortisol release under high ammonium concentrations.

However, results of several studies suggest that the physiology of fish can be affected by changes in the hematology and biochemistry of exposed fish. Therefore it is important to assess the extent of physiological changes of fish exposed to an anesthetic [26, 27]. 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 [28].

Water quality parameters, such as temperature, pH, salinity and hardness, can affect metabolic rate, acid-base regulation, osmoregulation and ion regulation [29-31]. From affective parameters, temperature is the most effective one and pH is the least one.

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 dosage [29, 31]. When possible, fish should be monitored for 24-48 h after anesthetic administration and the associated procedure to ensure full recovery. Although AQUI-S is an important component of the rested harvesting process, careful attention must also be paid to fish handling prior to and during application of the anesthetic.

There are few reports in which investigators systematically evaluated its physiologic, behavioral and pathologic effects. The behavioral and physiological differences need to examine the response of each fish species of interest to anesthetic exposure, prior to its recommendation for large-scale use. Also, more works are

required to assess immune parameters. Hence, serious considerations should be given to the use of AQUI-S as a replacement for ancient anesthetics.

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