

## **The Efficacy and Safety of AQUI-S® as an Anesthetic on Freshwater Fish**

**Molly Bowman**, Jim Bowker, Dan Carty, Bonnie Johnson, and Dr. Dave Erdahl

U.S. Fish and Wildlife Service

Aquatic Animal Drug Approval Partnership Program

4050 Bridger Canyon Road

Bozeman, MT 59715

[molly\\_bowman@fws.gov](mailto:molly_bowman@fws.gov) 406-587-9265 ext. 139, Fax: 406-582-0242

Anesthetics are physical or chemical agents that act on an animal by initially inducing a calming effect and subsequently inducing loss of equilibrium, mobility, consciousness, and reflex action (Summerfelt and Smith 1990). Anesthetics are commonly used in the culture of captive fish and in the handling of wild fish. As such, fish anesthetic research has been conducted on many compounds, e.g., carbonic acid, sodium bicarbonate, quinaldine, benzocaine, and 2-phenoxyethanol. Virtually all fish culturists, biologists, and fisheries managers are familiar with two U.S. Food and Drug Administration (FDA) approved anesthetics: Tricaine-S® and Finquel® (i.e., MS-222). Although both types of MS-222 are very effective fish anesthetics, their use requires a 21-day withdrawal period before harvestable fish can be released or slaughtered. Such a lengthy withdrawal period greatly limits the scope of their use; therefore, fisheries professionals have long recognized that there is an important need for a zero-withdrawal fish anesthetic. This need has led to research on clove oil, a naturally derived compound that contains 90 - 95% eugenol. 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 document (Guidance for Industry Document 150) describes FDA's current position on clove oil, that it is not legal to use. An alternative product, AQUI-S®, has recently emerged as a compound that has a good chance of gaining FDA-approval as a zero-withdrawal anesthetic. The U.S. 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.

The AADAP program has conducted efficacy studies on a variety of salmonid species at AQUI-S® concentrations ranging from 10 to 60 mg/L. Such studies have been conducted according to guidelines described in an FDA-approved research study protocol. Preliminary results from these studies demonstrated that fish become handleable when exposed to 60 mg/L AQUI-S® in times that were comparable to times for fish to become handleable when exposed to 80 mg/L MS-222. However, fish anesthetized with MS-222 recover more quickly than those exposed to AQUI-S. Results from these studies reinforce the premise that a new animal drug approval for AQUI-S® will not displace MS-222, but add another fish anesthetic to the tool chest for fish culturists and fisheries managers. Advantages of AQUI-S® over MS-222 include potentially fewer human health concerns and a potential zero-withdrawal period.

Gaining FDA approval of a drug for use in aquaculture requires the completion of five technical sections: product chemistry, efficacy, target animal safety, human food safety, and environmental safety. Although not required, it is highly recommended that research study protocols, which describe study procedures to follow, be approved by FDA before starting a study. It should be

noted that a research study protocol differs considerably from a compassionate Investigational New Animal Drug (INAD) protocol. Fish culturists and fisheries managers use AQUI-S<sup>®</sup> under compassionate INAD protocol #10-541. The AADAP program conducted all efficacy studies under research study protocol AQUIS-01-EFF. The experimental procedures followed by AADAP researchers included (1) anesthetizing 15 fish separately to and from the handleable stage when exposed to one of several concentrations of AQUI-S<sup>®</sup> or 80 mg/L MS-222 (active control); (2) testing two life-stages of each fish species tested; (3) testing each fish species at two water temperatures that differ by at least 5°C; (4) dose-verification of working solutions of AQUI-S<sup>®</sup> to ensure that actual concentrations differ from target concentrations by no more than  $\pm 25\%$ ; and (5) statistically analyzing data with the Kaplan Meier approach to survival analysis. Primary response variables that were measured were (1) time to handleable and (2) time to recovery from handleable. Initially, we considered generating data to support the following levels of anesthesia: anesthetized, handleable, sedation, and rested harvesting (calming fish before being slaughtered for market). However, it soon became clear that this was a monumental task, and we refocused our efforts to pursue an approval of handleable only. The handleable stage of anesthesia is suitable for many culture and management practices (e.g., sorting, tagging, spawning, weighing, and measuring, but not for surgery).

Results from these efficacy studies have demonstrated the following:

1. Fingerling and adult rainbow trout (RBT) *Oncorhynchus mykiss* became handleable and recovered in about 5 minutes when exposed to concentrations of 40 - 60 mg/L AQUI-S<sup>®</sup>. We found that there was no significant difference in times to become handleable between (1) fingerling and adult RBT, and (2) water temperatures tested. Although not consistently significant, fish recovered faster in warmer water than in cooler water.
2. Times to handleable and recovery for adult steelhead trout *Oncorhynchus mykiss* returning to the hatchery were comparable to times measured for RBT in the previous study.
3. At AQUI-S<sup>®</sup> concentrations of 20 - 60 mg/L, subadult pallid sturgeon *Scaphirhynchus albus* and shovelnose sturgeon *Scaphirhynchus platyrhynchus* took substantially longer to become handleable and recover from handleable than such times observed in studies with salmonids. Therefore, we tested sturgeon at 80 mg/L and found that times to handleable at this higher concentration were comparable to 80 mg/L MS-222 (i.e., < 5 min).
4. Similar studies have been conducted 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* x *M. saxatilis*, Tilapia *Oreochromis mossambica*, and carp goldfish hybrids *Cyprinus carpio* x *Carassius auratus*.

5. For all fish species tested, increasing concentrations of AQUI-S<sup>®</sup> resulted in significantly faster times to handleable. We also found that there is a substantial difference between coldwater species and cool- or warm-water species with respect to times to handleable.

We conclude, from the studies conducted to date, that concentrations of 40 - 60 mg/L rapidly anesthetize coldwater species to the handleable 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.

Studies conducted to date have shown that AQUI-S<sup>®</sup> is indeed an effective fish anesthetic. However, data must also be generated to demonstrate that AQUI-S<sup>®</sup> is safe to fish at the proposed treatment concentrations. Demonstrating the safety of AQUI-S<sup>®</sup> is a challenging task because no fish anesthetic has yet been approved under recent FDA guidelines. The AADAP program is working closely with FDA's Center for Veterinary Medicine's Aquaculture Review Team to establish current guidelines. Preliminary tests conducted following these new guidelines are intended to demonstrate that fish can survive for an acceptable period of time if accidentally overexposed (i.e., leaving fish in for longer than the time to handleable) or overdosed (i.e., too high a concentration of AQUI-S<sup>®</sup>). However, based on preliminary safety data and discussions with FDA, it appears that 60 mg/L AQUI-S<sup>®</sup> is not safe for salmonids. Therefore, it is anticipated that a concentration of 40 - 50 mg/L AQUI-S<sup>®</sup> 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<sup>®</sup> may be a safe concentration for use on cool- and warm-water fish.

Overall, results from efficacy and preliminary safety studies indicate that it may be possible to have the following label claim for AQUI-S<sup>®</sup>: 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. In addition, we hope that the following statement will be included: Fish may be stocked or harvested any time after exposure to AQUI-S<sup>®</sup>.

Summerfelt, R. C., and L. S. Smith. 1990. Anesthesia, surgery, and related techniques. Pages 213 - 272 in C. B. Schreck and P. B. Moyle, editors. *Methods for fish biology*. American Fisheries Society, Bethesda, Maryland.