



U.S. Fish & Wildlife Service

Aquatic Animal Drug Approval Partnership**DRUG RESEARCH INFORMATION BULLETIN****Use of AQUI-S®20E, Tricaine-S™, and AQUACALM™ to Sedate Rainbow Trout to Handleable**

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Fish sedatives are used to achieve various stages of sedation (Summerfelt and Smith 1990) to facilitate fisheries research and management, such as “light sedation” for transportation, “loss of equilibrium” for routine handling, and “deep sedation” for surgical procedures. Although numerous chemicals have been evaluated for their utility as fish sedatives, only Tricaine-S™ (tricaine methanesulfonate, tricaine, or MS222; Syndel USA., Ferndale, Washington USA) is approved by the U.S. Food and Drug Administration (FDA) as a fish sedative/anesthetic and available in the U.S. Tricaine-S™ is approved for temporary immobilization of ictalurid, salmonid, esocid, and percoid fishes. Tricaine-S™ is effective and widely used by fisheries professionals throughout the U.S.; however, a 21-day withdrawal period is required for treated fish that may enter the human food supply through stocking or slaughter. For many field applications, holding fish for 21 days post-sedation is not practical and may seriously compromise management or research activities.

In the U.S., efforts are underway to obtain FDA-approval of AQUI-S®20E (10% eugenol; AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand) as an immediate-release fish sedative. Considerable research has shown that AQUI-S®20E is efficacious for sedating freshwater and marine fishes to a handleable state (e.g., Trushenski et al. 2012a, 2012b, 2013).

In addition, the sponsor for Aquacalm™ (100% metomidate hydrochloride; Syndel, USA) has expressed interest in pursuing FDA-approval for the use of their product as a fish sedative/anesthetic. Aquacalm™ has been used in Canada and other countries outside the U.S. in ornamental fish for many years. In the U.S., Aquacalm™ is an FDA Indexed product that can be legally marketed for sedation/anesthesia of ornamental fish. Metomidate hydrochloride produces its sedation or anesthetic effect on the central nervous system (Ashton and Wauguier 1985), whereas anesthetics such as tricaine and benzocaine are sodium-channel blocking local anesthetics (Hedrick and Winmill 2002) that act first on peripheral nerves and secondarily on the central nervous system. In addition, metomidate has been shown to suppress steps in the synthesis of cortisol, the primary stress hormone in fish (Ross and Ross 2008).

To gain additional information on the effectiveness of these products, we sedated small fingerling Rainbow Trout *Oncorhynchus mykiss* with AQUI-S®20E, Aquacalm™, or Tricaine-S™ to assess time to handleable and recovery. In addition, information on the cost to prepare 378 L (100 gal) of each sedative solution used in the study was determined. The goal of this trial was not to make statistical comparisons of the dosage used for each sedative but rather to demonstrate that fish became sedated and recovered within periods of time that would be considered reasonable by fisheries professionals.

Methods

The trial was conducted at the U.S. Fish and Wildlife Service Bozeman Fish Technology Center (Bozeman, MT, USA) in July 2017. Fingerling Rainbow Trout (mean total length \pm 1 SD, 9.8 cm \pm 1.0 cm) were sedated to handleable with either 250 mg/L AQUI-S®20E (25 mg/L eugenol), 80 mg/L Tricaine-S, or 6 mg/L Aquacalm. Doses were selected based on results from preliminary testing that showed they consistently sedated fish to handleable within 2 min. A fish was deemed sedated to handleable when it lost equilibrium and the ability to swim, could easily be caught by and held in hand, and did not struggle while being weighed or measured.

Ninety fish were used in the trial: 30 fish were individually sedated under static conditions with each of the three sedatives. Working sedative solutions prepared in bulk (50 gal) were used to prepare individual sedation baths (~1.5 gal solution in 5-gal plastic buckets). Sedative solutions were replaced after sedating each fish (each bath used only once for a single fish). When a fish became handleable, it was removed from the sedative solution, weighed and measured, and transferred to a recovery tank of fresh, flowing water. Fish were considered recovered when they regained equilibrium, resumed normal swimming behavior, and could avoid a net handle placed in their path. Times to sedation and recovery were determined for each fish, and general fish behavior was assessed during sedation and recovery. Following recovery, fish were returned to a fish holding tank supplied with fresh, flowing water and monitored for survival for 24 h.

Water temperature and dissolved oxygen (DO) concentration were measured in each sedative bath before use. Water hardness, alkalinity, and pH were measured once in source water.

Cost comparisons were based on the largest quantity commercially available for purchase as a single unit because these had the lowest per unit volume prices (Figure 2).

Results and Discussion

Regardless of sedative, mean times for fish to become sedated were < 2 min and ranged from 0.8 to 3.1 min (Table 1). Mean recovery times for fish sedated with AQUI-S®20E, Tricaine-S™ and Aquacalm™ were 2.1, 1.2, and 5.0 min, respectively. Mean elapsed time for fish to become sedated and recover was longest for Aquacalm™ (6.4 min) and shortest for Tricaine-S™ (2.5 min) (Figure 1). Fish behavior was considered normal for all fish during exposure and recovery regardless of sedative used.

Mean water temperatures and DO concentrations during exposure and recovery were 15.6°C and 8.5 mg/L, and 15.1°C and 8.5 mg/L, respectively. Hardness (207 mg/L as CaCO₃), alkalinity (166 mg/L as CaCO₃), and pH (7.5) were within ranges suitable for rearing Rainbow Trout.

Costs for preparing 378 L (100 gal) of a 6 mg/L Aquacalm™ solution was the most expensive while the cost for preparing the same volume of a 250 mg/L AQUI-S20E solution was the least expensive (Figure 2).

Based on results from these trials, all sedative treatment doses tested under these conditions effectively sedated fish to handleable within a mean time of 1.9 min, all fish recovered, and no abnormal behavior was observed during or after sedation. Based on these results, selection of the appropriate sedative to use should be based on factors such as cost, withdrawal period, and whether cortisol suppression is desired.

Acknowledgments

We thank AQUI-S New Zealand Ltd and Tom Goodrich, AquaTactics Fish Health, for providing AQUI-S®20E and Syndel USA for providing Aquacalm™, and Jesse Trushenski (Idaho Department of Fish and Game Eagle Fish Health Laboratory) and Taylor Lipscomb (University of Florida Tropical Aquaculture Laboratory) for their critical review of this Drug Research Information Bulletin.

References

- Ashton, D., & Wauquier, A. (1985). Modulation of a GABA-ergic inhibitory circuit in the in vitro hippocampus by etomidate isomers. *Anesthesia & Analgesia*, 64(10), 975-980
- Hedrick, M.S. and R.E. Winmill. 2003. Excitatory and inhibitory effects of tricaine (MS-222) on fictive breathing in isolated bullfrog brain stem. *American Journal of Physiology – Regulatory, Integrative, and Comparative Physiology* 284:405-412.
- Ross, L.G., and B. Ross. 2008. *Anaesthetic and sedative techniques for aquatic animals*. Blackwell Publishing.
- 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.
- Trushenski, J.T., J.D. Bowker, B.R. Gause, and B.L. Mulligan. 2012a. Chemical and electrical approaches to sedation of hybrid striped bass: induction, recovery, and physiological responses to sedation. *Transactions of the American Fisheries Society* 141:455–467.

Trushenski, J.T., J.D. Bowker, B.L. Mulligan, and B.R. Gause. 2012b. Induction, recovery, and hematological responses of largemouth bass *Micropterus salmoides* to chemo- and electrosedation. *North American Journal of Aquaculture* 74:214–223.

Trushenski, J.T., J.C. Bowzer, J.D. Bowker, and M.H. Schwarz. 2013. Chemical and Electrical Approaches to Sedation of Cobia: Induction, Recovery, and Physiological Responses to Sedation. *Marine and Coastal Fisheries*. In print.

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Table 1. Mean (SD) times to handleable and recovery for fingerling Rainbow Trout sedated to handleable with AQUI-S®20E (250 mg/L), Tricaine-S™ (80 mg/L), or Aquacalm™ (6 mg/L).

	AQUI-S®20E	Tricaine-S™	Aquacalm™
Mean time (minutes) to sedation (SD)	1.9 (0.4)	1.3 (0.2)	1.4 (0.4)
Mean Time (minutes) to recovery (SD)	2.1 (0.7)	1.2 (0.3)	5.0 (1.5)

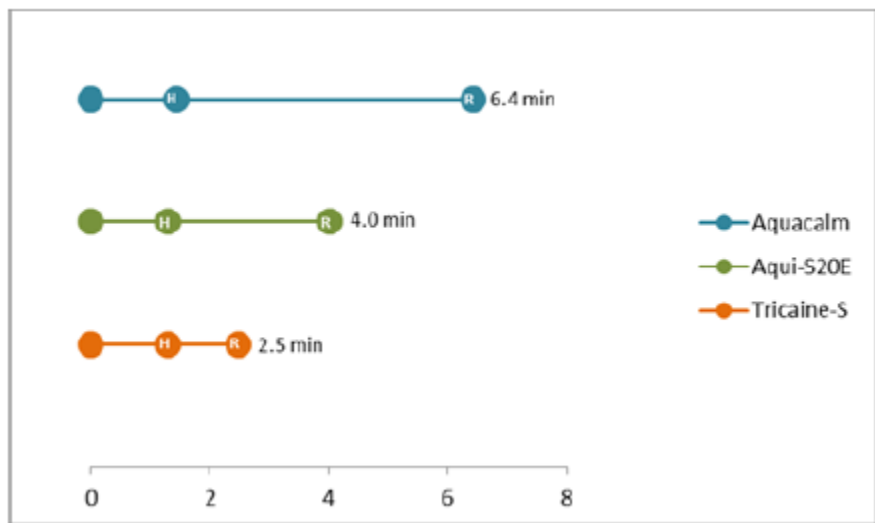


Figure 1. Mean elapsed times (min) for fingerling Rainbow Trout to become sedated to the handleable (H) stage of anesthesia with each sedative tested and recover (R) from sedation. X-axis = elapsed time in minutes.

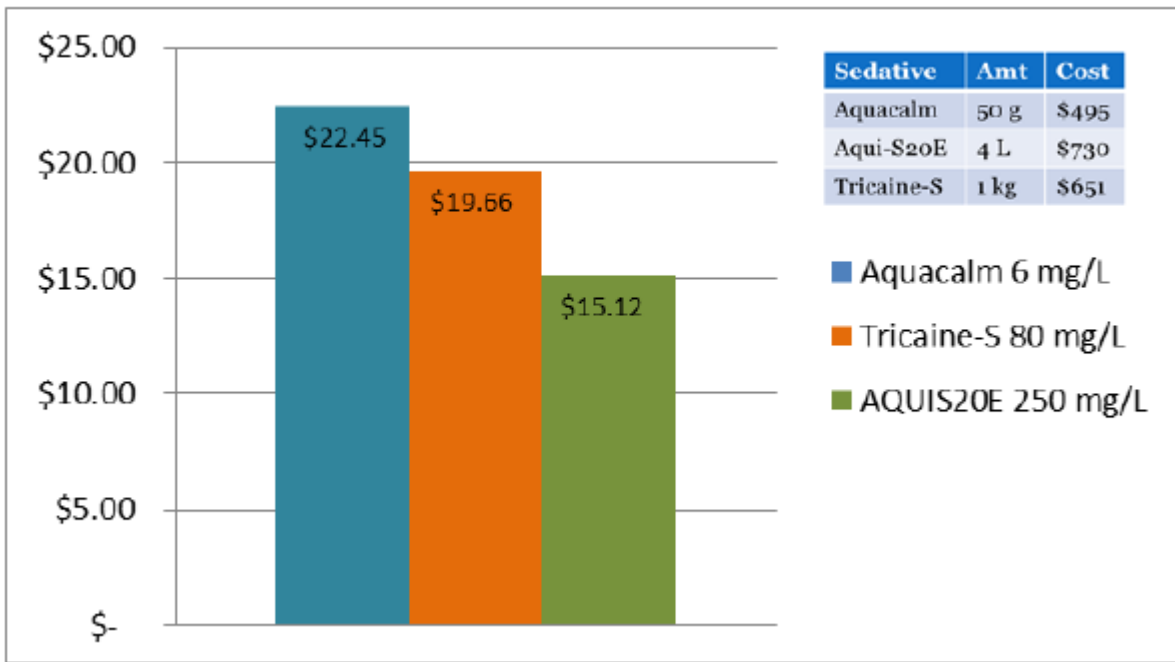


Figure 2. Cost (USD) of preparing 378 L of Aquacalm™, AQUIS20E, and Tricaine-S™ sedative solutions. Cost is based on purchasing the largest quantity available as a single unit purchase from the sponsors' websites.