U.S. Fish & Wildlife Service

Aquatic Animal Drug Approval Partnership

DRUG RESEARCH INFORMATION BULLETIN

Safety of AQUI-S®20E (10% Eugenol) as a Sedative for Striped Bass

Rod Getchell[1], Danni Scott[1] Julie Schroeter[2]*, Niccole Wandelear[2], Shane Ramee[2], & Marilyn Blair[2]

[1]Cornell University, College of Veterinary Medicine, Aquatic Animal Health Program
C4 177 VMC, Ithaca, New York 14853
[2]U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program
4050 Bridger Canyon Road, Bozeman, Montana 59715, USA

Sedatives are physical or chemical agents that initially induce a calming effect on vertebrate animals, and subsequently induce loss of equilibrium, mobility, consciousness, and reflex action. Fisheries professionals routinely sedate fish for a variety of purposes, including collection of tissue samples or morphometric data, implantation of tags or tracking devices, spawning, and transport. Sedating fish before handling can minimize stress and physical injury to the fish and also help protect the handler. Ideally, a fish sedative is safe, effective, easy to administer, and inexpensive. Also, it is desirable that the sedative have no mandated withdrawal period so that treated fish can be returned to, or released into, public waters immediately after treatment.

Currently, only TRICAINE-S is approved by the U.S. Food and Drug Administration (FDA) for the temporary immobilization of fish and other aquatic, cold-blooded animals. This drug is an effective sedative and widely used by fisheries professionals; however, a 21-d withdrawal period is required after use before treated fish may enter the human food chain through stocking or release. For many field applications, holding fish for 21 d postsedation is not practical and seriously compromises management or research activities.

Efforts are underway to generate data to support FDA approval of AQUI-S20E (10% eugenol; AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand) as an immediate-release fish sedative. Considerable research has shown that eugenol is efficacious for sedating freshwater and marine fishes to the handleable stage of anesthesia which is equivalent to stages 3-4 outlined in Summerfelt and Smith, 1990 (e.g., Trushenski et al. 2012a, 2012b, 2012c). Effectiveness and safety data have been generated by the U.S. Fish and Wildlife Service (USFWS) to support approval of AQUI-S20E for use to sedate all freshwater finfish to handleable (Bowker et al., 2013, 2015, and Wandelear et al., 2015), and now more data is needed to support a similar claim for use on marine fish in a saltwater environment. For this approval, FDA required data to demonstrate that fish could be safely exposed to 1) the proposed highest efficacious dose, 2) a dose 50% greater, and 3) for durations exceeding those necessary to sedate fish to handleable. As such, a study was conducted by Dr. Rod Getchell at the Veterinary Medical Center (VMC) to determine an adequate margin of safety associated with exposing fingerling Striped Bass Morone saxatilis to AQUI-S20E. Based on preliminary testing, the highest efficacious dose of 40 mg/L eugenol was selected and 60 mg/L eugenol was the dose that was 50% greater than the highest proposed efficacious dose. An adequate margin of safety was defined as an exposure dose and duration at which test fish survival was ≥95% when exposed for 3-4 min longer than the ET80[a] (effective time for 80% of the fish to become sedated) for the highest efficacious dose and 2-3 min longer than the ET80 for the dose 50% greater than that.

Methods

The study was conducted at the VMC Aquatic Facility at the Cornell University's College of Veterinary Medicine in Ithaca, New York in August 2019. Fingerling Striped Bass obtained from Delmarva Aquatics Facility (Smyrna, Delaware, USA) were exposed to AQUI-S20E at doses of 0, 40, or 60 mg/L eugenol. Mean ± SD total length and weight of 20 fish sampled

*Corresponding author: julie_schroeter@fws.gov
[a]Fifteen fish were individually sedated to handleable to determine the ET80 = (12^{th} time + 13^{th} time)/2

from the reference population before the start of the study for baseline fish health evaluation were 9.2 ± 0.9 cm and 10.3 ± 3.2 g.

One week before the start of the study, times to individually sedate 15 fish to handleable were measured for each dose, and the ET80 for each dose was calculated. Four exposure durations (T1 – T4) were selected for each exposure dose such that T1 and T2 exposure durations yielded survival data in the range of 95 - 100%; T3 yielded survival data in the 70 - 90% range; and T4 yielded survival data in the 50 - 70% range. The four exposure durations assigned to 0 mg/L were identical to those assigned to 40 mg/L, which ensured that groups of control fish were tested at the longest set of exposure durations used in the study. Hence, there were 12 exposure regimen combinations (3 doses × 4 exposure durations per dose).

Testing consisted of exposing four replicate groups (n = 15 fish per group) of test fish to each of the 12 exposure regimens, and each exposure event was followed by a 24-h recovery period. Fish were sedated in 18.9-L plastic buckets of aerated saltwater under static-bath conditions for predetermined durations and allowed to recover in 68.1-L plastic containers supplied with aerated saltwater. Water temperature and dissolved oxygen (DO) concentration were measured in each exposure container before placing fish in the solution. Sedative solution samples were collected from all exposure containers and analyzed to verify eugenol concentrations by UV-Vis spectrophotometry. Fish-response data included survival, general fish behavior during sedation and recovery, and fish health and histology recorded for dead fish collected within 30 min of transfer to recovery tanks and subsamples of live fish collected from each tank at 24 h postrecovery. To determine the mean and 95% CI for mean percent survival at each exposure concentration/duration, a generalized linear mixed model was used (RStudio Version 1.2.5033 software). All fish were examined visually during gross necropsy. Prevalence and severity of normal and abnormal histological changes (herein defined as lesions) observed microscopically in gill, liver, and posterior kidney of fish sampled from the T4 exposure groups were transformed to dichotomized versions of biologically important (scores of marked or severe) and not biologically important (scores of none, normal, mild, or moderate) lesions and summarized and tabulated.

Results and Discussion

All external and internal tissues examined from the reference population fish for baseline fish health analyses appeared normal.

All fish exposed to 0 mg/L eugenol survived with the exception of one fish that was injured during transfer and died during the first hour in the recovery container. At 40 mg/L eugenol, the ET80 was 1.167 min, and acceptable survival (≥95%) was observed among fish exposed for 6.0 min (T2; 5.1x the ET8040 (ET80 for the 40 mg/L dose); Table 1) but decreased to an unacceptable level when exposed for 9.0 min (T3; 7.7x the ET8040; Table 1). Based on these results, the margin of safety extended to at least 6.0 min and the safety break point for exposure in the AQUI-S20E solution was between 6.0 and 9.0 min. At 60 mg/L eugenol, the ET80 was 0.875 min, and acceptable survival was observed among fish exposed for 3.0 min (T1; 3.4x the ET8060 (ET80 for the 60 mg/L dose); Table 1) but decreased to an unacceptable level when exposed for 6.0 min (T2; 6.9x the ET8060; Table 1). Based on these results, the margin of safety extended to at least 3.0 min and the safety break point was between 3.0 and 6.0 min.

Gross examination of external and internal tissues of all fish sampled appeared normal regardless of exposure dose or duration and regardless of whether a fish was alive or dead when collected, except for the one fish exposed to 0 mg/L mentioned above. Prevalence and severity of lesions observed in live fish sampled from the 40 and 60 mg/L eugenol T4 exposure groups were similar to those observed in live fish sampled from the 0 mg/L T4 exposure group (Table 2). Although there was an increase in the frequency of marked/severe degeneration and necrosis of gill epithelium tissue in the dead fish in the 40 and 60 mg/L T1 – T4 exposure groups compared to the 0 mg/L group, the prevalence and severity of histological effects observed in surviving fish were not considered to be biologically important and did not appear to adversely affect fish health.

Fish behavior was observed to be normal upon immersion in AQUI-S20E at all doses and durations. Fish that recovered from sedation also displayed normal behavior.

Mean eugenol concentrations from the 40 and 60 mg/L exposure buckets were 45.4 (13.5% above target) and 67.9 (13.2% above target) mg/L eugenol, respectively. These concentrations were within the required ±25% of the target dose, and were therefore considered sufficient to use as treatment concentrations. No eugenol was detected in samples collected from the 0 mg/L exposure group.

Source water salinity used to fill test and recovery containers was approximately 35ppt. Mean water temperatures in exposure buckets and recovery tanks was 19.9oC and 19.6oC. Mean DO concentrations in exposure buckets before and after fish were sedated were 9.9 and 9.3 mg/L. Mean DO concentrations in recovery tanks at the beginning and end of the 24-h

recovery period were 9.0 and 9.6 mg/L. The mean pH measurement in the AQUI-S20E bulk working solution for 0 mg/L was 8.5, and for 40 and 60 mg/L eugenol batches, was 8.6. The mean pH measurement in recovery containers was 8.6.

Based on survival, there was an adequate margin of safety associated with overexposing fingerling Striped Bass to 40 or 60 mg/L eugenol. No gross or microscopic lesions were detected that indicated potential toxicity of AQUI-S20E to the test fish. Results from this study will be submitted to FDA to support a claim that AQUI-S20E is safe to use to sedate all freshwater and saltwater fishes to the handleable stage of sedation.

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Table 1. Relative survival of fingerling Striped Bass exposed to AQUI-S20E at doses of 40 or 60 mg/L eugenol for various durations. Acceptable survival was ≥95%.

Eugenol Dose (mg/L)	ET80 (min)	Exposure duration (min)			
		T1	T2	T3	T4
40	1.167	3.0	6.0	9.0	14.0
		100%	97%	85%	63%
60	0.875	3.0	6.0	9.0	12.0
		97%	68%	58%	58%

Table 2. Number of test fish with mild or moderate lesions (considered "not biologically important") and those with marked or severe lesions (considered "biologically important"). Where two numbers are listed (separated by "/"), the first number is the number of live fish from the T4 exposure groups observed with the lesion and the second number is the total number of dead fish from the T1 – T4 exposure groups observed with the lesion.

	Feature	Exposure dose (mg eugenol/L)					
		Mild or moderate lesions			Marked or severe lesions		
		0[1]	40[2]	60[3]	0[1]	40[2]	60[3]
Gill	Degeneration	0/0	0/0	0/4	0/0	0/25	0/42
	Necrosis	0/0	0/0	0/4	0/0	0/25	0/42
	Proliferation	0/0	0/0	0/0	0/1	0/0	0/0
Kidney	Degeneration	0/0	0/0	0/0	0/0	0/0	0/0
	Necrosis	0/0	0/0	0/0	0/0	0/0	0/0
Liver	Degeneration	0/0	0/0	0/0	0/0	0/0	0/0
	Necrosis	0/0	0/0	0/0	0/0	0/0	0/0

[1]n=16 live fish/1 dead fish
[2]n=16 live fish/26 dead fish
[3]n=16 live fish/48 dead fish