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

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Fish 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, in some cases, help protect the handler. Ideally, a fish sedative is safe, effective, easy to administer, inexpensive, and has a wide safety margin. 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 post- sedation 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. Bowker et al. (2013) demonstrated that 60 mg/L eugenol consistently sedated a variety of warmwater finfish to handleable within 2 min at a mean water temperature of 24° - 26°C, and this dose will be proposed as the *lowest* efficacious dose. A fish was determined to be handleable when it lost equilibrium and the ability to swim, could easily be caught by hand, and did not struggle while being measured for length. For approval, FDA also requires data demonstrating that fish can be safely exposed to (a) the proposed *highest* efficacious dose, (b) a dose 50% greater than that, and (c) for durations exceeding what is necessary to sedate fish to handleable. As such, we conducted a study to determine if there is an adequate margin of safety associated with exposing fingerling Channel Catfish (CCF) Ictalurus punctatus (a representative warmwater finfish) to AQUI-S20E, and selected 100 mg/L eugenol as the highest efficacious dose, and 150 mg/L eugenol as the dose 50% greater than that. 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 Bozeman Fish Technology Center, Bozeman, Montana in November 2012. Fingerling CCF were exposed to AQUI-S20E at doses of 0, 100, or 150 mg/L eugenol. Mean total length and weight of 20 fish sampled from the reference population before the start of the study for fish health evaluation were 5.3 (SD, 0.6) cm and 1.5 (SD, 0.5) g.

Three days 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 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

[a]Fifteen fish were individually sedated to handleable to determine the ET80 = (12th time + 13th time)/2

to 100 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 three 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 3.8-L plastic buckets under static-bath con- ditions for predetermined durations and allowed to recover in 57- L fiberglass tanks plumbed with flowing water. 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 60 min of transfer to recovery tanks and sub- samples of live fish collected from each tank at 24 h post- recovery. 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 at 24 h postexposure were transformed to dichotomized versions of biologically important (scores of marked or severe) and not biologically important (scores of normal, mild, or moderate) lesions and analyzed with a generalized linear model in SAS PROC GLIMMIX. Statistical significance was indicated when $P < 0.10$.

Results and Discussion

All fish exposed to 0 mg/L eugenol survived. At 100 mg/L eugenol, the ET80 was 0.4 min. Acceptable survival (≥95%) was observed among fish exposed for 4.5 min (T2; 4.1 min beyond the ET80100) but decreased to an unacceptable level (84%) when exposed for 8.0 min (T3; Table 1). Based on these results, the margin of safety extended to at least 4.5 min and the safety break point for exposure in the AQUI-S20E solution was between 4.5 and 8.0 min. At 150 mg/L eugenol, the ET80 was 0.3 min, and acceptable survival was observed among fish exposed for 4.0 min (T2; 3.7 min beyond the ET80150) but decreased to an unacceptable level (78%) when exposed for 6.0 min (T3; Table 1). Based on these results, the margin of safety extended to at least 4.0 min and the safety break point was between 4.0 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. With one exception, prevalence and severity of lesions observed in live or dead fish sampled from the 100 and 150 mg/L eugenol exposure groups were similar to those observed in live fish sampled from the 0 mg/L T4 exposure group (Table 2). A significant difference was detected in the number of fish with gill inflammation that was considered biologically important (scored as marked) between the the 0× T4 and 1× T4 exposure groups.

However, the prevalence of this lesion was higher in the 0× group than in the 1× group. No such difference was detected between the 0× T4 and 1.5× T4 exposure groups. Significant differences were not detected at any other time when comparing the frequency of biologically important vs. nonbiologically important lesions in 100 or 150 mg/L eugenol exposure groups to those in the 0 mg/L exposure group.

No abnormal fish behavior was observed upon immersion into the sedative solution. Fish that recovered from sedation resumed normal behavior.

Mean eugenol concentrations from the 100 and 150 mg/L exposure buckets were 101.5 (SD, 3.0) and 153.9 (SD, 4.0) mg/L eugenol. No eugenol was detected in samples collected from the 0 mg/L exposure group.

Mean water temperatures in exposure buckets and recovery tanks was 26.2oC and 27.8oC. Mean DO concentrations in exposure buckets before and after fish were sedated were 6.2 and 6.0 mg/L. Mean DO concentrations in recovery tanks at the beginning and end of the 24-h recovery period were 7.3 and 7.5 mg/L. Water alkalinity and hardness were 146 mg/L and 242 mg/L (both measured as $CaCO_3$); and pH was 8.6. Mean pH measurements in AQUI-S20E bulk working solutions for the 0, 100 and 150 mg/L eugenol batches were all 8.1.

Based on survival, there was an adequate margin of safety associated with overexposing fingerling CCF to 100 or 150 mg/L eugenol. No gross or microscopic lesions were detected that indicated toxicity to the test fish that could affect overall health. Results from this study were submitted to FDA to support a claim that AQUI-S20E is safe to freshwater finfish.

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References

Bowker, J., D. Carty, M.P. Bowman, and N. Wandelear. 2013. Use of AQUI-S20E and BENZOAK to sedate Sunshine Bass, Blue Catfish, and Nile Tilapia to Handleable. *Drug Research Information Bulletin No. 32*. U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program, Bozeman, Montana USA.

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Table 1. Relative survival of fingerling Channel Catfish exposed to AQUI-S20E at doses of 100 or 150 mg/L eugenol for various durations.

Eugenol Dose	ET80 (min)	Exposure Duration (min)			
		T1	T2	T3	T4
100	0.36	3.5	4.5	8.0	12.0
		96%	100%	84%	47%
150	0.31	2.5	4.0	6.0	10.0
		96%	96%	78%	38%

Table 2. Number of test fish with mild or moderate lesions and those with marked lesions (note that no tissue lesions were characterized as severe). 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 number of dead fish from the T4 exposure groups observed with the lesion.

Tissue	Feature	Exposure dose (mg eugenol/L)					
		Mild or moderate lesions			Marked lesions		
		0[1]	100[2/3]	150[4/5]	0[1]	100[2/3]	150[4/5]
Gill	Degeneration	2	1/2	0/4	0	0/0	0/0
	Necrosis	2	1/0	0/2	0	0/0	0/0
	Proliferation	11	12/11	10/11	1	0/1	0/1
	Scattered lamellar fusion	6	7/5	4/12	0	0/0	0/0
	Hypertrophy	0	0/0	0/0	0	0/0	0/0
	Inflammation	2	7/2	6/1	9	5/10	4/10
Kidney	Degeneration	9	11/11	8/8	0	0/1	0/0
	Tubule necrosis	9	11/12	8/8	0	0/0	0/0
	Hematopoietic cell proliferation	0	0/0	0/0	9	11/12	8/8
	Melanomacrophage centers	9	11/12	7/8	0	0/0	0/0
	Regenerating tubules	4	2/8	2/3	0/0	0/0	0/0
	Hypertrophy	0	0/5	0/5	0	0/3	0/0
	Inflammation	2	2/3	0/0	0	0/0	0/0
Liver	Degeneration	7	9/10	9/12	0	0/0	0/0
	Necrosis	2	3/6	1/11	0	0/0	0/0
	Nuclear pleomorphism	12	11/12	11/9	0	0/0	0/3
	Nuclear vacuolation	2	5/11	3/10	0	0/0	0/0
	Glycogen vacuolation	10	12/12	11/12	1	0/0	0/0
	Inflammation	6	7/11	8/9	0	0/0	0/0
	Melanomacrophages	2	8/4	7/4	0	0/0	0/0

[1]n=12 live fish with sufficient gill and liver for evaluation; n=9 live fish with sufficient posterior kidney for evaluation
[2]n=12 live fish with sufficient gill and liver for evaluation; n=11 live fish with sufficient posterior kidney for evaluation
[3]n=12 dead fish with sufficient gill, liver, and posterior kidney for evalution
[4]n=10 live fish with sufficient gill for evaluation, n=11 live fish with sufficient liver for evaluation; n=8 live fish with sufficient posterior kidney for evaluation
[5]n=12 dead fish with sufficient gill and liver for evaluation, n=8 dead fish with sufficient posterior kidney for evaluation