



U.S. Fish & Wildlife Service

Aquatic Animal Drug Approval Partnership

DRUG RESEARCH INFORMATION BULLETIN**The Efficacy of AQUI-S®20E (10% Eugenol) and BENZOAK® (20% Benzocaine) to Sedate Fish to Handleable: Individual Sedation versus Group Sedation**

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AQUI-S®20E (10% eugenol; AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand) and BENZOAK® (20% benzocaine; Europharma, Victoria, Minnesota) are candidates for U.S. Food and Drug Administration (FDA) approval for use as immediate-release fish sedatives. As part of the AQUI-S®20E and BENZOAK® approval processes, several efficacy trials were conducted on a variety of freshwater-reared finfishes (e.g., Bowker et al. 2013a, 2013b, and 2013c). In those trials, fish were sedated individually; however, it is common in aquaculture and fisheries management to sedate fish in small groups. Therefore, in 2011, additional trials were conducted with AQUI-S®20E and BENZOAK® to investigate whether time to handleable was similar between individually sedated and group-sedated (1) Lake Trout (LKT) *Salvelinus namaycush*, (2) Walleye (WAE) *Sander vitreus*, and (3) Channel Catfish (CCF) *Ictalurus punctatus*, which served as representative cold-, cool-, and warmwater finfish, respectively.

Methods

Test fish, nominal sedative concentrations, and study sites.—Lake Trout (120 ± 19 mm and 15 ± 7 g; mean \pm SD) were tested at 25 mg per L eugenol or 40 mg per L benzocaine at the U.S. Geological Survey Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin. Walleye (226 ± 12 mm) were tested at 60 mg per L eugenol or 150 mg per L benzocaine at the Iowa Department of Natural Resources Rathbun Fish Culture Research Facility, Moravia, Iowa. Channel Catfish (279 ± 33 mm and 135 ± 63 g) were tested at 60 mg per L eugenol or 150 mg per L benzocaine at the Southern Illinois University Fisheries and Illinois Aquaculture Center, Carbondale, Illinois. The nominal eugenol and benzocaine concentrations administered in each trial had been shown through pilot work to sedate the test fish to handleable within ~3 min, a time period acceptable to most fisheries professionals (Summerfelt and Smith 1990).

Experimental procedures.—In each trial, 40 fish were individually sedated to handleable, and 40 fish were sedated to handleable in four 10-fish groups (total, 80 fish tested per trial). Test fish were dipnetted from a reference population, transferred individually or as a 10-fish group into a container containing a sedative solution, and sedated under static conditions. A fish was determined to be handleable when it lost equilibrium and the ability to swim and could easily be caught by hand. When an individually sedated fish or a group-sedated fish became handleable, it was removed from the sedative solution and transferred to a recovery container. Fish were allowed to recover in fresh water under flow-through (LKT and CCF) or static (WAE) conditions. Following recovery, fish were placed in holding tanks plumbed with fresh, flowing water and monitored for survival for 24 h.

In each trial, time to handleable was recorded for each sedated fish, and fish behavior was evaluated during sedation. Mortality was recorded at 24 h postrecovery. Water temperature and dissolved oxygen concentration were measured during sedation, and source water hardness, alkalinity, and pH were measured once.

Statistical analysis.—For each trial, a SAS Proc Mixed model for equal variances was used to test for a significant ($P < 0.05$, two-sided) difference in mean time to handleable between individually sedated fish ($n = 40$ fish) and group-sedated fish ($n = 4$ groups).

Results

AQUI-S®20E.—In the *AQUI-S®20E* trials, time to handleable ranged from a low of 0.9 ± 0.1 min (mean \pm 95% CI) for individually sedated WAE to a high of 3.2 ± 0.1 min for individually sedated LKT (Figure 1). In the LKT trial, mean time to handleable for individually sedated fish was significantly longer (difference = 0.8 min) than that for group-sedated fish. In the WAE trial, mean time to handleable for individually sedated fish was not significantly different (difference = 0.1 min) from that for group-sedated fish. In the CCF trial, mean time to handleable for individually sedated fish was significantly longer (difference = 0.4 min) than that for group-sedated fish.

During sedation, some LKT exhibited head shaking and coughing behaviors, and some WAE and CCF appeared slightly agitated. However, these behaviors lasted only a few seconds, ceased as fish became sedated, and appeared to have had no adverse effects on fish during the 24-h postrecovery period.

BENZOAK®.—In the *BENZOAK®* trials, mean time to handleable ranged from a low of 0.7 ± 0.0 min for individually sedated WAE to a high of 3.0 ± 0.1 min for individually sedated LKT (Figure 2). In the LKT trial, mean time to handleable for individually sedated fish was significantly longer (difference = 0.7 min) than that for group-sedated fish. In the WAE trial, mean time to handleable for individually sedated fish was significantly shorter (difference = 0.3 min) than that for group-sedated fish. In the CCF trial, mean time to handleable for individually sedated fish was not significantly different (difference = 0.1 min) from that for group-sedated fish.

During sedation, all LKT and WAE appeared to behave normally. Most CCF appeared slightly agitated when first placed into the sedative solution; however, this behavior lasted only a few seconds, ceased as fish became sedated, and appeared to have had no adverse effects on fish during the 24-h postrecovery period.

Fish mortality and water chemistry.—No fish died during the *AQUI-S®20E* and *BENZOAK®* trials. Moreover, during each trial, water quality was maintained at levels suitable for rearing the species being tested (Table 1).

Discussion and Conclusions

Mean times to handleable sometimes differed significantly between individually sedated and group-sedated LKT, WAE, and CCF; however, there was no consistent pattern across trials, and the differences observed (range, 0.1 – 0.8 min) were not considered to be biologically important. Consequently, we concluded that the *AQUI-S®20E* and *BENZOAK®* concentrations administered were efficacious for sedating the fish species tested to handleable either individually or in small groups. The data generated in these trials were accepted by FDA as supporting the approval of *AQUI-S®20E* and *BENZOAK®* for use in the United States as immediate-release fish sedatives.

Acknowledgments

Mark Gaikowski, Jeff Meinertz, and their crew at the USGS Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin hosted the Lake Trout trials; Alan Johnson at the Iowa Rathbun Fish Culture Research Facility hosted the Walleye trials, and Jesse Trushenski and her students at Southern Illinois University hosted the Channel Catfish trials. Dave Erdahl, FWS AADAP Program, reviewed this bulletin.

References

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Figure 1. AQUI-S®20E: Mean ± 95% CI time to handleable for Lake Trout (LKT), Walleye (WAE), and Channel Catfish (CCF) individually sedated (Ind) or group-sedated (Grp). For each species, mean ± 95% CIs labeled with different letters are significantly ($P < 0.05$) different.

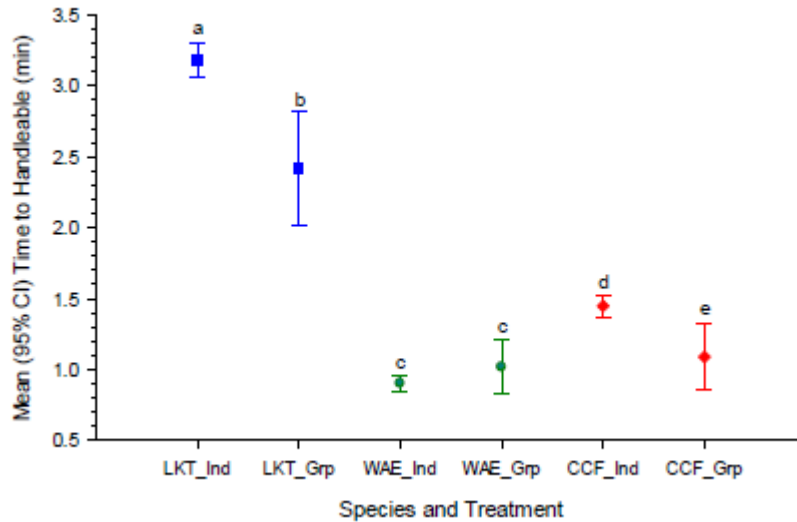


Figure 2. BENZOAK®: Mean ± 95% CI time to handleable for Lake Trout (LKT), Walleye (WAE), and Channel Catfish (CCF) individually sedated (Ind) or group-sedated (Grp). For each species, mean ± 95% CIs labeled with different letters are significantly ($P < 0.05$) different.

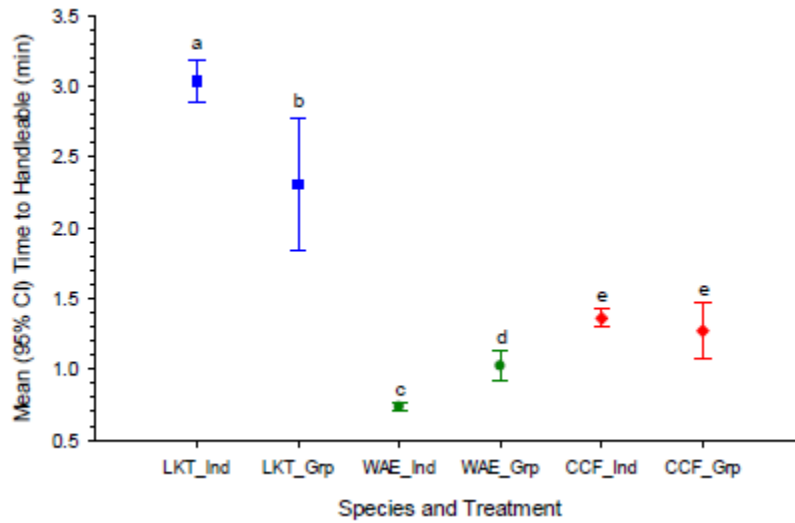


Table 1. Water quality variables measured during the AQUI-S®20E and BENZOAK® trials. Mean ± SD data are presented if available.

Fish Species	Water quality				
	During sedation		Source water		
	Temperature (°C)	Dissolved oxygen conc. (mg per L)	Hardness (mg per L as CaCO ₃)	Alkalinity (mg per L as CaCO ₃)	pH
Lake Trout	13.6 ± 0.2	10.0 ± 0.2	180	124	7.6
Walleye	20.9 ± 0.1	10.2 ± 0.1	97	64	9.9
Channel catfish	22.8 ± 0.9	8.7 ± 0.3	55	125	8.7