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Efficacy of Oxytetracycline Hydrochloride to Control Mortality in Rainbow Trout *Oncorhynchus mykiss* Infected with Coldwater Disease

Jim Bowker*, Molly P. Bowman, and Niccole Wandelear

U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program
4050 Bridger Canyon Road, Bozeman, Montana, 59715, USA

Bacterial coldwater disease (CWD; causative agent, *Flavobacterium psychrophilum*) causes high mortality in cultured freshwater-reared salmonids (LaFrentz et al. 2003). Salmonids ranging from yolk-sac fry to yearlings are susceptible to CWD; typically, the younger the fish, the more severe the disease (Leek 1987). To control mortality, systemic infections require antibiotic treatment (Noga 2000). In the U.S., two antibiotics are approved by the U. S. Food and Drug Administration (FDA) that are administered to fish in feed to control mortality of freshwater- reared salmonids due to CWD: Aquaflor® (florfenicol; Merck Animal Health, Summit, NJ) and Terramycin® 200 for Fish (oxytetracycline dihydrate; Phibro Animal Health, Teaneck, NJ). In spite of the approvals of these two antibiotics, there is interest by the aquaculture community to obtain approval of an antibiotic that can be administered as a bath solution. To gain U.S. Food and Drug Administration (FDA) approval, it must be demonstrated that the product is safe and effective.

In this bulletin, we summarize the results of a trial to demonstrate the effectiveness of Pennox 343® (oxytetracycline hydrochloride; OTC-HCl) to control mortality in Rainbow Trout (RBT) *Oncorhynchus mykiss* naturally infected with coldwater disease.

Methods

The trial was conducted in May-June 2014 at the U.S. Fish and Wildlife Service Bozeman Fish Technology Center (BFTC) in Bozeman, Montana. Test fish were RBT fingerlings (mean length, 11.3 cm). A single production tank of RBT fingerlings was used as the reference population.

After reference population fish were presumptively diagnosed with coldwater disease, completely randomized design procedures were used to assign fish and treatment conditions (treated vs. nontreated control) to test tanks. Circular fiberglass test tanks (46 cm. diameter; rearing volume, 31 L) were stocked with fish impartially collected from the reference population. Each treatment condition was replicated four times (n = 8 test tanks at 60 fish/tank). The trial comprised a 1-d acclimation period, 3-d treatment period, and 14-d posttreatment observation period.

During the treatment period, OTC-HCl was administered to the four treated tanks at a nominal concentration of 46 mg/L in a static bath for 60 min per day on three consecutive days, and the four control tanks received a hatchery water sham treatment under static-bath conditions. Mortality, general fish behavior, feeding behavior (i.e., non-aggressive, semi-aggressive, aggressive), water temperature, and dissolved oxygen concentration data were collected daily throughout the trial. A water sample was collected for OTC-HCl dose verification at 45- 55 min into the 60 min treatment period from one randomly se- lected treated tank and one randomly selected control tank on each of the three treatment days. Analytical dose verification was conducted the U.S. Geological Survey Upper Midwest Environmental Science Center and measure for OTC-HCl by high performance liquid chromatography.

The SAS PROC GLIMMIX (logit link) procedure was used to compare mean cumulative mortality in control tanks to that in treated tanks. Treatment levels were judged statistically significant if $P < 0.05$.

Results

At the end of the trial (Figure 1), mean percent cumulative mortality in treated tanks, 13.0 ± 3.71 (mean ± SD), was significantly less (P = 0.0326) than mean percent cumulative mortality in control tanks 21.6 ± 4.99 (mean ± SD).

Based on dose verification samples collected (n = 3 treated; n = 3 control), the overall mean OTC-HCl concentration administered to treated and control tanks was 53.1 (15.3% from the nominal dose) and 0.0 mg/L, respectively. The doses administered were within FDA-acceptable limits.

Mean water temperature (7.8oC; range 7.6–8.1°C) and mean dissolved oxygen concentration (8.7 mg/L; range, 8.1–10.0 mg/L) during the trial were suitable for rearing healthy RBT. General fish behavior was considered normal and fish in all test tanks fed semi-aggressively on each day of the study.

Discussion

In this trial, OTC-HCl administered at the analytically verified dose of 46 mg/L for 60 min daily in a static bath on three consecutive days was effective in controlling mortality in fingerling RBT caused by coldwater disease for a period up to 14 d posttreatment. Results will be submitted to FDA to support a supplemental approval for use of Pennox 343® to control mortality in freshwater-reared salmonids due to coldwater disease associated with *F. psychrophilum*.

Acknowledgments

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References

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***Corresponding author: jim_bowker@fws.gov**

Figure 1. Mean percent cumulative mortality during the trial of Rainbow Trout in treated (n=4) and control (n=4) tanks diagnosed with coldwater disease (error bars = ±1SD).