



U.S. Fish &amp; Wildlife Service

## Aquatic Animal Drug Approval Partnership

**DRUG RESEARCH INFORMATION BULLETIN****Efficacy of AQUAFLO<sup>®</sup> (50% Florfenicol) to Control Mortality in Freshwater-Reared Salmonids Diagnosed with Systemic Columnaris Disease**

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Columnaris (causative agent, *Flavobacterium columnare*) is an acute-to-chronic external or systemic bacterial disease affecting freshwater-reared finfish worldwide. Early in a disease outbreak, HALAMID<sup>®</sup> AQUA (100% chloramine-T) and 35% PEROX-AID<sup>®</sup> (35% hydrogen peroxide) can be used as drugs approved by the U.S. Food and Drug Administration (FDA) to control mortality in some freshwater fish species. However, neither drug is approved for use on freshwater-reared salmonids. These two drugs, as well as oxytetracycline hydrochloride can be used for such treatment under compassionate Investigational New Animal Drug (INAD) exemptions; and potassium permanganate can be used as an FDA “regulatory action deferred” drug. When the disease becomes systemic, options are even more limited. Until recently, no antibiotic was approved to control mortality caused by columnaris, and available treatments were limited to those that could be administered via a compassionate INAD exemption or by veterinary prescription for extra-label use of over-the-counter drugs approved for other uses in fish. As such, U.S. aquaculture needed an FDA-approved drug for use to control mortality in a variety of finfish due to systemic columnaris.

AQUAFLO<sup>®</sup> (50% florfenicol; Merck Animal Health Corporation, Roseland, NJ) is a medicated premix for inclusion in fish feed. Florfenicol is a broad-spectrum antibiotic with bacteriostatic and bactericidal properties. Prior to 2012, the FDA had approved AQUAFLO<sup>®</sup> as a veterinary feed directive (VFD) drug for a variety of claims, including use to control mortality in catfish caused by enteric septicemia (associated with *Edwardsiella ictuluri*), and in freshwater salmonids due to coldwater disease (associated with *F. psychrophilum*) or furunculosis (associated with *Aeromonas salmonicida*). In 2012, supplemental approvals for AQUAFLO<sup>®</sup> were granted by FDA and included treatment to control mortality of all warmwater finfish due to streptococcal disease (associated with *Streptococcus iniae*), and based in part by data reported in the bulletin, to control mortality caused by systemic columnaris in all freshwater finfish. In this bulletin, we summarize the results from two trials that were conducted to support FDA-approval of AQUAFLO<sup>®</sup> to control mortality in freshwater-reared salmonids diagnosed with systemic columnaris.

**Methods**

Trials were conducted at the Washington Department of Fish and Wildlife, Bellingham State Fish Hatchery (BSFH), Bellingham, Washington. Trial 1 (Jul 12 – Aug 05, 2002) was conducted on coho salmon *Oncorhynchus kisutch* fingerlings (mean length = 8.9 cm). Trial 2 (Jul 01 – 25, 2008) was conducted on rainbow trout *O. mykiss* fingerlings (mean length = 8.7 cm). In both trials, AQUAFLO<sup>®</sup> was administered orally in commercial fish feed at a target dosage of 10 mg florfenicol/kg fish/d for 10 consecutive days.

In each trial, six 6,300-L concrete raceways were used as test tanks. Test fish were sample-counted and weighed into tanks; thus, number of fish per tank differed within and between trials. Number of fish per tank averaged 7,355 (range, 6,546 – 9,073) in Trial 1 and 8,352 (range, 7,305 – 10,437) in Trial 2. Treatment conditions (treated vs. nontreated control) were allocated among tanks with a completely randomized design. There were three treated tanks and three control tanks per trial. Tanks were supplied with single-pass surface water from Lake Whatcom, WA.

Each 25-d trial comprised 1-d acclimation, 10-d treatment, and 14-d posttreatment periods. During trials, feed was administered to tanks at 1.5% (Trial 1) or 2.0% (Trial 2) mean body weight/d. During treatment periods, AQUAFLO<sup>®</sup>-medicated feed was administered to treated tanks and nonmedicated feed was administered to control tanks. During posttreatment periods, nonmedicated feed was administered to all tanks.

Mortality, general fish behavior, feeding behavior, water temperature, and dissolved oxygen concentration data were collected daily. During treatment and posttreatment periods, a necropsy was performed on selected moribund fish. Internal tissues from each of the selected fish were streaked and cultured on TYES media and cultures grossly examined and presumptively identified or streaked on a glass slide and examined with a light microscope to infer primary cause of mortality. Florfenicol concentrations in samples of medicated and nonmedicated feeds were analytically verified with high pressure liquid chromatography by Eurofins/AvTech Laboratories (Portage, MI).

In each trial, a SAS PROC GLIMMIX (logit link)-based model was used to compare mean cumulative mortality in control tanks to that in treated tanks on each day of the treatment and post-treatment periods. Treatment levels were judged statistically significant if  $P < 0.05$ .

## Results and Discussion

At the end of Trial 1 (Figure 1), mean cumulative mortality in treated tanks (47.3%; range, 43.5 – 54.9%) was significantly less ( $P = 0.038$ ) than that in control tanks (66.7%; range, 59.9 – 75.9%). At the end of Trial 2 (Figure 2), mean cumulative mortality in treated tanks (18.4%; range, 12.4 – 26.2%) was less than that in control tanks (30.4%; range, 27.4 – 32.1%), but the difference was not significant ( $P = 0.055$ ). In both trials, treated and control fish appeared to behave normally and were characterized as feeding semi-aggressively or aggressively.

*Flavobacterium columnare* was identified as the primary cause of mortality based on the presumptive identification of this pathogen in Trial 1 via cultures grown on TYES and in Trial 2 via stained impression smears of kidney and spleen tissues. No other pathogens were found.

In both trials, florfenicol doses administered were within FDA- acceptable limits (80 – 110% of target). In Trial 1, AQUAFLO<sup>®</sup>-medicated feed was administered at 10.2 mg florfenicol/kg fish/d (102% of target). In Trial 2, AQUAFLO<sup>®</sup>-medicated feed was administered at 9.5 mg florfenicol/kg fish/d (95% of target). In both trials, no florfenicol was detected in control feed.

Mean ( $\pm$  SD) water temperatures and dissolved oxygen concentrations in Trial 1 ( $20.8 \pm 1.06^{\circ}\text{C}$  and  $7.9 \pm 0.54$  mg/L) and Trial 2 ( $20.0 \pm 0.51^{\circ}\text{C}$  and  $8.1 \pm 0.59$  mg/L) were normal for BSFH and within the acceptable temperature range for rearing salmonids (Piper et al. 1982).

Trial 1 was accepted by FDA as demonstrating effectiveness of AQUAFLO<sup>®</sup> to control mortality in Coho salmon due to systemic columnaris when administered in feed at the target dose for 10 consecutive days. However, Trial 2 was not accepted by FDA primarily because mean cumulative mortality did not differ significantly between treated tanks and control tanks and because an insufficient number of moribund fish were sampled during the posttreatment period to reliably determine if *F. columnare* was the primary cause of mortality. Regardless, results from both trials were combined with results from florfenicol field effectiveness trials conducted on other species of fish (e.g., Straus et al. 2012, Bowker et al. 2013, Matthews et al. 2013) to support an FDA approval for use of AQUAFLO<sup>®</sup> to control mortality in all freshwater finfish due to systemic columnaris (<http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/UCM299146.pdf>)

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## References

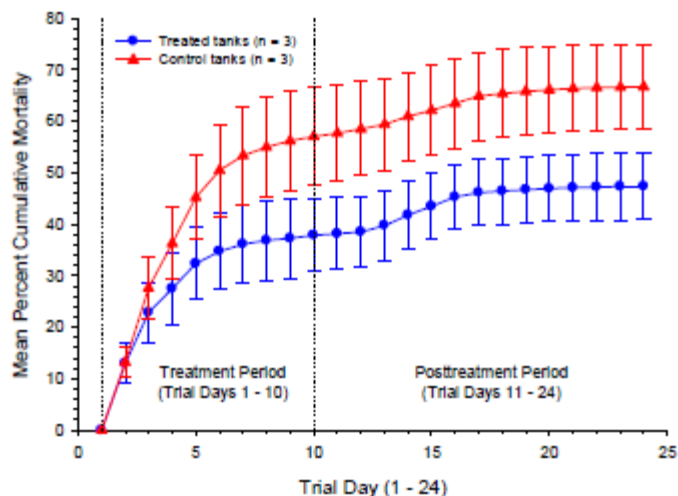
Bowker, J.D., D. Carty, M.P. Bowman. 2013. Safety of Aquaflo<sup>®</sup>-Medicated Feed to Yellow Perch. North American Journal of Aquaculture 75:517-523.

Matthews, M.D., J.D. Bowker, D.G. Carty, N. Wandelaar, M.P. Bowman, J.C. Sakmar, and K. Childress. 2013. Efficacy of Aquaflor (50% Florfenicol) to Control Mortality Associated with *Flavobacterium columnare* infection in Largemouth Bass and Bluegill. *North American Journal of Aquaculture* 75:385- 392.

Straus, D.L., J.D. Bowker, M.P. Bowman, D. Carty, A.J. Mitchell, and B. D. Farmer. 2012. Safety of Aquaflor®- Medicated Feed to Sunshine Bass. *North American Journal of Aquaculture* 74:1-7.

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**Figure 1. Trial 1: Mean ( $\pm$  SD) percent cumulative mortality (treated tanks vs. control tanks) of Coho salmon fingerlings diagnosed with systemic columnaris.**



**Figure 2. Trial 2: Mean ( $\pm$  SD) percent cumulative mortality (treated tanks vs. control tanks) of rainbow trout fingerlings diagnosed with systemic columnaris.**

