A CLINICAL FIELD TRIAL TO DETERMINE:

The Efficacy of Florfenicol-Medicated Feed to Control Mortality of Coho Salmon *Oncorhynchus kisutch* Caused by Furunculosis, Causative Agent *Aeromonas salmonicida*

Study Number: FLOR-01-EFF.3-27

ORIGINAL

Study Director

James D. Bowker
U.S. Fish and Wildlife Service
Bozeman Fish Technology Center - National INAD Office
4050 Bridger Canyon Road
Bozeman, MT 59715
Phone: 406-587-9265 ext. 126
FAX: 406-582-0242

Investigator

Randy Rickert
Makah National Fish Hatchery
PO Box 739
Neah Bay, WA 98357
360-645-2521

Testing Site: Makah National Fish Hatchery
PO Box 739
Neah Bay, WA 98357
360-645-2521

Study start date: July 28, 2006

Study end date: August 21, 2006

D. B.
Signature

11/16/06
Date

Daniel Carty
Signature

Nov 16, 2006
Date

Executive Summary

The U.S. Fish and Wildlife Service (FWS) Aquatic Animal Drug Approval Partnership (AADAP) program conducted a pivotal efficacy study to help obtain U.S. Food and Drug Administration approval for the use of Aquaflor®-medicated feed to control mortality in hatchery-reared salmonids diagnosed with furunculosis (causative agent, *Aeromonas salmonicida*). The study was conducted July 28 - August 21, 2006, at the FWS Makah National Fish Hatchery (NFH; Neah Bay, WA) by AADAP, Makah NFH, and FWS Olympia Fish Health Center personnel under Study Protocol Number FLOR-01-EFF.3 (3rd revision, revised and signed September 27, 2002) and in compliance with Good Clinical Practice standards. The test article, Aquaflor®, is a 50% active florfenicol premix in a palatable base for salmon (Schering-Plough Animal Health Corp., Summit, NJ). Test fish were coho salmon *Oncorhynchus kisutch* fingerlings (mean length, 9.9 cm; mean weight, 9 g) impartially drawn from a reference population diagnosed with furunculosis.

The study's null hypothesis was H_o: $\mu_{treated} \geq \mu_{not\ treated}$, and a randomized complete block design (RCBD) was used to allocate six replicates (i.e., six test tanks) of each treatment condition (treated vs. control) across two blocks of tanks. The experimental unit was the "test tank" (n = 160 to 163 fish per tank). The 25-day study was single-blinded and comprised a 1-d pre-treatment period (July 28), a 10-d treatment period (July 29 - August 7), and a 14-d post-treatment period (August 8 - 21).

Throughout the study, feed was administered to treated and control tanks at 1% mean fish body weight. Non-medicated feed was administered to all tanks during the pre- and post-treatment periods. During the treatment period, Aquaflor®-medicated feed was administered to treated tanks, and non-medicated feed was administered to control tanks. Target dose for treated tanks was 10 mg florfenicol/kg fish/d for 10 consecutive days. Actual dose administered to treated tanks was 10.4 mg florfenicol/kg fish/d (+4% of target), which was well within the FDA-acceptable limit of ±25% of target.

The primary response variable was "percent total mortality per test tank." At the end of the study, a RCBD analysis of variance revealed that mean percent total mortality in the six treated tanks (17.4%; range, 9 - 24% per tank) was significantly less ($P = 0.012$) than that in the six control tanks (30.0%; range, 17 - 35% per tank). In both treated and control tanks, general fish behavior was always characterized as normal; fish feeding behavior went from non-aggressive to semi-aggressive to aggressive during the pre-treatment and treatment periods and from aggressive back to semi-aggressive during the post-treatment period. These results—combined with (a) fish health data collected during the study, (b) the fact that feed assays showed that the medicated feed was only +4% of target, and (c) the fact that the pathogen of concern (*A. salmonicida*) was shown to be sensitive to florfenicol—demonstrated that the Aquaflor®-medicated feed treatment regimen administered in this study was efficacious in controlling mortality in coho salmon fingerlings diagnosed with furunculosis.