Bacterial diseases are a major problem in aquaculture and account for significant losses of fish (Clarke and Scott 1989; Frerichs and Roberts 1989; Bjornadal 1990). Therefore, the use of antimicrobial therapy plays an important role in aquaculture (Klontz 1987; Alderman 1988). Florfenicol (FFC) is a potent, broad-spectrum, antibacterial agent with bacteriostatic and bactericidal properties that is active against a variety of Gram-positive and Gram-negative bacteria (Horsberg et al. 1996).

AQUAFLOR® (Merck Animal Health Corp., Summit, New Jersey USA) is an aquaculture feed premix containing 50% FFC and is approved in more than 20 countries for use to control mortality in a variety of cultured fishes due to diseases associated with infectious bacterial pathogens. Currently in the U.S., AQUAFLOR® is a Veterinary Feed Directive drug approved by the U.S. Food and Drug Administration (FDA) for use to control mortality in (1) freshwater-reared salmonids due to furunculosis disease and coldwater disease, (2) catfish due to enteric septicemia, (3) freshwater-reared warmwater finfish associated with *Streptococcus iniae*, and (4) freshwater-reared finfish due to columnaris disease. Presently, some fish species can be treated at 10 mg FFC per kg fish per d for 10 d while others can be treated at 10 – 15 mg FFC per kg per d for 10 d.

The U.S. aquaculture community would like to expand the AQUAFLOR® label such that all freshwater-reared finfish can be treated at up to 15 mg FFC per kg fish per d to control mortality due to a variety of diseases. However, to obtain such an approval, data must be generated to show that this dose is safe to representative target animals. Consequently, we conducted a target animal safety study to evaluate the safety of AQUAFLOR® administered in feed to Yellow Perch *Perca flavescens*, a representative coolwater finfish.

**Methods**

The study was conducted February 11 – March 09, 2010, at the U.S. Fish and Wildlife Service (FWS) Bozeman Fish Technology Center (BFTC), Bozeman, Montana USA. The study consisted of a 6-d acclimation period, 20-d exposure period, and 1-d postexposure period. Test fish were from fertilized Yellow Perch eggs collected from a local pond and brought to the BFTC for incubation, hatching, and rearing of fish to desired size. At the time of the study, mean total fish length and weight (±SD) were 7.8 ± 1.6 cm and 5.0 ± 3.4 g, respectively. AQUAFLOR® was administered in feed at 0× (0 mg per kg), 1× (15 mg per kg), 3× (45 mg per kg), or 5× (75 mg per kg) the proposed maximum therapeutic dose of 15 mg FFC per kg fish per d. In all treatments, AQUAFLOR® was administered for 2× (20 d) the proposed therapeutic treatment duration of 10 d. Before study began, 20 reference population fish were collected and used to characterize baseline fish health and histological characteristics.

Completely randomized design procedures were used to assign each of the four exposures to 3 of 12 test tanks and to stock 15 fish into each test tank. The study was single-blinded such that personnel involved in day-to-day data collection did not know which exposures were assigned to which test tanks. Feed samples were collected and sent to Eurofins/AvTech Laboratories (Portage, Michigan USA) to analytically verify the concentration, homogeneity, and stability of FFC in the 1×-, 3×-, and 5×-medicated feeds and to determine if there was any FFC in the 0× (control) feed.

Mortality, general fish behavior, fish feeding (appetite) behavior, water temperature, and dissolved oxygen concentration were assessed daily. In addition, source water hardness, alkalinity, and pH were measured weekly.

On the postexposure day, all remaining test fish were collected from test tanks, euthanized, measured for total length and weight, and necropsied. During necropsy, 120 fish (10 per tank) were randomly selected and processed for histological evaluation of gill, liver, anterior kidney, and posterior kidney tissues. Concomitantly, a second randomization was used to select 24 of these fish (2 per tank) for additional histological evaluation of brain, heart, muscle, skin, spleen, pyloric intestine, and rectal intestine tissues.

Initially, only tissues from the 0× and 5× exposure groups were evaluated for histopathologies (lesions), which were scored via an ordinal scale (0 = none, 1 = normal, 2 = mild, 3 = moderate, 4 = marked, and 5 = severe). None of the lesions detected in the 5× exposure group met all three of the following criteria: (1) marked or severe, (2) apparently AQUAFLOR®-induced, and (3) not observed in the 0× exposure group. Consequently, as specified in the study protocol, we were not required to evaluate tissues from the 1× and 3× exposure groups.

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Mortality data (all exposure groups) and histology data (5× exposure group versus 0× exposure group only) were analyzed in SAS 9.1.3 with Proc Glimmix-based models (logit link). Before analysis of the histology data, lesions scored as 0, 1, 2, or 3 were coded 0 (not biologically important), and lesions scored as 4 or 5 were coded 1 (biologically important). Treatment effects for mortality and histology were tested at $\alpha = 0.10$ (two-sided).

Mean total length and weight were analyzed by one-way ANOVA. For these variables, treatment differences were considered significant if $P < 0.05$ (two-sided).

**Results and Discussion**

Mean percent total mortality was observed in 0× (6.7%), 1× (2.2%), and 3× (10.0%) exposure groups but not in the 5× exposure group. However, mortality differences among exposure groups were not significant ($P = 0.3134$). General fish behavior was characterized as normal in all tanks. Fish fed actively, broke the surface of the water while feeding, and consumed all feed offered. At the end of the in-life phase, fish had grown an average of 0.9 cm and 7.2 g, and overall mean total length and weight of test fish were 8.5 ± 1.75 cm and 7.2 ± 4.74 g, respectively. Mean total length ($P = 0.689$) and mean weight ($P = 0.786$) did not differ significantly among exposure groups.

Skeletal deformities were observed in 95% of the fish sampled from the reference population and 52–60% of fish sampled from each of the exposure groups. Although the prevalence of this abnormality was higher than expected, it was common to most of the fish necropsied in this study, and we speculate it might have been the result of incubation techniques or a nutritional deficiency during early larval development. No lesions were observed in brain, heart, muscle, skin, pyloric intestine or rectal intestine tissues of fish collected postexposure. Lesions not considered biologically important were observed in gill, liver, spleen, anterior kidney, and posterior kidney, and lesions considered biologically important were observed in gill, liver, spleen, anterior kidney, and posterior kidney in fish examined from the 0× or 5× exposure groups (Table 1). All biologically important lesions detected were ranked as “marked” while none were ranked as “severe,” and significant differences between 0× and 5× exposure group comparisons of these lesions were not detected.

Based on feeding test fish at 1% BW, analytically verified mean FFC doses delivered to the 1×, 3×, and 5× exposure groups were 15.4, 45.6, and 77.7 mg FFC per kg fish per d, respectively. No FFC was detected in the control feed.

Based on these results, we concluded that the margin of safety was at least five times greater than the proposed therapeutic treatment concentration of 15 mg FFC per kg fish per d. In addition, the FDA accepted the study as demonstrating an adequate margin of safety for the use of AQUAFLOR® on Yellow Perch at a dosage of 15 mg FFC per kg fish per d for 10 consecutive days.

**Acknowledgments**

We thank Merck Animal Health for supplying AQUAFLOR® and paying for feed analysis. Beth MacConnell (Headwaters Fish Pathology, LLC) did histological evaluations. Dave Erdahl, FWS AADAP, reviewed this bulletin.

**References**


Table 1. Relative frequency of pathological lesions detected in Yellow Perch in the 0× and 5× (75 mg FFC per kg fish per d) exposure groups.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Sample size</th>
<th>Flufenicol concentration (mg per kg fish per d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Spleen-melanomacrophages</td>
<td>6</td>
<td>17%</td>
</tr>
<tr>
<td>Liver-glycogen vacuolation</td>
<td>30</td>
<td>0%</td>
</tr>
<tr>
<td>Liver-degeneration</td>
<td>30</td>
<td>3%</td>
</tr>
<tr>
<td>Gill-epithelial lifting</td>
<td>30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20%</td>
</tr>
<tr>
<td>Anterior Kidney-inflammation</td>
<td>18/14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6%</td>
</tr>
<tr>
<td>Anterior Kidney-melanomacrophages</td>
<td>18/14</td>
<td>83%</td>
</tr>
<tr>
<td>Posterior Kidney-proliferation</td>
<td>24/23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100%</td>
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<tr>
<td>Posterior Kidney-tubule degeneration</td>
<td>24/23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>97%</td>
</tr>
<tr>
<td>Posterior Kidney-tubule necrosis</td>
<td>24/23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42%</td>
</tr>
</tbody>
</table>

<sup>a</sup>Tissue available from 29 of 30 fish from the 5× group.
<sup>b</sup>Tissue available from 18 of 30 fish from the 0× group and 14 of 30 fish from the 5× group.
<sup>c</sup>Tissue available from 24 of 30 fish from the 0× group and 23 of 30 fish from the 5× group.