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The Safety of AQUAFLOR® (50% Florfenicol; Type A Medicated Article) Administered in Feed to Sunshine Bass (Female White Bass *Morone chrysops* × Male Striped Bass *M. saxatilis*)

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Bacterial diseases are a major problem in aquaculture and account for significant losses of fish (Clarke and Scott 1989; Frerichs and Roberts 1989; Bjorndal 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 bacteriocidal properties that is active against a variety of Gram-positive and Gram-negative bacteria (Horsberg et al. 1996).

AQUAFLOR® (Intervet/Schering-Plough Animal Health Corp., Roseland, New Jersey USA) is an aquaculture feed premix containing 50% florfenicol. Worldwide, AQUAFLOR® has been approved in more than 20 countries including Norway, Japan, Chile, Canada, and the U.S., to control mortality in a variety of cultured fishes due to diseases associated with infectious bacterial pathogens. In the U.S., AQUAFLOR® has been approved by the U.S. Food and Drug Administration (FDA) for use to control mortality in (1) catfish due to enteric septicemia associated with *Edwardsiella ictaluri*, (2) freshwater-reared salmonids due to coldwater disease associated with *Flavobacterium psychrophilum*, and (3) freshwater-reared salmonids due to furunculosis associated with *Aeromonas salmonicida* (Bowker et al., in press). For all currently labeled uses in the U.S., the FDA-approved treatment regimen is to administer AQUAFLOR® in feed at 10 mg FFC/kg fish body weight/d for 10 consecutive days.

There is considerable interest within public and private aquaculture in the U.S. with respect to the expanded use of AQUAFLOR® to include therapeutic treatment of freshwater-reared, cold-, cool-, and warmwater finfish at 15 mg FFC/kg fish body weight/d for 10 consecutive days. To obtain such an approval, data must be generated to show that this treatment regimen (i.e., higher dose) is safe to representative target animals. Consequently, we conducted a target animal safety (TAS) study to evaluate the safety of AQUAFLOR® administered in feed to sunshine bass (female white bass Morone chrysops × male striped bass M. saxatilis).

Methods

The study was conducted March 17 – April 8, 2009, at the Harry K. Dupree Stuttgart National Aquaculture Research Center (U.S. Department of Agriculture, Agriculture Research Services, Stuttgart, Arkansas) and consisted of a 3-d acclimation period, 20-d exposure period, and 1-d postexposure period. Test fish (mean ± SD) total length and weight were 11.1 ± 0.42 cm and 13.6 ± 1.58 g, respectively. AQUAFLOR® was administered in feed at 0× (0 mg/kg), 1× (15 mg/kg), 3× (45 mg/kg), or 5× (75 mg/kg) the proposed maximum therapeutic dose of 15 mg FFC/kg fish body weight/d for 2× (20 d) the proposed therapeutic treatment duration of 10 d. Completely randomized design procedures were used to assign each of the four exposures to 3 of 12 test tanks, and to stock 20 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) to analytically verify the 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 test fish were collected from test tanks, euthanized, measured for total length and weight, and necropsied. During necropsy, 10 fish were randomly selected from each tank and tissues collected for histological examination. Of these, two fish/tank were randomly selected for evaluation of 11 tissues (gill, liver, anterior kidney, posterior kidney, brain, heart, muscle, skin, spleen, pyloric intestine, and rectal intestine). The other eight fish/tank were evaluated for four tissues (gill, liver, anterior kidney, and posterior kidney). All tissues were examined for lesions or cellular changes (hereafter referred to as lesions) that might provide evidence of AQUAFLOR®-induced toxicity. Lesions were scored on a six-point ordinal severity scale. Fish in 0× and 5× exposure groups were examined and compared first. If lesions were detected in one or more tissues of the 5×-exposure group that were (a) marked or severe, (b) not observed in the 0× exposure group, and (c) appeared to be AQUAFLOR®-induced, then all relevant tissue(s) were examined in all fish representing the 3× exposure group. If lesions were not detected in the 5× exposure groups, tissues from the 3× exposure groups were not evaluated.

No mortality occurred during the study; therefore statistical analysis of mortality data was not required. Gross necropsy and histopathology findings were described qualitatively. Secondary response variables (mean feed consumption, mean body weight) were analyzed by one-way ANOVA. Treatment differences were considered significant if $P < 0.05$.

Results and Discussion

During the in-life phase of the study, no fish died or exhibited signs of morbidity. Moreover, 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 within 10 sec; therefore, feeding (appetite) behavior was charac-terized as "100% of feed consumed."

At the end of the in-life phase, fish had grown an average of 1.4 cm and 7.3 g, and overall mean total length and weight of test fish were 12.6 ± 0.45 cm and 21.0 ± 2.29 g, respectively. Mean total length ($P = 0.493$) and mean weight ($P = 0.955$) did not differ among exposure groups.

No lesions of any kind were observed in the heart, liver, or rectal intestine tissues; no degeneration was observed in skin tissue; and no inflammation was observed in posterior kidney tissue or spleen tissue in fish examined from the 0× or 5× exposure groups. Lesions not considered to affect overall health of the fish (e.g., mild or moderate) were observed in all other tissues examined (Table 1). Only posterior kidney tissue was examined in fish from the 3× exposure group; however, results were nearly identical to those observed in the 0× and 5× exposure group fish. For example, mild to moderate tubule epithelial degeneration and necrosis was observed in 100% of the 30 fish examined from the 3× exposure group.

Based on histological data as reported above, mortality data, the fact that fish fed aggressively regardless of the FFC concentration in feed offered, and that general fish behavior was considered normal, we concluded that the margin of safety was at least five times greater than the proposed therapeutic treatment concentration of 15 mg FFC/kg BW/d. No fish health and/or histological lesions were detected that might indicate that 75 mg FFC/kg fish body weight/for 20 d was not safe to sunshine bass. In addition, reviewers for FDA's Center for Veterinary Medicine found that the data demonstrated there is an adequate margin of safety for the use of AQUAFLOR® at a dosage of 15 mg FFC/kg fish body weight/d for 10 consecutive days.

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Table 1. Relative number of sunshine bass per exposure condition in which mild to moderate lesions were detected in the 0× and 5× (75 mg FFC/kg fish BW) exposure groups.

Tissue	Sample	Exposure (mg FFC/kg fish BW/d)	
		0	75
Muscle degeneration of fibers	6	33%	67%
Spleen-melanomacrophages	6	100%	83%
Pyloric intestine- epithelial degeneration	6	33%	17%
Pyloric intestine-epithelial necrosis	6	33%	17%
Gill-epithelial lifting	30	97%	100%
Gill-telangectiasis	30	67%	47%
Gill-inflammation	30	93%	83%
Ant. Kidney-melanomacrophages	30	13%	3%
Post. Kidney-tubule epithelium degeneration	30	100%	100%
Post. Kidney-tubule epithelium necrosis	30	97%	100%