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ARTICLE

The Safety of Aquaflor (50% Florfenicol) Administered in Feed to Fingerling Yellow Perch

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Abstract

Aquaflor is an aquaculture feed premix containing 50% florfenicol and is approved for use in more than 50 countries to control mortality in a variety of cultured fishes caused by diseases associated with infectious bacterial pathogens. As part of an effort to expand the current approval in the United States, we conducted a study to evaluate the safety of Aquaflor to Yellow Perch *Perca flavescens* when 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 treatment dose of 15 mg florfenicol·kg fish⁻¹·d⁻¹ for 20 consecutive days, 2 × the proposed therapeutic treatment duration of 10 consecutive days. Fingerling Yellow Perch (7.8 ± 1.6 cm and 5.0 ± 3.4 g; mean ± SD) were stocked into flow-through test tanks at 15 fish per tank, and treatments were randomly assigned to tanks in triplicate. At the end of the 20-d exposure period, mean cumulative mortality in the 0 × and 3 × groups (6.7% for both) was greater than that in the 1 × and 5 × groups (2.2% and 0.0%, respectively); however, differences among the groups were not significant ($P = 0.3741$). Throughout the study, general fish behavior was characterized as normal, and fish consumed virtually all feed offered. Fish health and histology assessments revealed no signs or lesions associated with toxicity of florfenicol. In conclusion, there is an adequate margin of safety associated with administering Aquaflor-medicated feed to fingerling Yellow Perch at the proposed therapeutic treatment regimen of 15 mg florfenicol·kg fish⁻¹·d⁻¹ for 10 d.

Bacterial disease outbreaks can cause significant losses of captive-reared fish (Clarke and Scott 1989; Frerichs and Roberts 1989; Bjørndal 1990). Often, such outbreaks can be prevented or minimized by, for example, disinfecting and oxygenating incoming water, implementing appropriate nutrition, rearing, and health management practices, and regularly disinfecting equipment (Piper et al. 1982; Post 1987; Jeney and Jeney 1995; Wedemeyer 2001). In addition, there are ongoing efforts to develop efficacious vaccines (e.g., Bebak and Wagner 2012; Burbank et al. 2012; Shoemaker et al. 2012), but until then antimicrobials are needed.

Several antimicrobials, including three oral antibiotics, are approved by the U.S. Food and Drug Administration (FDA) for use to control mortality in captive-reared fish associated with a variety of diseases (Matthews et al. 2013). However, their use is restricted to specific disease indications and treatment regimens (FDA 2012). These restrictions limit the ability of fish culturists

to control bacterial disease outbreaks, and thus there is a need for new antimicrobials or expanded uses of the antimicrobials currently approved in the United States.

Florfenicol {[R-(R*, S*)]-2, 2-dichloro-N-[1-(fluoromethyl)-2-hydroxy-2-[4-(methylsulfonyl) phenyl] ethyl-acet amide]} 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). Because of its high potency and because it is not used in human medicine, florfenicol has become an important veterinary therapeutic drug, especially when administered in feed. Florfenicol can control mortality caused by furunculosis in Atlantic Salmon *Salmo salar* (Nordmo et al. 1994; Samuelson et al. 1998), pseudotuberculosis in Yellowtail (buri) *Seriola quinqueradiata* (Yasunaga and Yasumoto 1988), columnaris in Largemouth Bass *Micropterus salmoides*, and Bluegill *Lepomis macrochirus* (Matthews et al. 2013), and streptococcal disease

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in sunshine bass (female White Bass *Morone chrysops* × male Striped Bass *M. saxatilis*) (Darwish 2007; Bowker et al. 2010) and Nile Tilapia *Oreochromis niloticus* (Gaunt et al. 2010). In addition, florfenicol caused no mortalities, changes in fish growth, or clinical changes in Channel Catfish *Ictalurus punctatus* (Gaikowski et al. 2003) when fed for 20 d at doses up to 34.9 mg florfenicol·kg fish⁻¹·d⁻¹ or in sunshine bass (Straus et al. 2012) when fed for 20 d at doses up to 75 mg florfenicol·kg fish⁻¹·d⁻¹.

Aquaflor (Merck Animal Health, Summit, New Jersey) is an aquaculture feed premix containing 50% florfenicol that is approved for use in more than 50 countries to control mortality in a variety of cultured fishes caused by diseases associated with infectious bacterial pathogens. In the United States, the FDA has approved its use to control mortality in (1) freshwater-reared salmonids affected by furunculosis disease associated with *Aeromonas salmonicida* and coldwater disease associated with *Flavobacterium psychrophilum* (10 mg florfenicol·kg fish⁻¹·d⁻¹ for 10 d), (2) catfish affected by enteric septicemia associated with *Edwardsiella ictaluri* (10–15 mg florfenicol·kg fish⁻¹·d⁻¹ for 10 d), (3) freshwater-reared warmwater finfish affected by streptococcal septicemia associated with *Streptococcus iniae* (10–15 mg florfenicol·kg fish⁻¹·d⁻¹ for 10 d), and (4) freshwater-reared finfish affected by columnaris disease associated with *F. columnare* (10–15 mg florfenicol·kg fish⁻¹·d⁻¹ for 10 d for warmwater finfish and 10 mg florfenicol·kg fish⁻¹·d⁻¹ for 10 d for all other finfish).

The U.S. aquaculture community would like to expand the Aquaflor label such that all freshwater finfishes can be treated at up to 15 mg·kg fish⁻¹·d⁻¹ for 10 d to control mortality caused by a variety of diseases. To obtain such an approval, data must be generated to show that this treatment regimen 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 a representative coolwater finfish, Yellow Perch *Perca flavescens*, 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 florfenicol·kg fish⁻¹·d⁻¹ for 20 consecutive days, which is 2 × the proposed therapeutic treatment duration of 10 consecutive days. This exposure scheme allowed us to establish a margin of safety, which is herein defined as the dosage at which chronic or acute toxicity becomes evident. A water temperature of 23°C was selected as the test temperature because it was considered the upper end of the range at which oral antibiotic treatments (e.g., Terramycin 200 for Fish and Aquaflor) were administered to coolwater finfish under authorization of the U.S. Fish and Wildlife Service (USFWS) Investigational New Animal Drug (INAD) exemption (B. Johnson, USFWS, personal communication).

METHODS

Testing facility, test fish, and test article.—The Yellow Perch used in the study were approximately 10 months of age and

were hatched from wild-collected eggs incubated at the USFWS Bozeman Fish Technology Center (BFTC; Bozeman, Montana) in April 2009. After hatching, the resultant fry were reared under standard hatchery conditions by BFTC staff. Sex of fish was neither determined nor considered; however, it was assumed that males and females were present in roughly equal proportions. The reference population fish were held in one fiberglass circular tank (water volume, 756 L) with a water inflow of 30 L/min (single-pass, flow-through water), which produced a water exchange rate of 2.4 exchanges/h. One week before exposure fish were moved to test tanks to begin the acclimation period; 30 fish were collected from the reference population and measured for TL (7.8 ± 1.6 cm, mean \pm SD) and weight (5.0 ± 3.4 g). During the 6-d acclimation period, fish were fed non-medicated Silver Cup No. 3 Salmon/Trout Crumbles (Nelson and Sons, Murray, Utah) at 1% body weight (BW)/d via belt feeders (Zeigler Brothers, Gardners, Pennsylvania).

Aquaflor premix was provided by Merck Animal Health. Control and medicated feeds were prepared at the BFTC in a Marion model SPS-1224 Mixer (Marion Mixers, Marion, Iowa). Medicated feeds were prepared by top-coating the commercial feed with appropriate amounts of Aquaflor and fish oil (0.5% w:w) to administer doses of 0, 15, 45, and 75 mg florfenicol·kg fish⁻¹·d⁻¹ when fish were fed at 1% BW/d (representing 0 ×, 1 ×, 3 ×, and 5 × the proposed dose). Control feed was top-coated with fish oil only. Immediately after test feeds were prepared, one sample was collected from each of the top, middle, and bottom ($n = 3$ samples per batch total) to verify homogeneity of florfenicol in each batch of feed. On study days 1, 7, 14, and 20, one sample of feed was collected from each batch to verify drug stability. Control feed samples were collected to verify that it was not contaminated with florfenicol. Florfenicol concentrations were determined via HPLC by Eurofins Lancaster Laboratories, Portage, Michigan. We tested for no other antibiotics or contaminants in the feed.

Experimental design and procedures.—A completely randomized design procedure was used to (1) assign treatments to tanks ($n = 3$) and (2) stock 15 fish into each test tank. Hence, 12 test tanks and 180 test fish were used in the study. Test tanks were 19-L plastic buckets (water volume, 17.4 L). Three additional nonstudy tanks were also stocked with 15 fish per tank so that we could monitor growth and make weekly adjustments to the amounts of feed administered to test tanks. Feed amounts were also adjusted daily to account for mortality. Water flow (single-pass, flow-through water) to each test tank was 3.8 L/min, which produced a water exchange rate of 13.1 exchanges/h.

Fish were observed daily for mortality, general behavior, and feeding behavior. During the acclimation and exposure periods, fish were fed twice daily. Feeding behavior was assessed once daily during the acclimation period and during each of the two feeding events during the exposure period. The following five-point scale was used to score feeding behavior: 0 = approximately no feed was consumed, and fish show no interest in feeding; 1 = approximately 25% of the feed was consumed, and

fish showed little interest in feed; 2 = approximately 50% of the feed was consumed, and fish showed a moderate interest in feeding; 3 = approximately 75% of the feed was consumed, and fish showed moderate interest in feeding; and 4 = approximately 100% of the feed was consumed, and fish fed aggressively.

Water temperature ($23.3 \pm 0.4^\circ\text{C}$) and dissolved oxygen (DO) concentration (6.0 ± 0.2 mg/L) were measured once daily in each tank with a YSI model 550 dissolved oxygen and temperature meter (YSI, Yellow Springs, Ohio). Water alkalinity (276 ± 23 mg/L as CaCO_3) and hardness (168 ± 4 mg/L as CaCO_3) were measured with Hach reagents and equipment (Hach, Loveland, Colorado), and pH (7.9 ± 0.25) was measured with a YSI EcoSense pH pen four times during the study (once during the acclimation period and three times during the exposure period). Overhead lights were on for 9–10 h/d.

Fish health and histology.—Before the study started, 20 reference fish were collected and necropsied to characterize baseline fish health and histopathology associated with routine fish culture and handling procedures. After collection, fish were sedated in an ice–water slurry and then euthanized by spinal severance. Each necropsy consisted of visual examination of skin, gills, and internal organs and tissues for gross lesions or abnormalities. Ten of the 20 fish were randomly selected with a completely randomized design procedure, fixed in Davidson's fixative solution, stored in 70% ethyl alcohol, and later processed for histology.

At the end of the in-life phase, all live fish in all test tanks were collected, measured for TL and weight, sedated in an ice–water slurry, and then euthanized by spinal severance and necropsied. If a test tank held 10 or more live fish, then 10 fish were randomly selected with a completely randomized design procedure and processed for histology. If a test tank held fewer than 10 fish, then all fish were processed for histology. All but two fish that died during the study were too decomposed before being collected to be necropsied or used for histology.

Selected tissues were dissected and then processed in Fisher Omnissette tissue cassettes (Fisher Scientific, Pittsburgh, Pennsylvania). The tissues were infiltrated with paraffin by means of a Leica ASP 300 Advanced Smart Processor (Leica Microsystems, Nussloch, Germany), and the paraffin-infiltrated tissue samples were embedded in paraffin blocks by means of a Leica EG 1160 tissue embedding system. Tissues in selected paraffin blocks were sectioned with a Leica RM2255 rotary microtome. The 5- μm tissue sections were mounted on glass microscope slides, stained with hematoxylin and eosin using a Leica AutoStainer XL, and evaluated microscopically. As per FDA Center for Veterinary Medicine (CVM) guidelines, gill, liver, anterior kidney, posterior kidney, brain, heart, muscle, skin, spleen, pyloric intestine, and rectal intestine tissues were evaluated from two of the fish randomly selected from each tank for histology. Histological evaluations of the remaining fish were only for gill, liver, anterior kidney, and posterior kidney.

Tissues were submitted for histopathologic evaluation of florfenicol-induced toxicity. Tissues were scored under a six-

point ordinal severity scale: 0 = no change; 1 = normal (<5% of the tissue affected); 2 = mild (5–15% of the tissue affected); 3 = moderate (15–25% of the tissue affected); 4 = marked (25–50% of the tissue affected); or 5 = severe (>50% of the tissue affected). Only scores of 4 or 5 were considered severe enough to have adversely affected fish health. As per CVM guidance, to minimize the number of histological images needing to be scored, images from the 0 \times and 5 \times treatment groups were evaluated first. If significant differences were not detected between these two groups, then we were not required to evaluate differences between the 0 \times and 3 \times exposure groups or between the 0 \times and 1 \times exposure groups.

Statistical analysis.—Percent cumulative mortality and histology data (5 \times exposure group versus 0 \times exposure group only) were analyzed separately with SAS (2008) version 9.2, Proc Glimmix-based models (logit link). In both analyses, the test tank was the experimental unit. Inadvertent mortality occurred in one of the 3 \times treatment tanks on exposure day 4 when the water supply line was inadvertently disconnected for 24 h. Hence, data from this tank were excluded from analysis and there were only two replicates for this treatment group. Before the histology data were analyzed, lesions scored as 0, 1, 2, or 3 were coded as "0" (not biologically important), and lesions scored as 4 or 5 were coded as "1" (biologically important). Treatment effect on mortality and histology was tested at $\alpha = 0.10$ (two-sided). Mean length and weight of fish at the end of the study were analyzed with a one-way ANOVA (SYSTAT 2012). The treatment effect on fish size was tested at the significance level of $\alpha = 0.05$. Feeding behavior was summarized by adding the feeding score across replicates in each exposure group for each feeding event (e.g., day 1, first feeding) and plotting the results in a mosaic plot using Microsoft Office Excel software, 2010 version (Bowker et al. 2013).

RESULTS

Exposures

At the end of the 20-d exposure period, mean cumulative mortality in the 0 \times and 3 \times groups (6.7% for both) was greater than that in the 1 \times and 5 \times groups (2.2% and 0.0%, respectively). However, differences among groups were not significant ($P = 0.3741$). Throughout the study, general fish behavior was characterized as normal. Fish consumed all the feed that they were going to consume within 10–20 s of it being offered. Fish in the 1 \times , 3 \times , and 5 \times groups appeared to consume approximately 100% of the feed offered in all but three instances (Table 1). On study day 10, fish in two of the 1 \times treatment tanks appeared to consume approximately 75% of the feed offered, and on the last day of the study, fish in one of the 1 \times tanks appeared to consume approximately 75% of the feed offered. Fish in the 0 \times and nontrial groups appeared to consume less feed than fish in tanks offered medicated feed.

At the end of the 20-d exposure period, no significant differences were detected in mean length ($P = 0.642$) or mean weight

TABLE 1. Sum of feeding scores (based on five-point ordinal scale) across the three replicate tanks per exposure group of Yellow Perch during the first and second feeding periods on each study day. Areas with no shading indicate that fish in each of the replicate tanks in an exposure group appeared to consume approximately 100% of the feed offered. Sequentially darker shades of gray indicate less feed consumed. Note that in the 3 × exposure group, there were only two replicates.

Study day	Exposure group									
	0 ×		1 ×		3 ×		5 ×		Nontrial	
	First	Second	First	Second	First	Second	First	Second	First	Second
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										

Summary of feeding scores:
 For 0 ×, 1 ×, 5 ×, and nontrial groups
 White area = 12
 Very light gray area = 11
 Dark gray area = 10
 For 3 × group
 White area = 8

($P = 0.750$) in fish from among the four exposure groups. Test fish had grown an average of 0.8 cm and 2.3 g, and mean TL and weight ($n = 164$ fish in 12 test tanks) were 8.6 cm (± 1.8 cm) and 7.3 g (± 4.8 g). Water temperature, DO concentration, water hardness, alkalinity, and pH were within acceptable ranges for Yellow Perch culture (Hart et al. 2006).

Mean measured florfenicol concentration in the 1 ×, 3 ×, and 5 × feed samples indicated that fish were treated with 15.3 ± 0.5 (+3% from the target dose), 45.6 ± 2.6 (+1% from target), and 77.7 ± 2.1 (+4% from target) mg florfenicol·kg fish⁻¹·d⁻¹, respectively, at the beginning of the experiment. No florfenicol was detected in the 0 × feed samples.

Fish Health and Histology

Reference population.—External and internal tissues appeared normal, although skeletal or opercular deformities were noted in 95% of the 20 fish sampled. In the 10 fish evaluated for

histology, no lesions were observed in the skin, muscle, or pyloric intestine tissues. Lesions observed in other tissues were (1) mild to marked gill epithelial separation, mild to moderate proliferation of gill epithelium at the base of lamellae, and mild to moderate telangiectasia (aneurysms in lamellar blood vessels); (2) mild to moderate liver glycogen vacuolation, indicating the amount of carbohydrate storage in fish at time of sampling; and (3) presence of mild, moderate, or marked nephrocalcinosis and cellular changes (e.g., degeneration and necrosis of tubule epithelium) associated with this condition. The observed lesions did not appear sufficient to adversely affect fish health.

0 × exposure.—All fish ($n = 42$) appeared healthy at the end of the study, although skeletal or opercular deformities were noted in 57% of the fish. Lesions observed in the reference population fish were also observed in the 0 × fish. In addition, mild or moderate (Table 2), or marked (Table 3), melanomacrophage centers were observed in the anterior kidney of 18 fish and spleen

TABLE 2. Percentage of fingerling Yellow Perch treated with $0 \times$ or $5 \times$ of the standard dose of 15 mg florfenicol·kg fish⁻¹·d⁻¹ for 20 d and evaluated histologically where lesions were observed and scored as mild or moderate. These lesions were not considered biologically important. Where two numbers are listed, the first number refers to the number of $0 \times$ fish evaluated and the second number represents the number of $5 \times$ treatment fish evaluated.

Tissue lesion	Number of samples	Treatment	
		$0 \times$	$5 \times$
Spleen – melanomacrophage centers	6	50%	0%
Heart – inflammation	6	17%	0
Liver – degeneration	30	7%	17%
Liver – melanomacrophage centers	30	0%	3%
Liver – vacuolation	30	80%	83%
Gill – epithelial lifting	30	80%	79%
Gill – proliferation	30/29	53%	35%
Gill – aneurysms	30/29	7%	10%
Anterior kidney – melanomacrophage centers	18/14	89%	78%
Posterior kidney – proliferation	24/23	12%	4%
Posterior kidney – degeneration of tubule epithelium	24/23	63%	69%
Posterior kidney – necrosis of tubule epithelium	24/23	96%	61%
Posterior kidney – inflammation	24/23	12%	13%

TABLE 3. Percentage of fingerling Yellow Perch treated with $0 \times$ or $5 \times$ of the standard dose of 15 mg florfenicol·kg fish⁻¹·d⁻¹ for 20 d and evaluated histologically where lesions were observed and scored as marked. These lesions were considered biologically important. Note that no lesions were observed that were scored as “severe.” Where two sample numbers are listed, the first number refers to the number of $0 \times$ fish evaluated and the second number represents the number of $5 \times$ treatment fish evaluated.

Tissue lesion	Number of samples	Treatment	
		$0 \times$	$5 \times$
Spleen – melanomacrophage centers	6	17%	0%
Liver – degeneration	30	7.5%	5%
Liver – glycogen vacuolation	30	3%	0%
Gill – epithelial lifting	30/29	20%	14%
Anterior kidney – melanomacrophage centers	18/14	11%	21%
Anterior kidney – inflammation	18/14	6%	0%
Posterior kidney – degeneration of tubule epithelium	24/23	0%	4%
Posterior kidney – necrosis of tubules	24/23	0%	4%

of four fish, and mild inflammation of posterior kidney was observed in one fish (Tables 2, 3). No lesions considered severe enough to affect fish health were observed in other tissues.

1 × exposure.—All fish ($n = 44$) appeared healthy at the end of the study, although skeletal or opercular deformities were noted in 69% of the fish. No tissues from fish in this exposure group were examined histologically.

3 × exposure.—All fish ($n = 33$) appeared healthy at the end of the study, although skeletal or opercular deformities were noted in 64% of the fish. No tissues from fish in this exposure group were examined histologically.

5 × exposure.—All fish ($n = 45$) appeared healthy at the end of the study, although skeletal or opercular deformities were noted in 71% of the fish. Histological examinations showed that changes observed in the $5 \times$ exposure group fish were similar to those described for $0 \times$ exposure group fish (Tables 2, 3).

Observed lesions that were marked in the $0 \times$ and $5 \times$ exposure groups included (1) liver degeneration, (2) anterior kidney melanomacrophage centers, and (3) gill epithelial lifting (Table 3). Marked anterior kidney and spleen melanomacrophage centers, liver glycogen vacuolation, and anterior kidney inflammation were observed in fish from the $0 \times$ group. Each of these lesions was observed in a different fish. Marked posterior kidney degeneration and necrosis of tubules was observed in one fish from the $5 \times$ exposure group. No severe lesions were detected. Differences between prevalence of marked lesions in the $0 \times$ and $5 \times$ exposure groups were not significant (P -values > 0.1).

DISCUSSION

Our results indicated that the margin of safety for florfenicol administered in feed to fingerling Yellow Perch extends to at least 75 mg florfenicol·kg fish⁻¹·d⁻¹ for 20 d. This statement was based on the facts that there was no mortality among fish in this group and no dose–response trend in mortality was evident. Regardless of treatment there was no difference between groups in medicated feed consumption, behavior, or fish size. No fish health, lesions, or histological changes that indicated the highest florfenicol dosage was not safe to Yellow Perch were detected. The relative degree of skeletal or opercular deformities was higher than anticipated, and we speculate that it might have been attributed to feeding fish a commercial salmon–trout diet.

Our results are consistent with those found in similarly conducted studies to evaluate the safety of Aquaflor administered in feed to other freshwater-reared finfishes. Straus et al. (2012) observed no mortality, gross lesions, or microscopic lesions when sunshine bass were fed florfenicol doses ranging from 0 to 75 mg florfenicol·kg fish⁻¹·d⁻¹ for 20 d. In addition, fish consumed 100% of feed offered, often breaking the surface of the water while feeding. Similar results were reported in which fingerling Rainbow Trout *Oncorhynchus mykiss* were fed florfenicol doses ranging from 10 to 50 mg florfenicol·kg fish⁻¹·d⁻¹ for 20 d (FDA 2007). Rainbow Trout in each group consumed

>99% of the feed that was offered, and there was no mortality or clinically observable changes detected in fish behavior among the treated fish relative to the controls. In addition, no gross abnormalities of the internal organs were observed on necropsy and no morphological differences were detected during the histological examination. Similarly, Inglis et al. (1991) reported that no histological changes were observed in the kidneys of Atlantic Salmon parr when exposed to 100 mg florfenicol·kg fish⁻¹·d⁻¹ for 10 d. Gaunt et al. (2003) reported no mortality, microscopic lesions, histological changes, palatability issues, or adverse behavior among 5-month-old Channel Catfish associated with florfenicol doses ranging from 10 to 100 mg·kg fish⁻¹·d⁻¹ when administered for 10 d. In another study with Channel Catfish fed florfenicol at doses ranging from 10 to 50 mg florfenicol·kg fish⁻¹·d⁻¹ for 20 d, Gaikowski et al. (2003) reported no mortality, but did observe signs of inappetence and histological changes attributed to prolonged exposure to florfenicol. Those authors reported an increase in the amount of uneaten feed among fish exposed to 30 or 50 mg florfenicol·kg fish⁻¹·d⁻¹ and a “minimal to mild decrease” in hematopoietic–lymphopoietic (H&L) tissue in the anterior kidney, posterior kidney, and spleen. However, because of insufficient data, they were unable to determine whether the decrease in H&L tissue was an adverse effect. In a study with tilapia *Oreochromis* sp. fed florfenicol-medicated feed at the same dosages as administered in the our study, Gaikowski et al. (2013), reported a total of three mortalities that were considered incidental and evidence of inappetence among the 45- and 75-mg florfenicol·kg fish⁻¹·d⁻¹ groups during exposure days 11–19. Histopathological findings among the tilapia in the Gaikowski et al. (2013) study were more extensive than previously reported for other fish species and included lesions observed in gills, liver, anterior kidney, and posterior kidney. The authors concluded that these changes were likely to be of minimal clinical importance given the lack of mortality, but that feeding florfenicol-medicated feeds at 45 and 75 mg florfenicol·kg fish⁻¹·d⁻¹ for an extended period (> 10 d) will cause significantly decreased feed consumption and fish growth.

Although deleterious effects of Aquaflor treatment are possible, e.g., in untested species or sensitive life stages, we speculate that such events would be rare. As noted previously, several target animal safety studies have shown little to no effect of exposing fish to florfenicol concentrations well beyond the intended therapeutic dose of 15 mg florfenicol·kg fish⁻¹·d⁻¹ for 10 d. Further, over 100 million fish have been treated with Aquaflor since 2001 without adverse effects under the auspices of the USFWS National INAD Program (B. Johnson, USFWS, personal communication) and many more have been treated in other countries with existing approvals for this product. Accordingly, we conclude that Aquaflor-medicated feed administered at 15 mg florfenicol·kg BW⁻¹·d⁻¹ for 10 d is safe for use on Yellow Perch and is likely to be safe for use on all freshwater-reared finfishes.

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