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ARTICLE

The Safety of SLICE (0.2% Emamectin Benzoate) Administered in Feed to Fingerling Rainbow Trout

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Abstract
SLICE (0.2% emamectin benzoate [EB]) is an in-feed treatment that has been shown to be effective and safe for controlling infestations of several ectoparasitic crustacean copepods and branchiurans in a variety of seawater- and freshwater-reared fishes. Although the safety of EB (in a pre-SLICE formulation) for use with seawater-reared Rainbow Trout *Oncorhynchus mykiss* has been demonstrated, the safety of SLICE for freshwater-reared Rainbow Trout has not. Consequently, we conducted a trial to evaluate the safety of SLICE for freshwater-reared Rainbow Trout when administered in feed at a dose of 0 (0×), 50 (1 × the maximum proposed therapeutic dose [1×]), 100 (2×), or 150 (3×) μg of EB·kg of fish body weight $(BW)^{-1}\cdot d^{-1}$ for 14 d (2 × the proposed 7-d treatment duration). Medicated feed was prepared by top-coating commercially available feed with SLICE. Rainbow Trout fingerlings (mean TL ± SD = 7.4 ± 0.7 cm; mean weight ± SD = 4.4 ± 1.2 g) were stocked into 57-L flow-through tanks at 20 fish/tank. Diets were randomly assigned to four replicate tanks per treatment; fish in four additional, nontrial tanks were fed control diets and were weighed weekly to calculate the proper feeding quantities. Throughout the trial, water quality was maintained within ranges suitable for Rainbow Trout culture, fish were fed the assigned feeds at 4% BW/d divided equally between three feedings, and fish behavior was characterized as normal. Fish in the 0× , 1× , and 2× exposure groups consumed all of the offered feed at least 92% of the time, whereas fish in the 3× exposure group consumed all of the offered feed 75% of the time. No fish died, and gross and microscopic fish health evaluations revealed no chronic toxicity patterns. Based on these results, we conclude that there is an adequate margin of safety associated with administering SLICE-medicated feed to fingerling Rainbow Trout at the proposed therapeutic treatment regimen of 50 μg EB·kg fish $BW^{-1}\cdot d^{-1}$ for 7 d.

SLICE (0.2% emamectin benzoate [EB]) is an in-feed treatment that was originally developed to control infestations of sea lice (e.g., *Lepeophtheirus salmonis*, *Caligus elongatus*, and *Caligus rogercresseyi*) in seawater-reared salmon and trout (MSD Animal Health 2012). The active component of SLICE is EB. Emamectin is an avermectin (Lasota and Dybas 1991) that was developed initially as an insecticide for use with food crops; EB is derived synthetically from avermectins produced by fermentation of the soil bacterium *Streptomyces avermitilis* (MSD Animal Health 2012). Like other avermectins, emamectin interferes with neural transmissions by binding or blocking gamma aminobutyric acid (GABA) receptors and glutamate-gated chloride channels, causing neuromuscular paralysis (CAHS 2007). Vertebrates are generally unaffected by avermectins because of the absence of glutamate-gated chloride channels, reduced receptor affinities, and protection of GABA receptors by the blood–brain barrier; however, arthropods, platyhelminths, and nematodes do not possess these protective features, rendering these and other invertebrates vulnerable to the effects of avermectins, including EB (Lasota and Dybas 1991; Jansson et al. 1997). When EB is fed to fish, it is absorbed from the gut and distributed throughout the body of the fish via the circulatory system (Sevatdal et al. 2005). When sea lice or other parasitic crustaceans feed on the tissues or fluids of the host fish, EB is absorbed by the parasites, resulting in their paralysis and death (CAHS 2007). Emamectin benzoate has been shown to

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be an effective means for controlling and preventing sea louse infestations in Atlantic Salmon *Salmo salar* (e.g., Stone et al. 1999, 2000b, 2000c, 2002; Armstrong et al. 2000). Further, fish slowly metabolize EB, resulting in an extended period of protection from parasites long after treatment has been completed (Stone et al. 2000a). Protection can extend for up to 10 weeks posttreatment (Horsberg 2012), thus making SLICE a viable candidate for long-term parasite control in cultured fish. Currently, SLICE is approved for the control of sea lice in salmonid species within the United Kingdom, Ireland, Finland, Spain, Portugal, Iceland, Faroe Islands, Norway, Chile, and Canada.

Recently, SLICE has been shown to be effective for controlling infestations of freshwater ectoparasitic crustacean copepods and branchiurans in several freshwater-reared fishes. Examples include the control of *Salmincola edwardsii* in Brook Trout *Salvelinus fontinalis* (Duston and Cusack 2002), *Salmincola californiensis* in Rainbow Trout *Oncorhynchus mykiss* (Bowker et al. 2012; Gunn et al. 2012), *Argulus coregoni* in Rainbow Trout (Hakalahti et al. 2004), *Argulus foliaceus* in Common Carp *Cyprinus carpio domestica* (Braun et al. 2008), and *Argulus* sp. in Koi (ornamental variant of Common Carp) and Goldfish *Carassius auratus* (Hanson et al. 2011). The observed efficacy of SLICE against these freshwater parasites included, in most cases, the "extended period of protection" that was previously documented for the use of SLICE against sea lice (Stone et al. 2000a, 2002; Skilbrei at al. 2008; Horsberg 2012). Moreover, SLICE has been tested for controlling infections of the endoparasitic nematode *Anguillicoloides crassus* in freshwater-held American Eels *Anguilla rostrata* (Larrat et al. 2012), and EB has been tested for controlling infestations of two ectoparasitic crustacean copepods (*Lernanthropus kroyeri* in seawater-reared European Bass *Morone* [*Dicentrarchus*] *labrax*: Tokşen et al. 2006; *Caligus curtus* in seawater-reared Atlantic Cod *Gadus morhua*: Hamre et al. 2011) and for its in vitro effect against the endoparasitic monogenean *Acolpenteron ureteroecetes* collected from freshwater-reared Largemouth Bass *Micropterus salmoides* (Reimschuessel et al. 2011).

To obtain Food and Drug Administration (FDA) approval of SLICE for use on fish in the United States, data must be generated to demonstrate that the standard treatment regimen (50 μg of EB·kg of fish body weight $[BW]^{-1}\cdot d^{-1}$ administered for 7 consecutive days) is not only effective but also safe for application to the representative target animals. Several studies have demonstrated the safety of SLICE for seawater-reared Atlantic Salmon, Rainbow Trout, and Brown Trout *Salmo trutta* (Roy et. al. 2000; Stone et al. 2000a, 2002); one study has evaluated the safety of SLICE for use with freshwater-reared Common Carp (Braun et al. 2008), and a preliminary study has investigated the safety of SLICE for freshwater-held American Eels (Larrat et al. 2012). Nevertheless, additional data are needed to demonstrate the safety of SLICE for use with other freshwater-reared fishes and to establish an associated margin of safety. Accordingly, we conducted a trial to evaluate the safety of SLICE when administered in feed to freshwater-reared Rainbow Trout at 50 (1× the maximum proposed therapeutic dose [1×]), 100 (2×), or 150 (3×) μg EB·kg fish $BW^{-1}\cdot d^{-1}$ for 14 d (2 × the proposed 7-d treatment duration).

METHODS

Experimental design and procedures.—Test fish used in the trial were Rainbow Trout (~4–5 months in age) that were spawned from domestic broodstock held at the U.S. Fish and Wildlife Service (USFWS) Ennis National Fish Hatchery (Ennis, Montana); the fish were reared from the eyed egg stage at the USFWS Bozeman Fish Technology Center (BFTC; Bozeman, Montana). After hatching, the resultant fry were reared by BFTC staff via standard hatchery procedures. Sex of the fish was neither determined nor considered; however, it was assumed that males and females were present in roughly equal proportions and were sexually immature. Fish were ultimately held in two fiberglass circular tanks (86 cm in diameter × 58 cm high; ~200 fish/tank) with a water depth of 51 cm (water volume = 297 L/tank). Water inflow to each tank was 15 L/min, which produced a water exchange rate of 3.0 exchanges/h. As per our FDA-approved research protocol (Bowker and Erdahl 2010), 30 fish were randomly collected from the reference population tanks (15 fish/tank) 1 week before the fish were moved to test tanks to begin the acclimation period. The mean ± SD weight of the initial sample of fish was 4.4 ± 1.2 g; there was no significant difference in mean weight between fish from the two reference tanks (*t*-test: $t = 0.248$, df = 28, $P = 0.806$). Mean weight was determined so that feed amounts could be accurately administered. During the holding period, fish were fed nonmedicated Silver Cup 1.0-mm pellets (Skretting/Nelson and Sons, Inc., Murray, Utah) at 4% BW/d delivered with a belt feeder (Ziegler Brothers, Inc., Gardners, Pennsylvania).

The trial was masked, and only one nonmasked trial participant knew the treatment assignments of test tanks. Completely randomized design procedures were used to assign each of the four EB exposure treatments (0, 50, 100, and 150 μg EB·kg fish $BW^{-1}\cdot d^{-1}$) to 4 of the 16 test tanks and to stock 20 fish into each test tank (i.e., 10 fish from each reference population tank). Test tanks were circular and made of fiberglass (46 cm in diameter × 46 cm deep), with a water depth of 35 cm containing 57 L of flow-through well water. Four additional, nontrial tanks were similarly stocked for the purpose of determining proper feeding rates as described below. Water inflow to each test tank was set to approximately 3.8 L/min, which produced a water exchange rate of 3.7 exchanges/h. Fish were allowed to acclimate to the test tanks for 6 d.

Fish were observed daily for mortality, general behavior, and feeding behavior. Criteria for assessing general fish behavior included position in water column (e.g., crowding near the inlet or outlet pipe; at the water surface), gasping for air, flashing, hyperactivity or lethargy, loss of equilibrium, abnormal pigmentation, and any other unusual behaviors or signs. During the acclimation and exposure periods, fish were fed three times daily at

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4% BW/d divided equally between the three feedings. Feeding behavior was assessed once daily during the acclimation period and at each of the three daily feeding events during the exposure period. Feeding behavior was scored by using a five-point scale as follows: 0 = approximately no feed was consumed, and the fish showed no interest in feeding; 1 = approximately 25% of the feed was consumed, and the fish showed little interest in feeding; 2 = approximately 50% of the feed was consumed, and the fish showed a moderate interest in feeding; 3 = approximately 75% of the feed was consumed, and the fish showed moderate interest in feeding; and 4 = approximately 100% of the feed was consumed, and the fish fed aggressively. Daily rations for fish in each test tank were weighed no more than 2 d in advance into separate containers and were stored at ambient temperature. Fish in the nontrial tanks were weighed on treatment days 1 and 8 before feed was offered, and the amount of feed used for all treated tanks was determined based on these weights.

Water temperature (mean = 15.0°C; range = 14.9–15.2°C) and dissolved oxygen (DO) concentration (mean = 7.0 mg/L; range = 6.5–8.0 mg/L) were measured once daily in each tank with a YSI Model 550A Dissolved Oxygen and Temperature Meter (YSI Environmental, Yellow Springs, Ohio). Water hardness (240 ± 20 mg/L as $CaCO_3$ [mean ± SD]) and alkalinity (160 ± 7 mg/L as $CaCO_3$) were measured with Hach reagents and equipment (Hach, Loveland, Colorado), and pH (8.0 ± 0.4) was measured with a YSI EcoSense pH Pen three times during the trial (once during the acclimation period and twice during the exposure period). Lighting consisted of overhead lights turned on for approximately 9–10 h daily (i.e., 0700–1700 hours).

Medicated and control feeds.—The SLICE premix was provided by Merck Animal Health Corporation (Summit, New Jersey). Control and medicated feeds were prepared at the BFTC in a Marion Mixer (Model SPS-1244; Marion Mixers, Inc., Marion, Iowa). Medicated feeds were prepared by top-coating commercial feed with appropriate amounts of SLICE and menhaden fish oil (0.5% weight per weight) to administer target doses of 0, 50, 100, and 150 μg EB·kg fish $BW^{-1}\cdot d^{-1}$ when fed at 4% BW/d. Control feed was top-dressed with menhaden fish oil only. After each batch of medicated feed was prepared, samples of feed ($n = 5$) were collected to confirm the EB concentration as follows: (1) three samples were collected from each batch immediately after medicated feed preparation (samples collected from top, middle, and bottom of the feed container); (2) one sample was collected from each batch (at the top) on the first day of the treatment period; and (3) one sample was collected from each batch (at the top) on the last day of the treatment period. Control feed samples ($n = 3$) were also collected (from the top, middle, and bottom of the container) to verify that control feed was not contaminated with EB. Concentrations of EB in feed samples were determined via high-performance liquid chromatography by Eurofins AvTech Laboratories (Portage, Michigan).

Fish health and histology.—Before the trial started, reference fish ($n = 30$) were randomly collected and used to characterize baseline fish health and histopathology associated with routine fish culture and handling procedures. A gross necropsy was performed on all 30 fish. Ten of the 30 fish were randomly selected, fixed in Davidson's fixative solution, and subsequently processed for histological evaluation.

At the end of the trial (1 d posttreatment), the 20 fish in each test tank (320 fish total) were collected, measured for TL and weight, and euthanized by spinal severance. To assess general fish health, standard necropsy procedures were used to examine external and internal tissues for gross pathologies of all fish. Each fish health evaluation comprised visual examination of (1) skin for discoloration and the presence and severity of dermal lesions, (2) gills for pallor, and (3) internal tissues (liver, spleen, and kidney) for signs of gross lesions or abnormalities. While fish were being necropsied, 10 fish from each test tank (total = 160 fish) were randomly selected, fixed in Davidson's fixative solution, and stored in a 70% solution of ethanol for subsequent histological examination.

Selected fixed tissues were dissected and processed in Fisher Omnisette Tissue Cassettes (Fisher Scientific, Fair Lawn, New Jersey). The tissues were infiltrated with paraffin in a Leica ASP 300 Advanced Smart Processor (Leica Microsystems, Nussloch, Germany), and the paraffin-infiltrated tissue samples were embedded in paraffin blocks with a Leica EG 1160 Tissue Embedding System.

Tissues in paraffin blocks were sectioned on a Leica RM2255 Rotary Microtome. The 5-μm tissue sections were mounted on glass microscope slides and stained with hematoxylin and eosin by using a Leica AutoStainer XL; slides were evaluated microscopically. A histologist documented lesions (e.g., necrosis or degeneration), whether lesions were scattered or focal, and whether any lesions were artifacts of tissue dissection, preservation, or processing. As per our FDA-approved research study protocol (Bowker and Erdahl 2010), 2 of the 10 fish that were randomly selected from each tank for histology were used for an evaluation of gill, liver, anterior kidney, posterior kidney, brain, heart, muscle, skin, spleen, pyloric intestine, and rectal intestine tissues. Histological evaluations of the remaining 8 fish/tank were only focused on the gill, liver, anterior kidney, and posterior kidney.

The tissues were evaluated histologically for evidence of SLICE-induced toxicity. Tissues were scored under a six-point ordinal severity scale: 0 = no change; 1 = normal (<5% of the tissue was affected); 2 = mild (5–15% of the tissue was affected); 3 = moderate (15–25% of the tissue was affected); 4 = marked (25–50% of the tissue was affected); or 5 = severe (>50% of the tissue was affected). Only scores of 4 or 5 were considered severe enough to adversely affect fish health. Before analysis, lesions with a score of 0, 1, 2, or 3 were coded as 0 (not biologically important), and lesions with a score of 4 or 5 were coded as 1 (biologically important). As per FDA guidance, to minimize the number of histological images that had to be scored, images from the pooled 0- and 150-μg EB·kg fish $BW^{-1}\cdot d^{-1}$ treatment groups were evaluated first. If significant differences were not detected between these two

TABLE 1. Relative frequency (%) of Rainbow Trout fingerlings with normal to moderate histology findings for tissue samples from the target animal safety study on administration of SLICE-medicated feed. Histology was not performed on fish in the 50- and 100-μg emamectin benzoate (EB)·kg fish body weight $(BW)^{-1}\cdot d^{-1}$ treatment groups.

Tissue and histological feature	Number of samples[a]	Treatment (μg EB·kg fish $BW^{-1}\cdot d^{-1}$)		
		Reference	0	150
Spleen: melanomacrophage centers	2, 7, 8	100	100	75
Heart: inflammation	2, 8, 8	0	13	0
Liver: degeneration	10, 40, 40	10	20	20
Liver: necrosis	10, 40, 40	0	20	18
Liver: vacuolation	10, 40, 40	40	52.5	37.5
Liver: inflammation	10, 40, 40	30	23	7.5
Gill: hypertrophy	10, 40, 40	30	2.5	5
Gill: proliferation	10, 40, 40	70	98	98
Gill: fusion	10, 40, 40	60	98	100
Posterior kidney: melanomacrophage centers	10, 40, 40	100	95	95
Posterior kidney: proliferation	10, 40, 40	90	98	98
Posterior kidney: degeneration of tubule epithelium	10, 40, 40	60	75	50
Posterior kidney: necrosis of tubule epithelium	10, 40, 40	60	63	40
Posterior kidney: regenerating tubules	10, 40, 40	90	75	83
Posterior kidney: calculi	10, 40, 40	30	23	23
Posterior kidney: hyaline droplet degeneration	10, 40, 40	0	0	2.5

[a]Number of samples from the reference population, the 0-μg EB·kg fish $BW^{-1}\cdot d^{-1}$ exposure group, and the 150-μg EB·kg fish $BW^{-1}\cdot d^{-1}$ exposure group, respectively.

groups, we were not required to test for differences between the 0- and 50-μg EB·kg fish $BW^{-1}\cdot d^{-1}$ treatment groups or between the 0- and 100-μg EB·kg fish $BW^{-1}\cdot d^{-1}$ treatment groups (Bowker and Erdahl 2010).

Statistical analysis.—Binomial variables (histopathology data) were analyzed with a PROC GLIMMIX-based model (logit link; Wolfinger and O'Connell 1993) in the Statistical Analysis System version 9.2 (SAS 2008) by using fish (the observation unit) nested within tanks (the experimental unit). As per Bowker and Erdahl (2010), histology results were tested at an α of 0.10 (two-sided). Choosing this conservative significance level enhanced our ability to identify potentially subtle deleterious side effects associated with the use of EB. For qualitative purposes (i.e., Tables 1, 2), fish were considered the experimental unit (Gaikowski et al. 2009). Mean weight and TL of fish at the end of the trial were analyzed by one-way ANOVA (SYSTAT 2012), and the significance level (two-sided) for these comparisons was the conventional α of 0.05. Feeding behavior was summarized by adding the feeding scores across replicates in each exposure group for each feeding event (e.g., day 1, first feeding) and graphing the results in a mosaic plot (Microsoft Excel 2010).

TABLE 2. Relative frequency (%) of Rainbow Trout fingerlings with marked or severe histology findings for tissue samples from the target animal safety study on the administration of SLICE-medicated feed. Histology was not performed on fish in the 50- and 100-μg emamectin benzoate (EB)·kg fish body weight $(BW)^{-1}\cdot d^{-1}$ treatment groups.

Tissue and histological feature	Number of samples[a]	Treatment (μg EB·kg fish $BW^{-1}\cdot d^{-1}$)			P[b]
		Reference	0	150	
Liver: degeneration	10, 40, 40	0	7.5	5	0.7379
Liver: vacuolation	10, 40, 40	60	47.5	62.5	0.2902
Gill: hypertrophy	10, 40, 40	0	0	2.5	0.9734
Gill: proliferation	10, 40, 40	0	0	2.5	0.9734
Posterior kidney: degeneration of tubule epithelium	10, 40, 40	0	2.5	0	0.9734
Posterior kidney: regenerating tubules	10, 40, 40	0	18	15	0.8693

[a]Number of samples from the reference population, the 0-μg EB·kg fish $BW^{-1}\cdot d^{-1}$ exposure group, and the 150-μg EB·kg $BW^{-1}\cdot d^{-1}$ exposure group, respectively.
[b]The P-value represents significance of differences between the 0- and 150-μg EB·kg fish $BW^{-1}\cdot d^{-1}$ exposure groups.

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TABLE 3. Sum of feeding scores (based on a five-point ordinal scale) for fingerling Rainbow Trout across the four replicate tanks per exposure group during the first, second, and third feeding periods on each study day (exposure groups: 0 [0×], 50 [1×], 100 [2×], and 150 [3×] μg of emamectin benzoate·kg of fish body weight^{-1}·d^{-1}; the nontrial group was used to determine feeding rates). White indicates that fish in each of the four replicate tanks within an exposure group appeared to consume approximately 100% of the feed offered. Sequentially darker shades of gray indicate that less feed was consumed. The five sequentially darker shades of gray indicate scores of 15 to 11, respectively, indicating that successively less feed was consumed.

	0×			1×			2×			3×			Nontrial		
Study day	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
12															
13															
14															

RESULTS

No fish died during the trial, and general fish behavior was characterized as normal throughout the trial. At each feeding event, fish consumed all of the feed that they were going to consume within 20–30 s of it being offered. There was an apparent trend of decreasing feed consumption with increasing EB concentration in the feed (Table 3). The minimum total feeding score observed in the 0× and 2× exposure groups during a single feeding event was 15, indicating that fish in three replicate tanks appeared to consume approximately 100% of the feed (3 tanks × feeding score of 4 for each replicate) and fish in one replicate tank consumed approximately 75% of the feed (1 tank × feeding score of 3). For the 1× exposure group, there were two instances in which the minimum total feeding score was 14. Feeding scores for the 3× exposure group ranged from 11 to 16, and scores for the nontrial group ranged from 12 to 16. With two exceptions, fish in all tanks appeared to consume at least approximately 75% of the feed offered. On study day 9, fish in two 3× tanks appeared to consume only 50% of the feed offered. Lower total feeding scores were documented more frequently at the first daily feeding event than at any other time.

During the trial, test fish grew an average of 2.8 cm in length and 8.7 g in weight. Mean TL and weight of fish in test tanks (n = 320 fish in 16 test tanks) at the end of the trial were 10.2 cm (range = 7.0–12.5 cm) and 13.1 g (range = 4.0–21.3 g), respectively. No significant differences were detected in mean TL ($P = 0.941$) or mean weight ($P = 0.920$) among the four exposure groups. Water temperature, DO concentration, water hardness, alkalinity, and pH were within acceptable ranges for Rainbow Trout culture (Piper et al. 1982).

The measured mean EB doses ± SD for the 50-, 100-, and 150-μg EB·kg fish BW^{-1}·d^{-1} treatment groups based on feed samples collected to confirm EB concentration were 43.0 ± 2.9 (–14% from the target dose), 100.7 ± 12.5 (1% from target), and 155.9 ± 48.3 (12% from target) μg EB·kg fish BW^{-1}·d^{-1}, respectively. No EB was detected in the control feed.

Fish Health and Histology

Reference population.—External and internal tissues appeared grossly normal in the 30 reference fish that were necropsied for fish health; however, shortened opercula were noted on three of these fish. For the 10 reference fish that were subjected to histological examination, no lesions were observed in the heart, liver, skin, muscle, spleen, and pyloric intestine tissues. Lesions observed in other tissues included (1) mild proliferation of epithelium and fusion of lamellae primarily at filament tips in gill tissue, with foci of cartilage and epithelial hyperplasia denoting previous areas of gill injury; (2) moderate to marked glycogen vacuolation of hepatocytes; (3) mild numbers of melanomacrophages in anterior kidney and the interstitial tissue of posterior kidney; and (4) mild to moderate proliferation of hematopoietic cells and numbers of regenerating tubules (Tables 1, 2). Early stages of nephrocalcinosis were observed in three fish. The observed lesions did not appear to adversely affect the health of the fish.

The 0× group.—All 40 of the 0× fish (0-μg EB·kg fish BW^{-1}·d^{-1} group) appeared to be healthy at the end of the trial. Observations during necropsy indicated that 1 fish had a fatty liver, 10 fish had cream-colored livers, and 4 fish possessed shortened opercula. Histological examinations showed that

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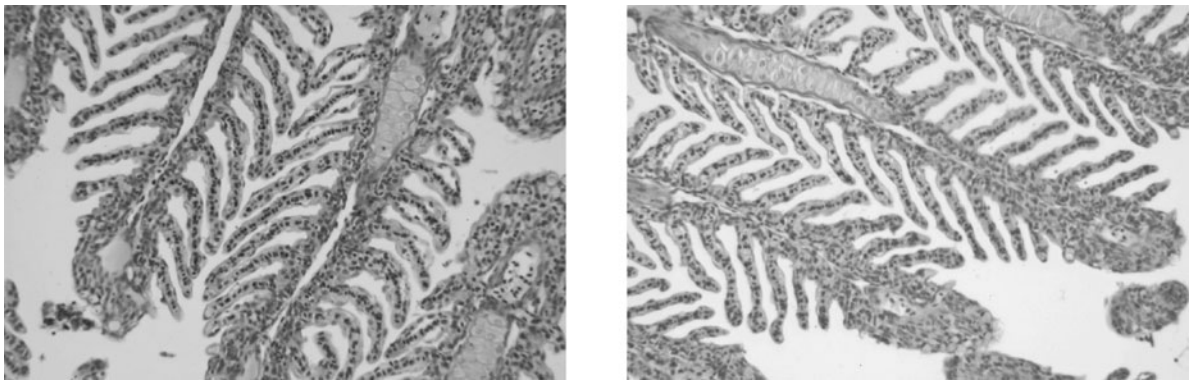

FIGURE 1. Gill tissue of a Rainbow Trout fingerling that was sampled from the reference population before the start of the study (left panel) and gill tissue of a fish that was sampled after treatment with 150 μg emamectin benzoate·kg fish body weight^{-1}·d^{-1} in feed for 14 d (right panel).

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changes observed in the 0× fish (Tables 1, 2) were similar to those described for fish from the reference population. Observations included increased severity of glycogen vacuolation of hepatocytes in the liver and changes related to nephrocalcinosis in the kidney. In addition, two fish from one of the 0× tanks showed marked degeneration and necrosis of liver hepatocytes. No histopathologies observed in other tissues were considered severe enough to affect the health of these fish.

The 1× group.—All 40 of the 1× fish (50-μg EB·kg fish BW^{-1}·d^{-1} group) were healthy in appearance, and all external and internal tissues that were examined appeared to be normal. Observations during necropsy indicated that three fish had fatty livers, nine fish had cream-colored livers, and two fish had shortened opercula. No tissues from fish in the 1× exposure group were examined histologically.

The 2× group.—All 40 of the 2× fish (100-μg EB·kg fish BW^{-1}·d^{-1} group) appeared to be healthy, and all of the external and internal tissues examined were normal in appearance. Observations during necropsy showed 13 fish with cream-colored livers and 4 fish with shortened opercula. No tissues from fish in the 2× treatment were examined histologically.

The 3× group.—All 40 of the 3× fish (150-μg EB·kg fish BW^{-1}·d^{-1} group) were healthy in appearance, and all external and internal tissues that were examined appeared to be normal. During necropsy, three fish were observed to have fatty livers, seven fish had cream-colored livers, and three fish had shortened opercula. Histological examinations showed that the changes observed in the 3× fish (Tables 1, 2) were similar to those described for reference fish and 0× fish (Figures 1, 2). Observations included increased severity of glycogen vacuolation of hepatocytes in the liver and changes related to nephrocalcinosis in kidney tissue. In addition, one fish showed marked degeneration and necrosis of liver hepatocytes.

Lesions observed that were marked or severe (Table 2) in both the 0× and 3× exposure groups included (1) liver degeneration, (2) liver vacuolation, and (3) posterior kidney regenerating tubules. Marked gill hypertrophy and proliferation were observed in one fish from the 3× group. Marked posterior kidney degeneration was observed in one fish from the 0× group. Differences in the prevalence of marked or severe lesions between the 0× and 3× exposure groups were not significant (Table 2).

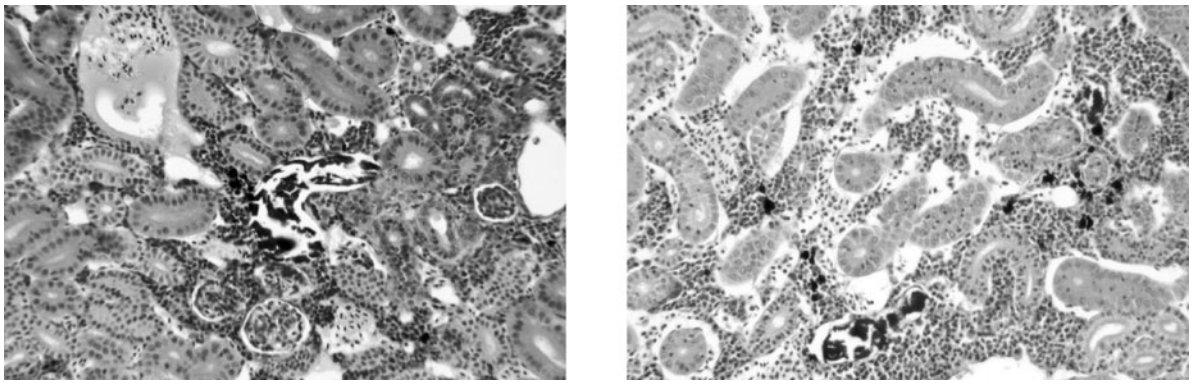

FIGURE 2. Kidney tissue of a Rainbow Trout fingerling that was sampled from the reference population before the start of the study (left panel) and kidney tissue of a fish that was sampled after treatment with 150 μg emamectin benzoate·kg fish body weight^{-1}·d^{-1} in feed for 14 d (right panel). In both panels, nephrocalcinosis is indicated by the darkly pigmented areas.

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DISCUSSION

Based on (1) survival results, (2) the fact that fish feeding behavior was characterized as mostly aggressive regardless of the EB concentration in the offered feed, and (3) the fact that fish behavior was considered normal, with no dose-related differences detected, we conclude that administering SLICE-medicated feed to fingerling Rainbow Trout at a target dosage of 150 μg EB·kg fish $BW^{-1}\cdot d^{-1}$ for 14 d is safe. Although there was an apparent trend indicating a decrease in feeding with increasing EB concentration in feed, the fish still appeared to consume most of the feed offered. One concern with administering top-coated, medicated feed to fish is the potential for the drug to leach into the water. We know of no reports of EB leaching from top-coated feed; however, Yanong et al. (2005) reported that a considerable amount of florfenicol can be lost from feed that is top-coated with Aquaflor if the feed remains in the water for several minutes. In our study, fish oil was applied to feed immediately after it was top-coated with SLICE, and fish appeared to consume the feed within 20–30 s after it was offered. Regardless, it is possible that some EB leached from the feed before it was consumed; thus, the intended dose might not have been received by every fish. Furthermore, the fact that fish were fed within 30 min of the lights being turned on in the study building might have resulted in decreased feed consumption during the morning feeding period. Despite the evidence of apparent decreased feed consumption as EB concentration increased from 0 to 100 μg·kg fish $BW^{-1}\cdot d^{-1}$, the fish in the treatment groups appeared to consume more feed than fish in the nontrial tanks. However, it was clear that fish in the 3× exposure group appeared to consume less feed than fish in the other exposure groups and that the greatest degree of decreased feeding occurred during study days 6–10. It has been speculated that diminished feed consumption is the first sign of intoxication to avermectins, which limits further toxic effects (Richard Endris, Merck Animal Health, personal communication).

No fish health or histopathological lesions were detected to indicate that the administration of SLICE at 150 μg EB·kg fish $BW^{-1}\cdot d^{-1}$ for 14 d was not safe for fingerling Rainbow Trout. Such findings are consistent with those of similarly conducted studies evaluating the safety of SLICE administered in feed to Rainbow Trout and Atlantic Salmon. Roy et al. (2000) conducted a study in which Atlantic Salmon and Rainbow Trout were exposed to nominal EB doses of 0, 100, 250, or 500 μg EB·kg fish $BW^{-1}\cdot d^{-1}$ for 7 d in seawater. After a 7-d postexposure period (also in seawater), no mortalities were reported and no pathognomonic signs of EB toxicity were observed during gross necropsy or histopathological examination. Roy et al. (2000) reported that unequivocal signs of toxicity were only observed at the highest exposure dose (10× the standard dose), and these signs included lethargy, dark coloration, and inappetence. Further, Roy et al. (2000) reported that mortalities occurring during their study were not treatment related, that fish behavior was characterized as normal in all exposure tanks, and that pathology findings were minimal. In the experiment with Atlantic Salmon (Roy et al. 2000), one fish in the high-dose exposure group died at 2 d postexposure. Pathological findings included focal necrosis, ceroid accumulation in the spleen, and melanin accumulation in the kidney, all of which are signs consistent with prolonged anorexia. In the experiment with Rainbow Trout, one fish in the low-dose exposure group died on exposure day 1. When that fish was examined histologically, findings included (1) scattered melanin granules in the spleen, suggesting toxic effects like those seen with bacterial toxins; and (2) high levels of melanin in the kidney, indicating that the fish had been anorexic for some time. Stone et al. (2002) conducted a study in which Atlantic Salmon smolts were exposed to nominal EB doses of 0, 50, and 250 μg EB·kg fish $BW^{-1}\cdot d^{-1}$ for 7 d in freshwater and then were moved to seawater and observed for an additional 14 d. Stone et al. (2002) reported that among smolts receiving the high dose, approximately 50% exhibited darker coloration and one fish (1%) exhibited uncoordinated swimming behavior. There was no mortality, and no histopathological changes indicative of EB toxicosis were found. In the studies by Roy et al. (2000) and Stone et al. (2002), dark coloration, abnormal swimming behavior, and inappetence were reported among fish that were exposed to EB doses much higher than those administered in our trial. Our results were similar to their results with respect to a lack of mortality, lesions and cellular changes, and inappetence at higher doses of EB administered in feed. Although a decrease in feed consumption was observed during an intermediate period in our study, it appeared that the fish resumed consumption of feed and thereafter consumed 100% of the offered feed for a majority of the time until the study ended.

We conclude that there is a substantial margin of safety associated with treating Rainbow Trout with the standard treatment regimen of 50 μg EB·kg fish $BW^{-1}\cdot d^{-1}$ for 7 d to control infestations of susceptible freshwater copepods and branchiurans. Furthermore, we hypothesize that other freshwater salmonids can be safely treated with the prescribed therapeutic dosage when administered to control infestations of susceptible freshwater copepods and branchiurans.

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