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

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SLICE® is an in-feed treatment developed for the control of sea lice (e.g., *Lepeophtheirus salmonis* and *Caligus elongates*) infestations in farmed salmon and trout. SLICE® has been tested to evaluate environmental safety as well as efficacy and tolerance in seawater-reared Atlantic salmon *Salmo salar*, rainbow trout *Oncorhynchus mykiss*, and brown trout *Salmo trutta* (Armstrong et. al. 2000; Roy et. al. 2000; Stone et al. 1999, 2000a, 2000b, 2000c, and 2002) and in freshwater-reared rainbow trout. Currently, SLICE® is approved for the control of sea lice in salmonid species in the United Kingdom, Europe, Norway, Chile, and Canada.

The active component of SLICE® is 0.2% emamectin benzoate (EB). When SLICE® is administered in feed to fish, EB is absorbed from the fish's gut and distributed to a variety of it's tissues. When sea lice (or other parasitic crustaceans) feed on the skin, mucus, blood, and muscle of fish, EB is taken into the tissues of the parasite. The EB then binds to ion channels of nerve cells and disrupts transmission of nerve impulses, which results in paralysis and death of the parasite. Furthermore, EB is slowly metabolized by fish, resulting in protection from sea lice that extends up to 9 weeks after treatment has been completed (Stone et al. 2000c).

Recently, SLICE® has been used to control mortality caused by a variety of freshwater parasites (Duston and Cusack 2002; Hakalahti et al. 2004; Bowker et al. 2012; Gunn et al. 2012). SLICE® is effective for the treatment of *Argulus coregoni* and *Salmincola californiensis* in rainbow trout, as well as for the treatment of *S. edwardsii* in brook trout *Salvelinus fontinalis*. Approval of SLICE® by the U.S. Food and Drug Administration (FDA) for use to control infestations of *Salmincola* spp. in freshwater-reared finfishes would help to optimize fish health and performance.

To help obtain FDA approval of a new drug, data must be generated to show that there is an adequate margin of safety associated with the proposed treatment regimen to target animals (i.e., safe to fish at 2× the proposed treatment regimen). Consequently, we conducted a target animal safety study to evaluate the safety of SLICE® administered in feed to disease-free rainbow trout at 0× (0 µg EB per kg fish per day), 1× (50 µg EB per kg fish per day), 2× (100 µg EB per kg fish per day), or 3× (150 µg EB per kg fish per day) the proposed maximum therapeutic dose of 50 µg EB per kg fish per day for 14 consecutive days (2× the proposed therapeutic treatment duration of 7 consecutive days).

Methods

The study was conducted at the U.S. Fish and Wildlife Service's Bozeman Fish Technology Center (Bozeman, Montana) on March 14 – April 02, 2012. Completely randomized designs were used to (1) assign each exposure dose to four fiberglass tanks (total, 16 tanks) and (2) allocate 20 fish into each tank (total, 320 fish). Mean ± SD length and weight of rainbow trout in the reference population were 7.4 ± 0.7 cm and 4.4 ± 1.2 g, respectively.

Each test tank contained 57 L of water, and first-pass inflow water was set to 3.7 L per min. Feed samples were collected throughout the study and sent to Eurofins/AvTech Laboratories, Inc. (Portage, Michigan) for analysis to verify the homogeneity and stability of EB in the 1×-, 2×-, and 3×-treated feeds and ensure the 0× (control) feed was not contaminated with EB.

The in-life phase of the study consisted of a 6-day acclimation period, 14-day exposure period, and 1-day postexposure period. During the study, feed was administered to all tanks three times daily by hand at a feeding rate of 4% mean body weight per day; feed amounts were adjusted weekly for growth. During the exposure period, treated feed was administered to treated tanks and control feed was administered to control tanks. During the acclimation and postexposure periods, control feed was administered to all tanks.

Mortality, general fish behavior, fish feeding behavior, water temperature, and dissolved oxygen (DO) concentration were monitored daily. Water hardness, alkalinity, and pH were measured once per week during the study.

At the end of the in-life phase, all test fish were collected, euthanized, and necropsied. During necropsies, 160 fish (10 per tank) were randomly selected for histological evaluation of gill, liver, anterior kidney, and posterior kidney tissues. Concomitantly, a second randomization was used to select 32 of these fish (2 per tank) for histological evaluation of brain, heart, muscle, skin, spleen, pyloric intestine, and rectal intestine tissues.

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Initially, only tissues from the 0× and 3× exposure groups were evaluated for pathologies, which were scored via an ordinal scale (0 = no change, 1 = normal, 2 = mild, 3 = moderate, 4 = marked, and 5 = severe). None of the lesions detected in the 3× exposure group met all three of the following criteria: (1) marked or severe, (2) apparently SLICE®-induced, *and* (3) not observed in the 0× exposure group. Consequently, as specified in the study protocol, we did not examine tissues from the 1× and 2× exposure groups.

Histology data (3× exposure group versus 0× exposure group only) were analyzed with a SAS 9.2, Proc Glimmix-based model (logit link). Before analysis, 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 effect was tested at $\alpha = 0.10$ (two-sided).

Results and Discussion

No fish died during the study, and general fish behavior was characterized as normal throughout the study. Fish in the 0×, 1×, and 2× exposure groups consumed approximately 100% of the feed 93 – 96% of the time. Fish in the 3× exposure group consumed approximately 100% of the feed 75% of the time and consumed approximately 75% of the feed 24% of the time.

Lesions of concern observed in 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 between lesions in the 0× and 3× exposure groups were not significant.

Mean EB doses in feed collected to demonstrate homogeneity in the 1×, 2×, and 3× batches of feed were 88%, 100%, and 118% of respective target doses. Mean EB doses in feed collected to demonstrate stability in the 1×, 2×, and 3× batches of feed were 82%, 102%, and 83% of respective target doses. Based on FDA criteria, EB was mixed homogenously in each batch of feed and was stable over the course of the study.

Overall mean water temperature and DO concentration were 15.0°C (range, 14.9 – 15.2°C) and 7.0 mg per L (range, 6.5 – 8.0 mg per L). Overall mean water hardness (241 mg per L as $CaCO_3$), alkalinity (160 mg per L as $CaCO_3$), and pH (8.0) in test tanks were suitable for rearing healthy rainbow trout.

Based on these results, we concluded the margin of safety associated with administering EB-treated feed to fingerling rainbow trout reared at a water temperature of approximately 15ºC extends to at least 150 µg EB per kg fish per day when administered for 14 consecutive days. Results have been submitted to FDA in support of approval of SLICE® for use in the U.S. to control infestations of *Salmincola* spp. in all freshwater-reared salmonids.

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