SLICE® (emamectin benzoate) Clinical Field Trials - INAD 11-370

Year 2012 - 2014 Annual Summary Reports on the Use of SLICE® (emamectin benzoate) in Clinical Field Efficacy Trials

Prepared by:

Bonnie Johnson, Biologist
U. S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, Montana

Summary

SLICE®(emamectin benzoate) has been used effectively in the U. S. under compassionate INAD Exemption #11-370 to control mortality in a variety of fish species caused by ectoparasites. In calendar years 2012 - 2014 (CY12-14), the efficacy of SLICE® (emamectin benzoate) was evaluated in 72 ectoparasite trials involving approximately 6.2 million fish to control mortality in a variety of fish species caused by ectoparasites. Trials were conducted at a total of 12 fish culture facilities, including one U.S. Fish and Wildlife Service National Fish Hatchery (NFH), six state hatcheries, four private hatcheries, and one tribal facility. The compassionate study protocol under which treatments were administered allowed the investigator to use SLICE® daily for 7 consecutive days at a dosage of 50 ug/kg/day. Overall, results of trials conducted in CY12-14 indicated that treatments appeared efficacious in 96% of the trials and ineffective in 4% of the trials.

Introduction

External parasites (ectoparasites) form one of the largest groups of pathogenic organisms of cultured aquatic species (Post 1987). Affected species include finfish (freshwater and marine) and invertebrates. Environmental conditions such as temperature change, poor water quality, and high organic loading due to intensive fertilization and feeding levels increase the incidence and spread of many external parasites. Stress (i.e., seining, handling, sorting, grading, vaccinating, anesthesia, crowding, and transport) is also a major contributor to most parasitic outbreaks in fish (Lasee 1995). Additionally, tissue damage induced by external parasites increases susceptibility to secondary bacterial and/or fungal infections (Lasee, 1995).

The organisms responsible for major parasitic infections on fish are, for the most part, protozoan and metazoan. These parasites are highly opportunistic and have tremendous reproductive capabilities. Under normal conditions (e.g., in wildstock populations) these organisms cause little pathology. However, under intensive culture where fish densities are typically high, many of these organisms can cause serious disease problems.

If parasitic infections are left untreated, they can cause substantial economic losses to commercial aquaculture, and severely impact the restoration, recovery, and preservation of depleted stocks of fish cultured by Federal and State agencies. The extent of losses of fish from parasites depends upon the severity of the primary cause

of infection. Morbidity can vary from less than 10% to total loss of the population (Post 1987). Historically, immersion treatments (static and flush) using a variety of compounds have been used to control mortality caused by parasite infestations. A number of these compounds have been found, both experimentally and under production settings, to be relatively effective.

SLICE® is an in-feed treatment that was developed specifically for the control of sea lice infestations in farmed salmon and trout. Control of sea lice (including *Lepeophtheirus salmonis*, *Caligus elongatus*, *C. rogercressyi*, and *C. teres*) on farmed fish is essential as lice feeding activity may result in mortalities, as well as susceptibility to a variety of other pathogens. SLICE® has been extensively tested in trials to evaluate environmental safety, efficacy, and tolerance in Atlantic salmon, *Salmo salar*, rainbow trout, *Oncorhynchus mykiss*, and brown trout, *Salmo trutta*, in the marine environment (Stone et al., 1999; Stone et al., 2000a; Stone et al., 2000b; Stone et al., 2000c; Stone et al., 2002; Roy et. al., 2000; and Armstrong et. al., 2000). Currently, SLICE® is approved for the control of sea lice in salmonid species in the UK, Europe, Norway, and Chile.

Purpose of Report

The purpose of this report is to summarize the results of CY12-14 supplemental SLICE® field efficacy data. Similar data have been submitted by the Service in previous years. We anticipate that CY12-14 data will be used to enhance the existing SLICE® database established from previous years, and will be considered in the "body of

evidence" for the purpose of developing an appropriate label claim for the use of SLICE® in aquaculture.

Facilities, Materials, Treatment Procedures

1. Facilities

A total of 72 field efficacy trials were conducted at 12 fish culture facilities, including one U.S. Fish and Wildlife Service National Fish Hatchery (NFH), six state hatcheries, four private hatcheries, and one tribal facility. Note: the study location for all Matapeake trials occurred at NRG Energy Co (Mirant Power Company - Chalk Point Generating Station), Aquasco, MD. Treatments were used to control mortality caused by ectoparasites in various fish species. Water temperature during treatments at the various testing facilities ranged from 42.4 - 85.1 °F, with a mean treatment temperature of 61.4°F.

2. Chemical material

The SLICE® premix used in CY12-14 trials consisted of 0.2% emamectin benzoate in an inert carrier, consisting of GM-free cornstarch, maltodextrin, antioxidant, and solvent. The premix has been formulated specifically for incorporation of emamectin benzoate onto fish feeds. All SLICE® used was supplied by Merck Animal Health, 556 Morris Avenue, Summit, NJ. SLICE® medicated feed was prepared either by top-coating SLICE® onto commercial fish

feed at the testing site by the Investigator, Monitor, or their designee, or prepared by commercial fish feed manufacturers.

3. Drug dosages and duration

As described in the Study Protocol for INAD #11-370, Investigators were allowed to use SLICE® daily for 7 consecutive days at a dosage of 50 ug/kg/day.

Fish Species Treated and Fish Ectoparasites Involved in CY12-14 Trials

1. Species and size of fish treated

Four fish species, including three species of salmonids and one marine non-salmonid species were treated with SLICE® during CY12-14. Treated fish ranged in length from 7.5 - 53.5 in. and the mean length of all treated fish was 16.4 in. Fish species treated included:

Salmonids:

chinook/spring chinook salmon *Oncorhynchus tshawytscha*

rainbow trout *O. mykiss*

steelhead trout *O. mykiss*

Marine non-salmonids:

Atlantic sturgeon *Acipenser oxyrhinchus*

2. Ectoparasite treated

Test fish were treated with SLICE® to control mortality caused by ectoparasites of the genera *Argulus* and *Salmincola*.

Data Collected

1. Pathologist's report

Fish health pathology reports provide essential information with respect to parasite confirmation and general fish health. A pathology report was submitted with 4% of the CY12-14 trials.

2. Treatment response and drug accountability data

Drug receipt reports, drug use reports, diagnosis, treatment, and mortality reports (including adverse effects/toxicity observations), and fish disposition reports were prepared by study investigators through the online INAD database. Such reports were routed through the study monitor for review, and then sent to the AADAP Office for review, data analysis and report writing, and archiving in permanent files.

Discussion of Study Results

1. General observations on the efficacy of SLICE® for the control of ectoparasites in a variety of fish species (Note: Table 1 provides a summary

of all trials in which treatment appeared efficacious; Table 2 provides a summary of all trials in which treatment was not efficacious; and Table 3 provides summary data for all trials conducted during CY12-14 under INAD #11-370).

A. Efficacy of SLICE® at 50 ug/kg/day

Atlantic sturgeon, rainbow trout, steelhead trout, and chinook/spring chinook salmon were treated with SLICE® at 50 ug/kg of fish biomass for 7 consecutive days in 42 trials (Tables 1 & 2). SLICE® treatments appeared to be effective in 96% of the trials and ineffective in 4% of the trials.

2. Observed Toxicity

No toxicity or adverse effects relating to SLICE® treatment were reported in any of the trials.

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded.

Current Study Protocol for SLICE® (emamectin benzoate) INAD #11-370

No changes have occurred to the current study protocol for SLICE® (emamectin benzoate) INAD #11-370.

Facility Sign-up List

Please see "Table 4. Facilities and Names of Investigators" for facilities that signed-up to participate in the SLICE® (emamectin benzoate) during CY12-14. All facilities that conducted trials during CY12-14 were compliant with their reporting requirements to the NPDES authority; and have been approved by CVM's environmental team to participate under the SLICE® INAD. A copy of their NPDES Authority has been attached to this report.

Correspondence sent to SLICE® (emamectin benzoate) INAD #11-370 Participants

Please see the attached correspondences that were sent to all SLICE® participants after the AADAP Office received their sign-up form for CY12-14.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY12-14 was 6,184,316. The total number of treated fish to count against the current treatment use authorization dated May 21, 2010 is 10,451,363.

Summary of Study Results

SLICE® was used at a dosage of 50 ug/kg of fish biomass for 7 consecutive days in 72 trials. Atlantic sturgeon, chinook/spring chinook salmon, rainbow trout and steelhead trout were the only fish species treated and trials involved approximately 6.2 million fish. Treated fish ranged in size from 7.5 - 53.5 in. Water temperature during

treatment ranged from 42.4 - 85.1°F, with a mean treatment temperature of 61.4°F. Overall, results showed that treatment appeared to be effective in 96% of the trials and ineffective in 4% of the trials. There was no evidence of toxicity or adverse effects related to SLICE® treatment reported in any of the trials. Data from the CY12-14 trials indicate that the SLICE® treatment regimen recommended in INAD Protocol #11-370 is safe and effective to control mortality in a variety of fish species caused by ectoparasites. As a result of the lack of quality criteria, such as dose verification, use of controls, replicates, and randomization, it is understood that these data will be considered as ancillary data, and that pivotal efficacy studies are needed to definitively demonstrate SLICE® efficacy for the treatment of ectoparasites. However, the ancillary data described above should provide useful, corroborative data to help support a label claim for the use of SLICE® to control mortality associated with ectoparasites in a variety of fish species. Although it is anticipated that the majority of future efficacy data collected under INAD #11-370 will also be ancillary data, efforts will be directed towards the continued generation of high quality data.

References

Armstrong, R., D. MacPhee, T. Katz, and R. Endris. 2000. A field efficacy evaluation of emamectin benzoate for the control of sea lice in Atlantic salmon. Canadian Veterinary Journal. 41: 607-612.

Lasee, B. A., editor. 1995. *Introduction to Fish Health Management*, 2nd edition. U.S. Fish and Wildlife Publication. Washington, D.C. 139 pp.

Post, G.W. 1987. Textbook of fish health. Revised and expanded edition. TFH Publications, Inc., Ltd., Neptune City, New Jersey. 288 pp.

Roy, W.J., I.H. Sutherland, H.D.M. Roger, and K.J. Varma. 2000. Tolerance of Atlantic salmon, *Salmo salar L.*, and rainbow trout, *Oncorhynchus mykiss* (Walbaum), to emamectin benzoate, a new orally administered treatment for sea lice. Aquaculture. 184: 19-29.

Stone, J., I.H. Sutherland, C. Sommerville, R.H. Richards, and K.J. Varma. 1999. The efficacy of emamectin benzoate as an oral treatment of sea lice, *Lepeophtheirus salmonis* (Kroyer), infestations in Atlantic salmon, *Slamo salar L.* Journal of Fish Diseases. 22: 261-270.

Stone, J., I.H. Sutherland, C. Sommerville, R.H. Richards, and K.J. Varma. 2000a. Field trials to evaluate the efficacy of emamectin benzoate as an oral treatmentof sea lice, *Lepeophtheirus salmonis* (Kroyer), and *Caligus elongatus* Nordman, infestations in Atlantic salmon, *Salmo salar L.* Aquaculture. 186: 205-219.

Stone, J., I.H. Sutherland, C. Sommerville, R.H. Richards, and K.J. Varma. 2000b. Commercial trials using ememectin benzoate to control *Lepeophtheirus salmonis* (Kroyer), and *Caligus elongatus* Nordman, infestations in Atlantic salmon, *Salmo salar L.* Diseases of Aquatic Organisms. 41: 141-149.

Stone, J., I.H. Sutherland, C. Sommerville, R.H. Richards, and R.G. Endris. 2000c. The duration of efficacy following oral treatment with emamectin benzoate against infestations of sea lice, *Lepeophtheirus salmonis* (Kroyer) in Atlantic salmon, *Salmo salar L.* Journal of Fish Diseases. 23:185-192.

Stone, J., W.J. Roy, I.H. Sutherland, H.W. Ferguson, C. Sommerville, R.H. Richard, and R.G. Endris. 2002. Safety and efficacy of emamectin benzoate administered in feed to Atlantic salmon, *Salmo salar L.*, smolts is freshwater, as a preventative treatment against infestations of sea lice, *Lepeophtheirus salmonis* (Kroyer). Aquaculture. 210: 21-34.

Table 1. Summary of Year 2012 - 2014 SLICE® Efficacy Results - Efficacious Studies

Hatchery	Number of efficacious trials	Fish Species	Fish Size (in.)	Number of Fish	Ectoparasite	Dose (ug/kg)	Number of treatment days	Temp. (°F)
Matapeake	13	Atlantic Sturgeon	10.0 - 53.5	546	Argulus	50	7	74.7 - 85.1
Little White Salmon NFH	1	Spring Chinook Salmon	13.50	405	Salmincola	50	7	42.7
	2	Chinook Salmon	14.1 - 18.5	1,083	Salmincola	50	7	42.4 - 46.6
Crystal Lake Hatchery	1	Rainbow Trout	20.00	1,000	Salmincola	50	7	56.0
Crystal River Hatchery	1	Rainbow Trout	14.10	7,728	Salmincola	50	7	52.0
Idaho Springs	7	Rainbow Trout	9.3 - 10.1	341,655	Salmincola	50	7	58.0 - 59.0
Magic Springs	30	Rainbow Trout	9.0 - 10.5	4,492,220	Salmincola	50	7	58.0 - 59.0
Maramec Spring Hatchery	1	Rainbow Trout	7.50	55,000	Salmincola	50	7	57.0
Pristine Springs/Blue Lakes	10	Rainbow Trout	7.9 - 9.9	1,119,135	Salmincola	50	7	58.0 - 59.0
San Joaquin Hatchery	1	Rainbow Trout	20.00	32,000	Salmincola	50	7	55.0
Sunburst Trout Farm	2	Rainbow Trout	9.0 - 13.0	98,500	Salmincola	50	7	58.0

Table 2. Summary of Year 2012 - 2014 SLICE® Efficacy Results - Ineffective Studies

Hatchery	Number of ineffective trials	Fish Species	Fish Size (in.)	Number of Fish	Ectoparasite	Dose (ug/kg)	Number of treatment days	Temp. (°F)
Oak Springs Fish Hatchery	1	Rainbow Trout	26.00	2,262	Salmincola	50	7	53.0
San Joaquin Hatchery	1	Rainbow Trout	15.00	32,774	Salmincola	50	7	52.0
Methow Steelhead Kelt Facility	1	Steelhead Trout	23.70	8	Salmincola	50	7	50.9

Table 3. Summary Data Regarding Year 2012 - 2014 SLICE® Efficacy Studies

Total Number of Fish Treated:	**6,184,316**
Number of fish treated in efficacious trials	6,149,272
Number of fish treated in ineffective trials	35,044
Total Number of Studies:	**72**
Efficacious trials	69
Ineffective trials	3

Treatment Regimens and Frequency Used:

50 ug/kg; 7 days	72 trials

Treatment Water Temperature (°F):

Temperature Range	42.4 - 85.1
Mean Temperature	61.4

Size of Treated Fish (in.):

Size Range	7.5 - 53.5
Mean Length	16.4

Species Treated:

Salmonids:
chinook/spring chinook salmon *Oncorhynchus tshawytscha*
rainbow trout *O. mykiss*
steelhead trout *O. mykiss*

Non-salmonids:
Atlantic sturgeon *Acipenser oxyrhinchus*