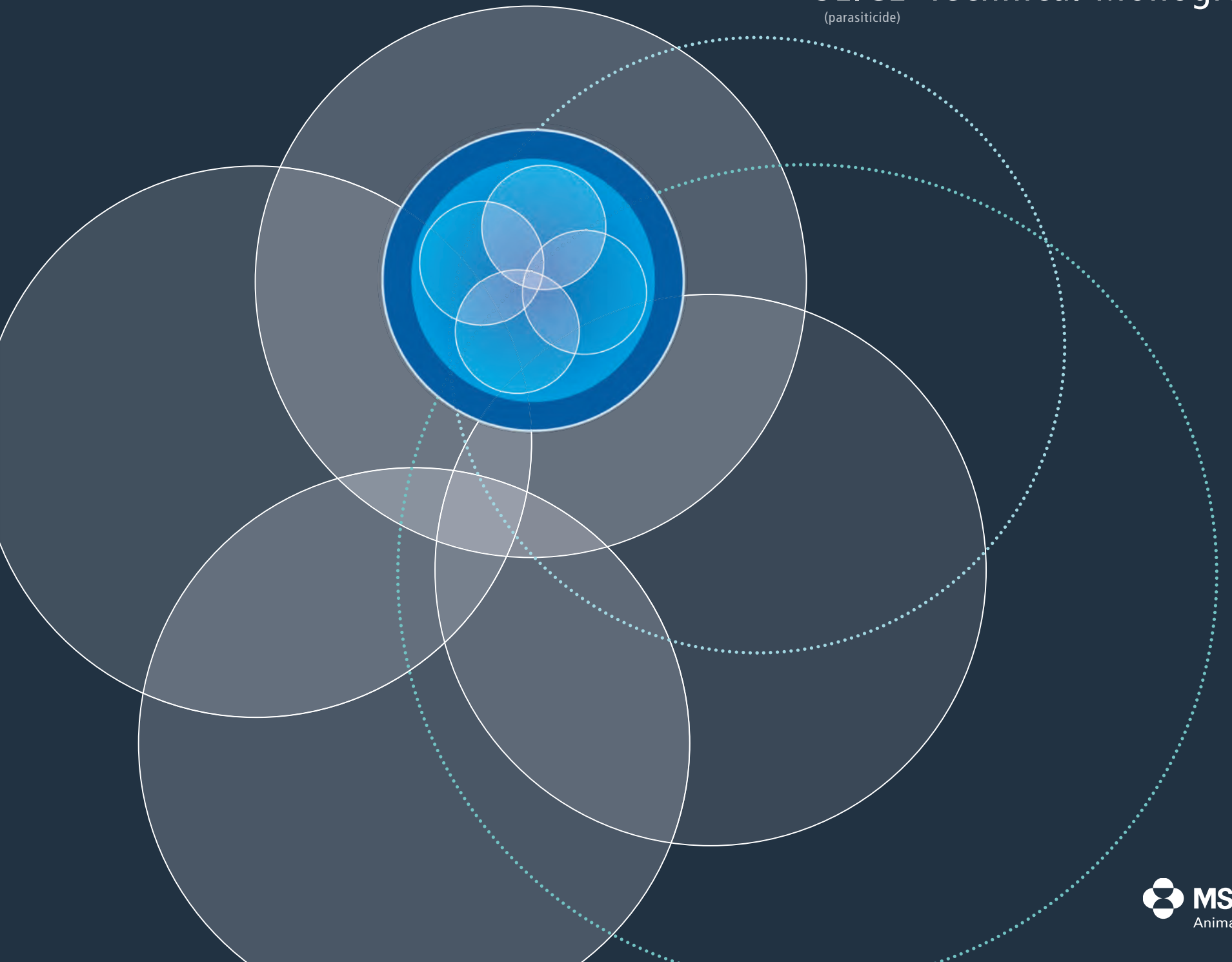
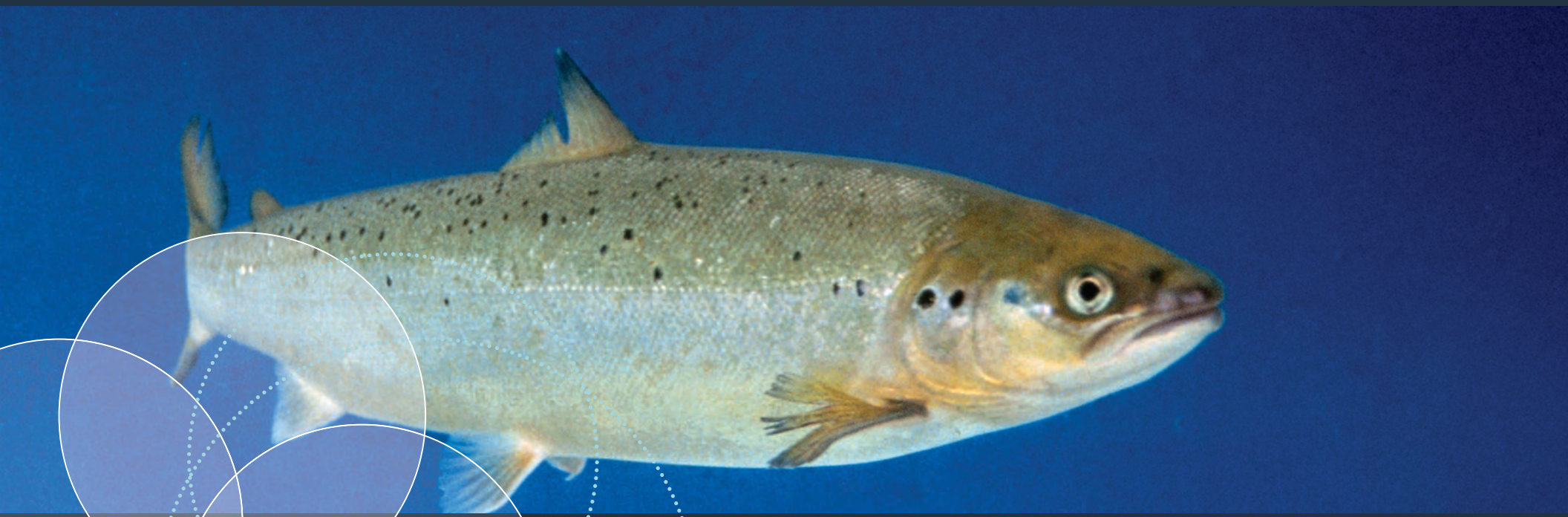
SLICE® Technical Monograph

(parasiticide)



MSD Animal Health



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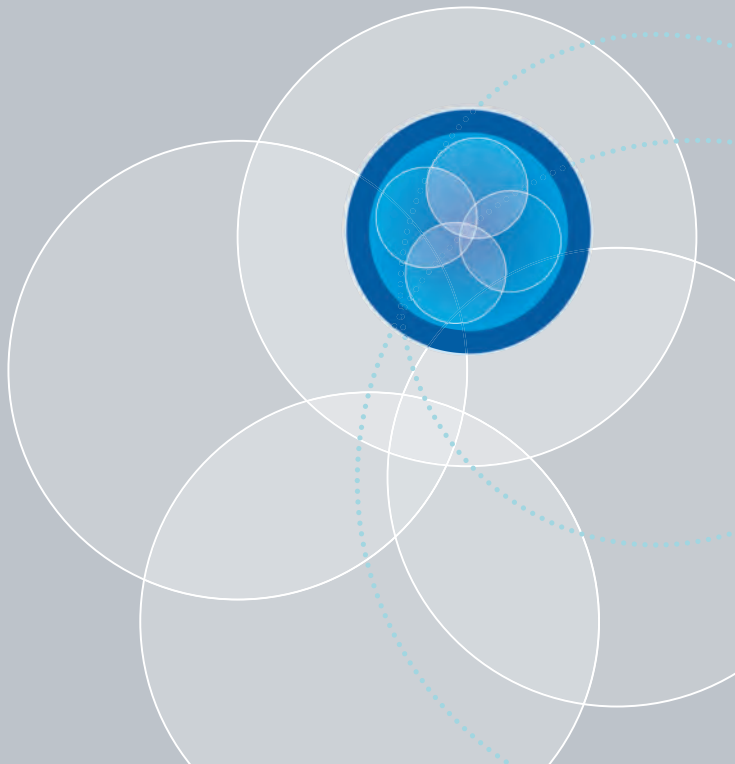


1 Introduction

Sea lice infestations represent the most significant disease problem currently affecting sea-farmed salmon and trout around the world. These external parasites have been recognized as long as man has fished for salmon. However, they have become a serious concern only since salmonids have been reared in increasingly large numbers at commercial production facilities.[1-2] The expansion of salmon and trout aquaculture in Europe, Canada and Chile has been accompanied by increasing infestations of sea lice. Sea lice feed on fish skin, mucus and blood, especially on the head, back and perianal region. Untreated infestations may lead to death from severe erosion and exposure of subcutaneous tissues, secondary bacterial infections, osmotic imbalance and extreme stress.

SLICE® is a feed premix containing the avermectin, emamectin benzoate, in a 0.2% formulation for the control of sea lice (*Lepeophtheirus salmonis* and *Caligus* spp.) on salmon and trout. Emamectin benzoate administered to salmonids in feed at a dose rate of 50 µg/kg/day for 7 consecutive days kills all parasitic stages of sea lice, i.e., chalimus, pre-adults and adults, including gravid females.

Key characteristics

- **Administered in feed**
- **Well tolerated by fish**
- **Kills all parasitic stages of sea lice**
- **Effective at all sea temperatures**
- **Sustained efficacy for up to 10 weeks**
- **Minimal environmental effect**

Emamectin is a member of the chemical class of avermectins, macrocyclic lactones, produced by fermentation of the soil actinomycete, *Streptomyces avermitilis*. Chemical modification of this fermentation product has yielded hundreds of analogues[3] including ivermectin, abamectin and doramectin which are widely used for control of animal and human parasites as well as insects and mites on crops.[4] The first member of the avermectin family, ivermectin, to be developed as an antiparasitic agent for livestock possessed both contact and systemic activity against immature and adult ectoparasites. Thus, it was logical that it would be tested in salmon for the control of sea lice. Ivermectin administered in feed proved efficacious against both chalimus and motile stages.[5-8] Ivermectin used for sea lice control has a duration of efficacy of approximately 1 month and can be toxic to fish, thus requiring a pattern of use that allows only twice weekly applications.[9-12] In many of the salmon-producing areas, ivermectin has been employed as a lousicide for salmon without regulatory approval for this application.[2] This has resulted in veterinarians recommending an extended withdrawal period because a maximum residue limit (MRL) for ivermectin in salmon tissues has been neither requested nor approved. When the manufacturer made a considered decision not to support the development of ivermectin for aquaculture, it was in the belief that its research effort on second-generation avermectins would yield a compound with significant advantages over ivermectin for use in aquaculture. Emamectin benzoate, the active principal ingredient of SLICE, is such a compound.

Other registered products available for control of sea lice either require the use of bath treatments, or they do not effectively control all parasitic stages of sea lice, or they provide poor sustained efficacy. Teflubenzuron, a benzoylphenylurea insect growth regulator, that is a non-specific inhibitor of chitin synthesis, is another available product that is administered in feed.

SLICE was developed by the research division of what is now MSD Animal Health (Merck Animal Health in the US and Canada) specifically to provide producers with a product that combines highly effective control of all parasitic stages of sea lice with safety for fish, ease of administration and sustained duration of efficacy for up to 10 weeks.

Introduction: Chemistry

Emamectin benzoate is the active ingredient in SLICE. Emamectin is a 16-member macrocyclic lactone, 4"–deoxy–4"–epi–methylamino–avermectin B1, which is a mixture of two active homologous compounds:

- **4"–deoxy–4"–epi–methylamino–avermectin B_{1a}** (minimum 90%)
- **4"–deoxy–4"–epi–methylamino–avermectin B_{1b}** (maximum 10%)

The mixture of these two homologues is termed emamectin. Emamectin is obtained synthetically from the natural avermectins B_{1a} and B_{1b} (collectively, abamectin or avermectin B_1), which differ from emamectin by the presence of a hydroxyl group at the 4" position rather than the 4"–epimethylamino group.

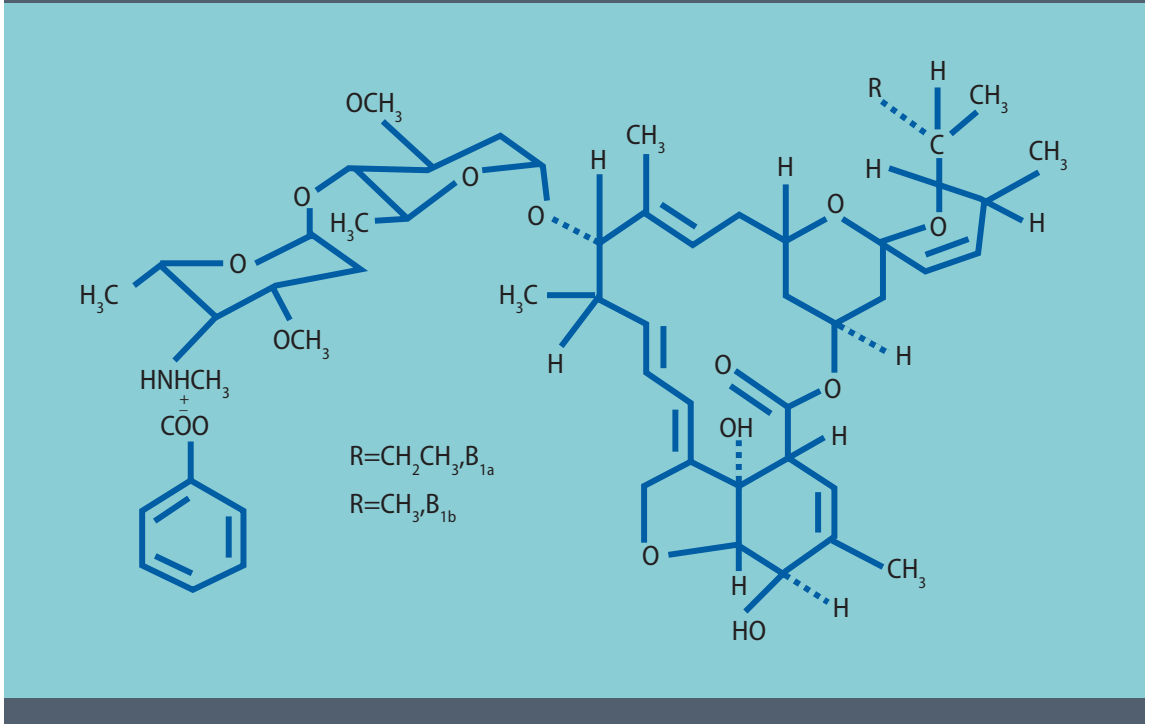
Scientific name

(4"R)-5-O-demethyl-4"deoxy-4"(methylamino) avermectin A_{1a} and (4"R)-5-O-demethyl-25-de (1-methylpropyl)-4"-deoxy-4"-(methylamino)-25-(1-methylethyl) avermectin A_{1a} (9:1)

Generic name

Emamectin benzoate

Figure 1. Chemical structure of emamectin benzoate



Molecular formula

B_{1a} component $C_{49}H_{75}NO_{13}C_7H_6O_2$
B_{1b} component $C_{48}H_{73}NO_{13}C_7H_6O_2$

Molecular weight

B_{1a} component: 1008.26 g/mole
B_{1b} component: 994.24 g/mole

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Introduction: Dosage Form

SLICE Premix

- Supplied in 2.5-kg sachets containing 5 g of emamectin benzoate (0.2% w/w)

- SLICE (emamectin benzoate) Aquaculture Premix 0.2% has a shelf life of 36 months.

Table 1. Slice components

Components	Percent (%w/w)
Emamectin benzoate	0.2
Inert ingredients	99.8

Medicated feed production (see label for explanation)

- The medicated feed can be manufactured by either a dry-coating method or a wet-coating method.

Dose rate

- Approved dose rate is 50 µg/kg of fish biomass per day for 7 consecutive days.

- SLICE should be included in 100% of the daily feeding ration.

- A 1-day withholding of feed prior to treatment is recommended.

- Suggested feeding rate for medicated feed = 0.5% of total weight of fish per pen. For example, 1,000 kg of fish should receive 5.0 kg of medicated feed per day (35.0 kg per week).

- If the feeding rate differs from 0.5% biomass/day, then the concentration of SLICE in feed must be adjusted proportionately as shown in Table 2.

- Rate of incorporation into non-medicated feeds for a 0.5% feeding rate:

  One [2.5 kg] sachet of SLICE Premix + 497.5 kg feed = 500 kg medicated feed

  Two [2.5 kg] sachets of SLICE Premix + 995 kg feed = 1,000 kg medicated feed

Table 2. SLICE Premix incorporation rates for medicated feed preparation when used at different feed rates (determined by fish biomass and temperature)

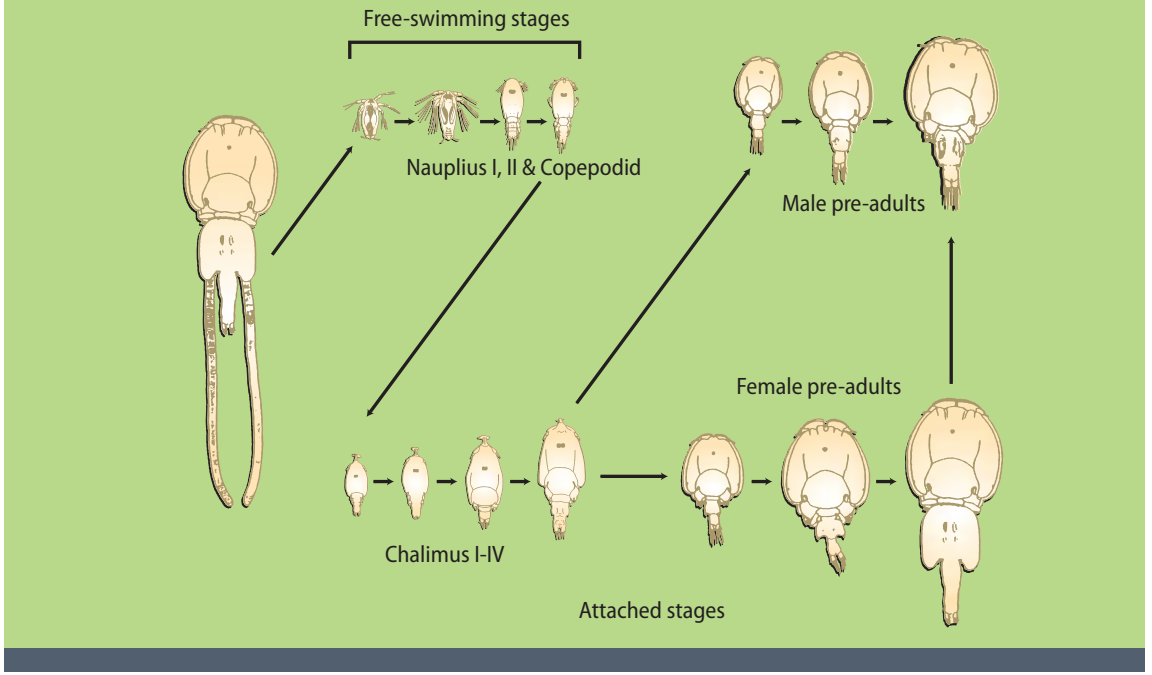
Feed rate	Amount of premix per ton of feed
0.25%	10.00 kg
0.50%	5.00 kg
0.75%	3.33 kg
1.0%	2.50 kg
1.5%	1.67 kg
2.0%	1.25 kg

Introduction: Mechanism of Action

Sea lice feed on the mucus, skin, plasma and, in severe infestations, the subcutaneous tissue of fish.[13-16] In a radiolabeled residue study, it was demonstrated that SLICE residues are present at very low concentrations in all of these tissues.[17] The chalimus stages attach to the skin by a frontal filament and are not motile, so it is most likely that they are exposed to SLICE during their feeding on mucus, skin and plasma. The pre-adult and adult stages are motile, so they move in the mucus covering the skin in addition to ingesting mucus, skin, plasma and subcutaneous tissue. Therefore, the motile stages of sea lice most likely are exposed to SLICE by contact with mucus and by feeding on various fish tissues and fluids.

The precise mechanism by which SLICE (emamectin benzoate) kills sea lice has not been fully elucidated, but through extensive research, the general mode of action for the avermectin class of compounds has been determined. The mechanism of avermectin killing is disruption of chloride ion movement in nerves, and thus, neurotransmission through competitive binding to glutamate-gated chloride channels of invertebrate nerves.[18-22] This mode of action differs from that of organophosphates that inhibit neurotransmission in sea lice by disruption of cholinesterase activity and of insect growth regulators that inhibit chitin synthesis to disrupt cuticle formation. This unique mode of action should reduce the potential for cross-resistance with other approved products used for sea lice control.

Figure 2. Stages of sea lice cycle (*Lepeophtheirus salmonis*)



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Pharmacokinetics

Absorption, distribution, metabolism and excretion studies using radiolabeled emamectin benzoate were conducted in rats, bluegill sunfish, salmon, chickens, goats and other species.[17, 23-26] Conclusions of these studies were consistent for all the species, in that emamectin benzoate was: a) well absorbed; b) rapidly excreted, nearly all in the feces; and c) the major residue with the minor metabolite (desmethylamino emamectin). Results of repeat dosing studies in rats and bluegill sunfish further confirmed that emamectin benzoate is not a bioaccumulative compound.

Studies in salmon

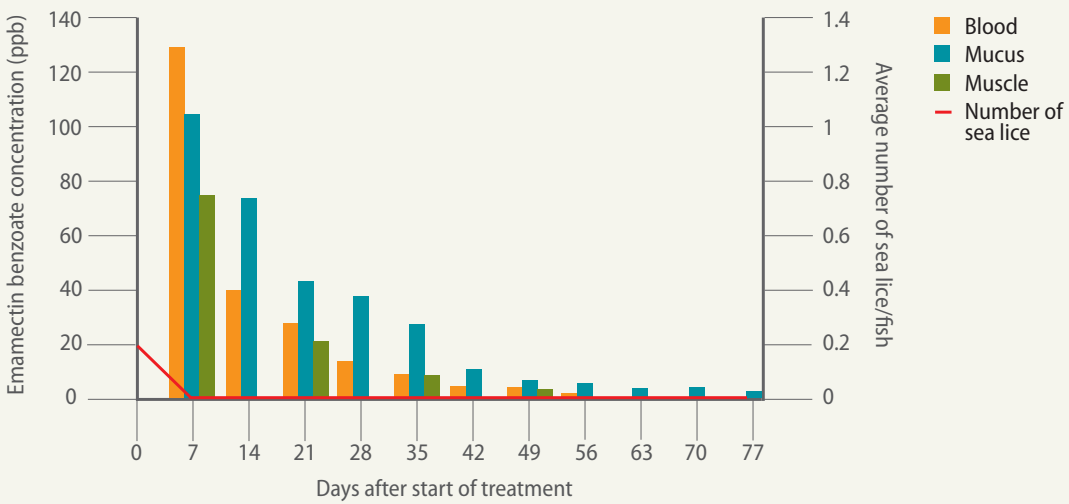
As mentioned earlier, sea lice feed on the mucus and skin of fish, as well as plasma and sometimes subcutaneous tissues. Therefore, emamectin benzoate must be able to penetrate into these secretions/tissues and persist for adequate time to be ingested/contacted by lice. Atlantic salmon, *Salmo salar*, was the representative salmonid species used for two studies to determine the fate of emamectin benzoate: a whole-body autoradiography study[27] and a radiolabeled residue depletion study conducted at both 5° C and 10° C.[17]

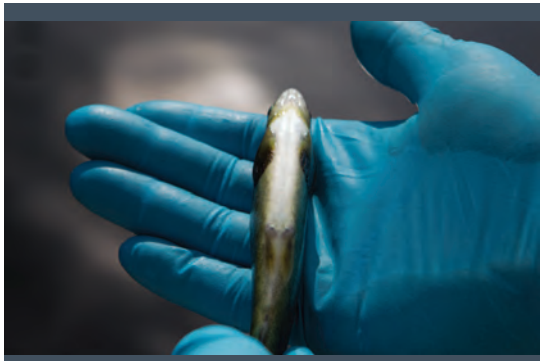
Table 3. Concentration (ppb) of emamectin benzoate in blood, mucus and muscle of Atlantic salmon (SLICE administered Days 1-7)

	Concentration (ppb) of emamectin benzoate		
Day	Blood	Mucus	Muscle
0	0	0	0
7	128.3	104.6	74.8
14	39.7	74.1	
21	27.9	42.7	20.9
28	13.1	37.6	
35	8.6	27.4	8.5
42	4.2	10.5	
49	3.5	6	3.2
56	1.7	4.9	
63	0	3	0
70	1	3.5	
77		1.4	

Results from the whole-body autoradiography study indicated that emamectin benzoate was: a) absorbed from the gastrointestinal tract and transferred to other tissues, b) widely distributed in salmon tissues including the skin, c) present in higher concentrations in skin than in muscle and d) excreted slowly because of enterohepatic circulation. The study also reported emamectin benzoate concentrations in mucus following field administration of SLICE at the recommended dose of 50 µg emamectin benzoate/kg/day for 7 days. Results summarized in Table 3 and Figure 3 show that emamectin benzoate concentrations reached maximum mean levels of 75 ppb (ng/g) in muscle on Day 7, the last day of administration. Weekly assessments showed that the concentration of emamectin benzoate in mucus decreased gradually from a maximum of 105 ppb (ng/g) at the end of treatment (Day 7) through Day 77, with a half-life of 11.3 days. Drug concentration in mucus was higher ($p < 0.05$) than that of plasma on all but one post-treatment sample dates. The ability of emamectin benzoate to sequester in mucus and skin, as demonstrated by this study, is likely a major contributor to product efficacy.

Figure 3. Average concentration (ppb) of emamectin benzoate in blood, mucus and muscle of Atlantic salmon after recommended dose of SLICE (50 µg/kg/day for 7 days)





The ability of emamectin benzoate to sequester in mucus and skin is likely a major contributor to product efficacy.

In the radiolabeled residue depletion study, salmon were fed medicated feed with radiolabeled emamectin benzoate at a target dose rate of 50 µg/kg for 7 consecutive days, but the actual daily dose received was ~33 µg/kg. Results showed that: a) maximum radioactivity concentrations in muscle and skin occurred within 72 hours after administration and declined thereafter; b) tissue radioactivity declined faster at 10° C than at 5° C, indicating that emamectin benzoate is cleared from tissues faster at higher temperatures; c) radioactivity concentrations were lower in muscle than in skin and depleted somewhat faster from muscle than from skin; d) emamectin benzoate was present at low concentrations in the mucus covering the skin for an extended period of time; and e) at either water temperature, mean radioactivity concentrations in the edible tissues (muscle/skin) never exceeded 80 ppb (microgram equivalents of emamectin per kg) at all time intervals tested from 3 hours to 90 days after fish received the final dose of medicated feed. Because fish were under-dosed by about 30%, tissue concentrations would be expected to be higher when fish are dosed accurately.

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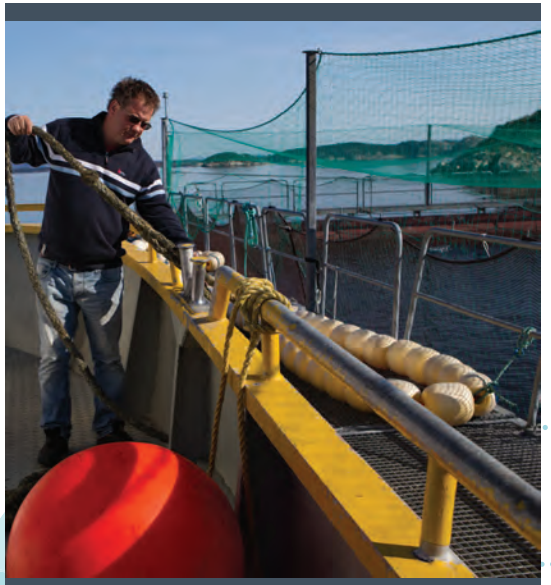
Toxicology

A complete toxicological evaluation has been conducted with emamectin benzoate. This includes extensive unpublished and published studies in mice, rats, birds and other species.[28] Results from 1-year and 2-year sub-chronic and chronic studies demonstrated that emamectin benzoate was not carcinogenic, and as a result, a No Observed Effect Level (NOEL) of 0.25 mg/kg was established. Results from another series of studies conducted to evaluate potential mutagenic and teratogenic effects showed that emamectin benzoate was not mutagenic and caused no teratogenic effects. Studies on reproductive and developmental toxicity resulted in a NOEL of 0.6 mg/kg.



Studies show that emamectin benzoate is not carcinogenic, mutagenic nor teratogenic.

Toxicology: Withdrawal Period

Maximum residue limit (MRL) — Europe: An Annex I, MRL, of 100 µg/kg (ppb) emamectin B_{1a} (marker residue) has been established for finfish, with the target tissues being muscle and skin in natural proportions.[29]

The establishment of withdrawal times for SLICE was investigated and considered by countries where the product is approved, typically based on the recommended European MRL of 100 µg/kg and the results of both radiolabeled and non-radiolabeled residue studies in salmon. As a result, no withdrawal period (0 days) is required for use of SLICE in the UK (Scotland), Ireland, Iceland, Finland, Spain, Portugal, Canada and Chile. A withdrawal period of 175 degree-days has been established in Norway and the Faroe Islands.

Toxicology: Salmon and Trout Safety Studies

Target animal safety and tolerance studies were performed with SLICE on two salmonid species: Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). A summary of the results for three studies is presented below.

Salmon safety study

This study measured the tolerance of Atlantic salmon (*Salmo salar*) to an orally administered feed medicated with SLICE.[30] Each treatment group contained 50 Atlantic salmon with a mean bodyweight of 382 g. The treatment groups were fed a diet medicated with SLICE at nominal dose rates of 0, 100, 250 and 500 µg/kg/day, respectively, for 7 consecutive days.

All fish were observed daily for 13 days, with data recorded for mortality, behavior and overall appearance. Following completion of the study on Day 13, all salmon in the trial were killed and examined by gross necropsy and histopathology.

Distinct signs of toxicity were observed only at the highest dose rate (Table 4). The signs observed were dark coloration, inappetence, lethargy and, in about 10% of fish, a loss of coordination. No pathognomonic signs of emamectin benzoate toxicity were identified during gross necropsy or histopathological examination. No treatment-related mortality was observed.

Table 4. Results of Atlantic salmon tolerance study

Daily rates* in µg/kg/day (for 7 consecutive days)	Multiple of target dose (based on 50 µg/kg/day)	Observed results
0	0x	No adverse reaction
70	1.4x	No adverse reaction
173	3.5x	No adverse reaction
356	7.1x	Progressive signs of toxicity — lethargy, dark coloration, inappetence, loss of coordination

*Actual dose rates were calculated based on the measured feed consumption and analysis of feed for emamectin benzoate concentration.

Results from this salmon safety study showed that feed medicated with SLICE, when administered at actual dose rates (based on feed analysis) of up to 3.5x the recommended label dose rate of 50 µg emamectin benzoate/kg/day, is safe for salmon.

Extended feeding

A study was conducted to evaluate the safety of SLICE when seawater-reared Atlantic salmon (*Salmo salar*) were over-dosed at up to 2.26x the approved 50 µg/kg/day rate for an extended 14-day period (double the recommended treatment duration of 7 days).[31] The study involved 240 fish (mean weight 196.6 g) that were stocked into 12 seawater tanks (20 fish/tank) and allocated to four treatment groups (three tanks/group, 60 fish/group). After an acclimation period, fish were fed either unmedicated feed or medicated feed for 14 consecutive days. Feed treated with SLICE was provided to the four groups at 0, 50, 100 or 150 µg/kg/day, representing dose rates 0x, 1x, 2x and 3x the nominal dose rate of 50 µg/kg/day. Fish were monitored for feeding activity, mortality and morbidity during the 14-day treatment period, after which all surviving fish were euthanized, necropsied and examined for gross pathology and by histopathology.

Actual dose rates proved to be 0, 42, 88 and 113 µg/kg/day, or 0%, 84%, 88% and 75% of the experimental targeted 0x, 1x, 2x and 3x treatment-group dose rates, respectively. SLICE administered at a dose rate of up to 1.76x the label dose rate of 50 µg active/kg/day for 14 days (twice the

Table 5. Results of Atlantic salmon extended-feeding tolerance study

Dose rates* in µg/kg/day (for 14 consecutive days)	Multiple of target dose (based on 50 µg/kg/day)	Observed results
0	0x	No adverse reaction
42	0.84x	No adverse reaction
88	1.76x	No adverse reaction
113	2.26x	Progressive signs of toxicity — inappetence, dark coloration, hepatic focal granuloma

*Actual dose rates were calculated based on the measured feed consumption and analysis of feed for emamectin benzoate concentration.

recommended duration of 7 days) had no detectable adverse effects on Atlantic salmon (Table 5). No mortality occurred and feeding activity was vigorous in the untreated, 0.84x and 1.76x groups. No treatment-related mortality was observed in the 2.26x group, but feeding activity declined from Study Days 9 to 14, and increased frequencies of dark skin coloration and hepatic focal granuloma were observed. Study results indicate that the feeding of SLICE at a dose of 50 µg emamectin benzoate/kg bodyweight for the recommended treatment period of 7 days would be well tolerated by Atlantic salmon.

Trout safety study

Trials involving 128 rainbow trout (*Oncorhynchus mykiss*) with a mean weight of 295 g were conducted to determine their dietary tolerance to emamectin benzoate.[30] Sixteen of these seawater-adapted trout were placed in each of eight experimental tanks, with two tanks selected for each of the following feeding regimens of feed medicated with SLICE: (nominal dose rates) 0, 100, 250 and 500 µg/kg/day for 7 consecutive days. The dose rates of this medicated daily diet represented multiples of 2x, 5x and 10x, respectively, of the recommended therapeutic dose rate of 50 µg/kg/day.

Daily observations were made for appearance, behavior and mortality for 13 consecutive days. All trout were then killed and examined by gross necropsy. In addition, five apparently healthy trout were also examined histopathologically. Results of the gross necropsy examinations were negative.

The results of this study (Table 6) showed that the diet of feed medicated with SLICE was safe for trout

Table 6. Results of rainbow trout tolerance study

Dose rates* in µg/kg/day (for 14 consecutive days)	Multiple of target dose (based on 50 µg/kg/day)	Observed results
0	0x	No adverse reaction
88	1.8x	No adverse reaction
218	4.4x	No adverse reaction
413	8.3x	Progressive signs of toxicity — lethargy, dark coloration, inappetence, loss of coordination

*Actual dose rates were calculated based on the measured feed consumption and analysis of feed for emamectin benzoate concentration.

even when fed at dosage rates of up to 4.4x the prescribed label dose rate. Distinct signs of toxicity were observed only at the highest dose. Signs of toxicity included increased melaninization (dark coloration), lethargy and inappetence. No pathognomonic signs of emamectin benzoate toxicity were identified during gross necropsy or histopathological examination. No treatment-related mortality was observed.

Environment

All chemotherapeutants currently available for the control of sea lice have the potential of causing environmental damage, depending on the exposure level[2] (with the possible exception of hydrogen peroxide baths). Environmental exposure is dependent upon a variety of factors, including the amount of active ingredient(s) used for treatment, the frequency of use, the biological activity of the active ingredient, the biological activity of any metabolites or degradation products, the degree of deposition and the sensitivity of the surrounding biota.

SLICE is administered in pelleted feed and there is typically little wastage. As a result, deposition into the surrounding environment may occur by two routes:

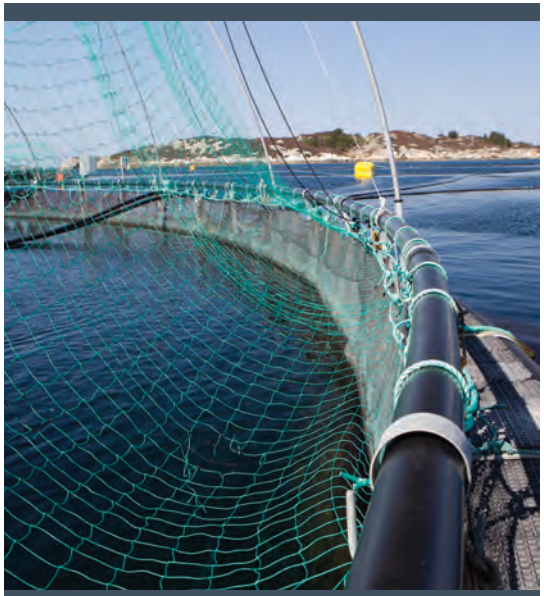
- Emamectin benzoate in uneaten feed that falls to the sea floor

- Emamectin benzoate and the desmethylamino metabolite excreted in feces of treated fish

Environmental risk assessment included evaluation of all available data for emamectin benzoate and generation of additional data specific for the use of SLICE in the marine environment. Extensive data relative to the potential environmental impact of emamectin benzoate use in terrestrial environments[32-37] have been generated during the development of emamectin benzoate for control of insects on high-value food crops intended for human consumption.[38-42]

From these studies and others undertaken by MSD Animal Health, a comprehensive data set has been developed on the toxicity of emamectin benzoate to invertebrates, fish, birds and mammals. This has enabled predictive risk assessments to be undertaken in which 100-fold assessment (safety) factors have been applied to the toxicity data for the most sensitive of the species appropriate for the environments considered. Comparison of the predicted no effect concentrations (PNEC) with the predicted environmental concentrations (PEC) in water during, and following, treatment indicates that the therapeutic use of emamectin benzoate should not affect invertebrates or vertebrates in the water.[43]

While the administration of emamectin benzoate in feed to fish reduces the overall inputs to the environment, it increases the potential for deposition in sediments. "Worst-case" models have been applied to commercial use scenarios, allowing for uneaten feed and excretion of parent compound and the primary metabolite from fish. The resultant PECs have indicated that any potential for adverse effects on sensitive sediment-dwelling biota would be limited to the immediate vicinity of the treated farm.



There is typically little wastage when fish are provided with pelleted feed medicated with SLICE.

To determine the extent of any impact, an extensive monitoring program was undertaken in a Scottish sea loch over 12 months, following the use of SLICE to treat salmon under commercial conditions.[44] Emamectin benzoate was detected in settling particulate material downstream of the cages, but the levels detected in sediments were lower than predicted by the models, indicating that it was not accumulating but being dispersed at low concentrations. Levels in sediments exceeding the PNEC for sediment-dwelling organisms were detected only within 10 meters of the cages, and lower levels were found at 12 months versus the 4-month time point. The PNEC for sediment-dwelling organisms is more than 4x the limit of detection in sediments, thus ensuring that any potential risk can be identified by the validated methods. Monitoring of the sediment-dwelling populations in the vicinity of the treated farm did not detect any changes in communities that could be attributed to treatment. Similarly, no interference was found with the seasonal rhythms in surface fauna or macrobenthic fauna. While emamectin benzoate was detected in mussels immediately following treatment, it was never found at quantifiable levels and was rapidly depurated such that within 1 month it could only be detected 10 meters downstream of the cages. Emamectin benzoate was detected in fish and invertebrates in the vicinity of the treated farm but never at levels greater than 4% of the established MRL for salmon muscle/skin. The concentrations declined such that no quantifiable levels could be detected by 4 months after treatment. No toxic effects were observed on captured crustaceans, which included three species of crabs.

Emamectin benzoate is absorbed in salmon and extensively metabolized with approximately 50% of the ingested dose being metabolized before excretion. Metabolism is rapid, with the metabolite being detected in settling material and sediment following treatment. The reductions in emamectin benzoate levels in sediments were accompanied by the appearance of the degradation metabolite, the desmethylamino metabolite, at non-quantifiable levels in samples taken in the field at 12 months. The toxicity of the metabolites of avermectins has been found to be less than those of the parent compounds.

Results from field efficacy studies indicate that SLICE can provide effective sea lice control for up to 10 weeks following a single treatment. Compared with other sea lice control agents that may have to be applied as often as twice per month, the use of SLICE should result in fewer applications for effective sea lice control. The duration of efficacy, together with exceptional control of all parasitic stages, should reduce future sea lice populations and minimize the quantities of compound delivered into the marine environment.

Field studies conducted at sites of SLICE use have demonstrated no adverse impacts upon zooplankton, pelagic crustaceans, molluscs or benthic invertebrates.

In summary, field studies conducted at sites of SLICE use have demonstrated no adverse impacts upon zooplankton, pelagic crustaceans, molluscs or benthic invertebrates. Although effects of localized organic enrichment in the "footprint" of the net pens have been observed, these investigations have not found any evidence of a toxic impact from SLICE. It can be concluded that the use of SLICE to control sea lice in accordance with label directions poses no short-term or long-term unacceptable risk to the marine ecosystems associated with commercial fish farms.

Efficacy

Overview

The efficacy of SLICE (emamectin benzoate) as a treatment for sea lice infestations on Atlantic salmon was evaluated through an extensive series of clinical studies and field trials. Initially, emamectin benzoate was used in a non-formulated state and incorporated in an edible oil for application as a coating on pelleted feed. Dose rate determination and dose rate confirmation studies were conducted at several locations throughout Scotland.

Small field trials were conducted in Scotland where fish were fed a commercial feed treated with SLICE (0.2% emamectin benzoate) Aquaculture Premix. A daily diet of medicated feed was administered at the recommended dose rate of 50 µg/kg biomass/day for 7 consecutive days.

Commercial field trials were conducted in Scotland, Norway, US, Canada and Chile. In Norway, the efficacy of SLICE was compared with that of another in-feed treatment, teflubenzuron, a chitin synthesis inhibitor known commercially as Ektobann® (Skretting A/S) or Calicide®. Results from four study sites in Norway showed feed medicated with SLICE provided better sustained efficacy against sea lice when compared with Ektobann-treated diets.

Prevention of reinfestation was demonstrated in two controlled challenge studies in Scotland with seawater-adapted smolts as well as smolts treated in freshwater prior to transfer to seawater.

Characteristics of SLICE

- High-level efficacy: SLICE rapidly killed all parasitic stages (motile and non-motile) of sea lice.
- Duration of efficacy: SLICE killed all stages of sea lice including gravid adult females for up to 10 weeks.
- Effective under a wide range of environmental conditions (e.g., water temperatures of 5-15° C and salinity from 23-35 ppt).
- Well tolerated: SLICE was well tolerated with no mortality or significant reduction in feeding associated with treatment.

The clinical evaluation of efficacy for the control of sea lice using emamectin benzoate was conducted as follows:

- Dose Titration and Dose Confirmation in Salmon
- Efficacy Field Trials (Scotland)

SLICE

Results from four study sites in Norway showed feed medicated with SLICE provided better sustained efficacy against sea lice when compared with Ektobann-treated diets.

- Dose Confirmation in Trout (Chile)
- Commercial Field Trials (Scotland, Norway, US, Canada and Chile)
- Duration of Efficacy Trial (Scotland)
- Prevention of Infestation Trial (Scotland)

5

Efficacy: Dose Titration and Dose Confirmation in Salmon

Four trials were conducted in Scotland over a 2-year period to determine and confirm the optimum dose rate of emamectin benzoate.[45] Atlantic salmon (*Salmo salar*) ranging from 150–400 g bodyweight were given a pelleted feed coated with emamectin benzoate in fish oil.

During each of these trials, treatment groups were fed the emamectin benzoate-medicated diet at various dose rates for 7 consecutive days from Day 0 to Day 6. Control groups were fed the same non-medicated commercial feed at the same rate. Efficacy was assessed by counting the number of sea lice on all fish at Day 7, Day 14 and Day 21.

A total of 580 Atlantic salmon were utilized in the dose titration and dose confirmation studies, with 414 fish in treatment groups that received medicated feed and 166 fish in the control groups fed a non-medicated diet.

Results and significant findings

Results of the studies are summarized in Table 7. Trials 1a and 1b indicated that an emamectin benzoate-medicated diet given to fish for 7 consecutive days (at dose rates between 20 and 100 µg/kg/day) resulted in effective control of both non-motile (chalimus) and motile (pre-adult and adult) stages of sea lice.

Trial 2, designed to determine the optimal dose rate of emamectin benzoate, indicated that a medicated diet fed at a dose rate of 25 to 50 µg/kg/day for 7 days provided control of all parasitic stages of the sea louse, *Lepeophtheirus salmonis*. This was further validated in subsequent dose confirmation trials (3 and 4), which resulted in the selection of 50 µg/kg/day as the dose rate for commercial product development.

Fish consumption of the emamectin benzoate-medicated feed equaled or exceeded feed intake rates of non-medicated control groups, thus indicating similar palatability. Emamectin benzoate treatments were physiologically well tolerated by all groups, with no adverse reactions or mortality observed at or above the selected dose regimen.

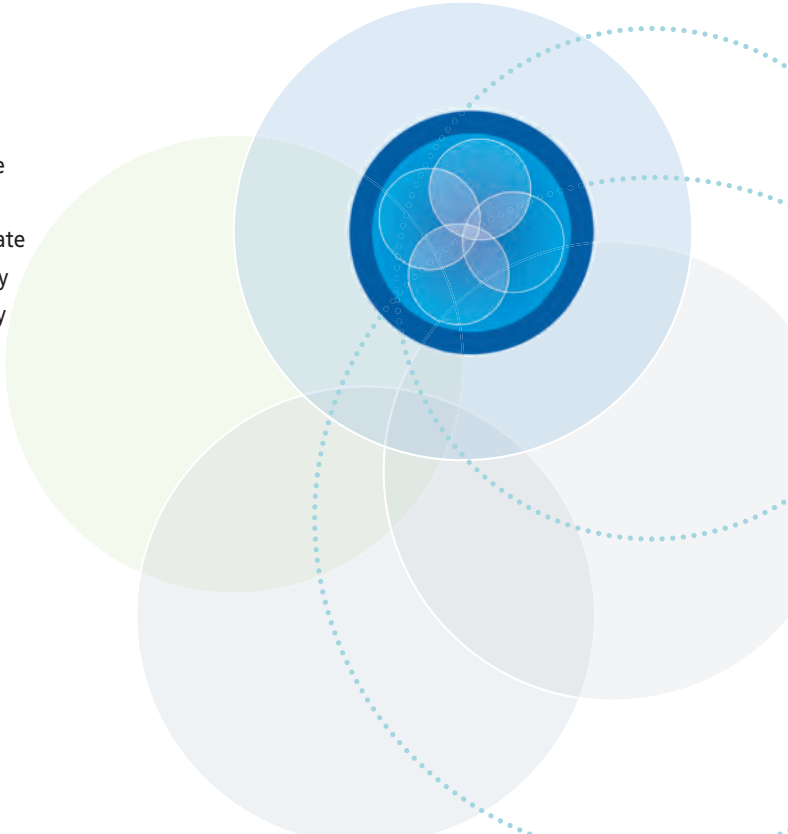


Table 7. Dose tritation and confirmation studies

Trial	Description / Objective	Sample size	Dose rate (µg/kg/day)	% Efficacy*			Summary results
				Day 7	Day 14	Day 21	
1a	Dose range-finding studies 30 fish in each of 6 tanks 15 fish per tank with induced infestation of the chalimus stage of *L. salmonis*	n = 15	0 5 10 20 100	18.2 8 36 25 61	19.2 45 82 71 97	14.5 48 87 72 100	Excellent palatability and feed consumption 87%-100% effective against the chalimus stage at dose rates between 10 and 100 µg/kg/day
1b	15 fish per tank with induced infestation of pre-adult/adult stages of *L. salmonis*	n = 15	0 5 10 20 100	7.2 0 8 13 36	8.9 0 53 58 88	9.3 18 73 83 97	83%-97% effective against pre-adult/adult stages of sea lice at dose rates between 20 and 100 µg/kg/day
2	Dose titration study 20 fish in each of 8 tanks Induced *L. salmonis* infestation (all stages)	n = 20	0 25 50 100	51.0 36.2 37.5 35.4	44.9 70.8 70.3 66.4	34.4 89.8 95.2 95.8	SLICE proven highly effective at dose rates of 25, 50 and 100 µg/kg
3	Dose confirmation, Study 1 16 fish in each of 9 tanks. Induced *L. salmonis* infestation (all stages)	n = 16	0 25 50	57.6 43.8 53.8	39.5 76.0 88.0	26.4 81.9 94.3	Proved 50 µg/kg/day for 7 days best dose rate Near complete efficacy
4	Dose confirmation, Study 2 16 fish in each of 6 tanks. Induced *L. salmonis* infestation (all stages)	n = 16	0 50	70.6 45.9	47.6 70.7	38.0 94.6	Confirmed high efficacy results at 50 µg/kg/day dosage with no adverse reactions

Note: Treatments were prepared by mixing selected test concentrations of emamectin benzoate with fish oil applied as a coating on commercial feed.
* Percent efficacy results shown for all treatment groups were calculated using Abbott's formula, based on the geometric mean number of sea lice per fish.

Shaded areas indicate the mean number of sea lice per untreated control fish.

5

Efficacy: Efficacy Field Trials (Scotland)

These extensive field studies conducted in Scotland on approximately 69,200 Atlantic salmon (ranging from 150 g–2.6 kg bodyweight) were the first in which emamectin benzoate was administered as SLICE Premix (0.2% active ingredient concentration).[46-47] During the trials, all treatment groups were fed feed medicated with SLICE at a daily dose rate of 50 µg/kg/day for 7 consecutive days and feeding rate of 0.5% biomass/day.

In contrast to previous studies conducted in tanks, this series of efficacy trials was conducted under typical production conditions, i.e., salmon were held in seawater cages. SLICE effectiveness against another common sea louse species, *Caligus elongatus,* was also assessed, and the efficacy against the sea louse species *L. salmonis* was confirmed.

In addition to the natural, continuous infestation by these two sea lice species (with constant reinfestation pressure from both copepodite and motile stages), the efficacy of SLICE was evaluated under a variety of diverse field conditions. Seawater temperatures ranged from a high of 15.5° C in the August trials to a low of 5.8° C during the winter field trials held in February. The salinity during these studies varied between 23.5 and 35.0 ppt.

Results summary

Efficacy: The efficacy of SLICE treatment against *Lepeophtheirus salmonis* in four studies (Table 8) increased from 21% to 63% one day after treatment (Study Day 7) to approximately 90% by 15 days after treatment (Study Day 21). This efficacy pattern was observed even as sea lice populations were rapidly increasing, two to three-fold on control fish (Trials 6 and 7). The efficacy was unaffected by variations in water temperature and salinity. As verified by these results, administration of feed medicated with SLICE consistently proved to be nearly 90% effective or greater at controlling sea lice even under the most adverse conditions.

Clinical appearance: The clinical appearance of sea lice-infested fish was improved after treatment with feed medicated with SLICE.

Mortality: No mortality associated with treatment was observed.

In several studies, SLICE efficacy was unaffected by variations in water temperature and salinity.

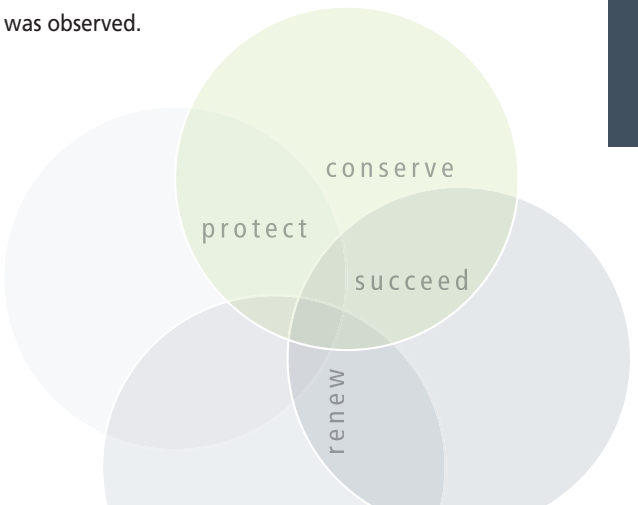


Table 8. Efficacy field trials: Scotland

Trial	Description / Objective	Sample size	Dose rate (µg/kg/day)	% Efficacy*			Summary results
				Day 7	Day 14	Day 21	
5	Field trial 1 1 x 1 study alongside commercial sea cages, 180 fish per cage [**against *L. salmonis***]	n = 30	0 50	17.0 45	12.8 80	13.8 91	91% efficacy Improved appearance 25% greater feed consumption 15% reduction in fish mortality
	[**against *C. elongatus***]	n = 30	0 50	39.3 58	24.5 89	96.8 84	89% efficacy
6	Field trial 2 2 x 2 study alongside commercial sea cages, 149 fish per cage [**against *L. salmonis***]	n = 20	0 50	34.3 63.3	48.8 93.4	73.3 99.3	99.3% efficacy No *L. salmonis* found on 25% of treated fish by Day 21
	[**against *C. elongatus***]	n = 20	0 50	20.4 45.6	13.9 79.9	11.6 81.9	81.9% efficacy; no *C. elongatus* found on 21% of treated fish Improved appearance
7	Field trial 3 2 x 2 study beside commercial sea cages, 360 fish per cage [**against *L. salmonis***]	n = 20	0 50	25.6 25.4	38.8 74.2	68.0 89.7	Infestation increased 271% in untreated control cages Treatment group cages decreased 90%
8	Field trial 4 4 x 4 study 16,000-18,000 per cage *Note: Severe worms occurred during the medicated-feed administration period.*	n = 15	0 50	40.9 20.8	36.0 36.1	38.9†† 59.4	Efficacy increased to 74.3% and 89.3% Day 28 and Day 35, respectively Unmedicated: 33-80% of fish had dorsal, ventral, cranial lesions Treated: Little evidence of any sea lice damage

Note: Salmon were naturally infected with *L. salmonis* and *C. elongatus.*

* Percent efficacy was calculated using Abbott's formula, based on the geometric mean number of sea lice per fish. †† Control fish treated with hydrogen peroxide

Shaded areas indicate the mean number of sea lice per untreated control fish.

5

Efficacy: Dose Confirmation in Trout (Chile)

A sea-pen efficacy study conducted with rainbow trout (*Oncorhynchus mykiss*) in Chile further validated the efficacy of emamectin benzoate against natural infestations of two other *Caligus* spp.: *C. flexispina* and *C. teres*.[48]

Study guidelines

Housing: Six 7m x 7m x 7m trial cages (500 trout per cage)

Treatment design: Allocated into 3 x 2 replicates (each replicate consisted of a pen treated with SLICE and a non-treated pen)

Seawater temperature: 11.0° C–12.5° C

Salinity range: 30–32 ppt

Infestation: Naturally infested with sea lice, *C. flexispina* and *C. teres*

Study design:
Treatment group: Fed diet medicated with SLICE at a target dose rate of 50 µg/kg/day for 7 consecutive days, from Day 0 to Day 6

Table 9. Efficacy of SLICE (emamectin benzoate) administered daily in feed for 7 days at a dose rate of 50 µg/kg/day against sea lice† (*Caligus flexispina* and *Caligus teres)* on rainbow trout (*Oncorhynchus mykiss*)

Trial	% Efficacy*						
	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Untreated control	(10.2)	(13.8)	(21.9)	(42.7)	(60.3)	(46.9)	(50.7)
SLICE	(11.3)	39.1	88.6	94.6	95.0	96.1	93.5

†Trout were naturally infested with chalimus, pre-adult and adult stages.
* Percent efficacy was calculated by Abbott's formula, based on the arithmetic mean number of sea lice/fish.

Control group: Received non-medicated feed throughout the trial

Data-gathering criteria: Trout were randomly selected from each cage on Study Days 3, 7, 14, 21, 28, 35 and 42. Sea lice were counted on 15 fish from each cage.

Results: SLICE efficacy against sea lice on trout was equivalent to that observed against sea lice on salmon (Table 9). The efficacy of SLICE was approximately 90% by 8 days after treatment and remained > 93% for at least 36 days after treatment.

Efficacy: Commercial Field Trials (Scotland)

The performance of SLICE for sea lice control was confirmed under commercial conditions in each of the major salmon-producing countries: Scotland, Norway, Canada and Chile. A total of approximately 870,000 Atlantic salmon were treated in these trials. In addition, a lengthy 9-year study was conducted in the US, involving over 34 million Atlantic salmon.

Commerical field trial: Scotland

Parameters and results of this study are detailed below.[47]

Housing: Sixteen 15m x 15m x 9m commercial pens with 14,163–15,961 Atlantic salmon (*Salmo salar*) per pen

Treatment design: 12 treated pens (total of 184,908 fish) and four non-treated pens (total of 62,435 fish)

Seawater temperature: 9.8° C–14.0° C

Salinity range: 13.0–31.5 ppt (at the surface)

Infestation: All fish naturally infested with *L. salmonis* and *C. elongatus* with continual reinfestation pressure during the entire study

Study design:

Treatment group: Fed feed medicated with SLICE at 50 µg/kg/day for 7 consecutive days, Day 0 to Day 6, then non-medicated feed throughout the remaining trial period

Control group: Fed non-medicated feed at rate of 1.0% biomass/day

Feed preparation: Feed medicated with SLICE was prepared at a commercial feed mill by coating SLICE Premix onto pelleted feed, with the addition of a final coating of fish oil.

Data-gathering criteria: Ten fish were randomly selected from four treated pens and four non-treated pens for counting of sea lice on Study Days -1, 13, 27 and 77. In addition, sea lice were counted on five fish from one non-treated pen and one treated pen on Study Days 34, 42, 49, 54, 64 and 72.

Results:

- Feeding a diet medicated with SLICE proved to be over 90% effective in the control of sea lice for 58 days after treatment (Table 10).
- Reduced skin damage: 70 days after treatment, approximately 50% of non-treated fish had dermal lesions from sea lice infestation, while less than 10% of salmon medicated with SLICE showed sea lice damage.
- Reduced sea lice reproductive potential by 80%: For up to 70 days after treatment, the percentage of gravid (egg-bearing) sea lice females on salmon medicated with SLICE was reduced by 80%. This reduction in gravid females may have dramatic impact on future sea lice populations within the SLICE treatment area.
- Well tolerated: Fish fed a diet medicated with SLICE exhibited no adverse health effects or mortality related to this treatment.

Table 10. Efficacy of SLICE (emamectin benzoate) administered daily in feed for 7 days (Day 0 to Day 6) at a dose rate of 50 µg/kg/day against sea lice (*Lepeophtheirus salmonis)* on Atlantic salmon (*Salmo salar*) naturally infested with chalimus, pre-adult and adult stages, Scotland, 1997

Study Day	No. pens sampled	No. fish/pen sampled	Treatment group	% Efficacy*	% Fish with lice	% Gravid females
-1 Pre-treatment	4	10	SLICE	(3.5)	95.0	56.2
	4	10	Untreated	(2.4)	72.5	57.9
13	4	10	SLICE	77.4	52.5	20.0
	4	10	Untreated	(3.1)	95.0	56.5
27	4	10	SLICE	89.3	25.0	35.3
	4	20	Untreated	(2.8)	90.0	87.9
34	1	5	SLICE	91.2	20.0	0
	1	5	Untreated	(6.8)	100	100
42	1	5	SLICE	97.8	20.0	0
	1	5	Untreated	(9.0)	100	75.0
49	1	5	SLICE	97.6	20.0	0
	1	5	Untreated	(8.4)	100	33.0
54	1	5	SLICE	94.0	40.0	0
	1	5	Untreated	(16.6)	100	36.0
64	1	5	SLICE	95.8	40.0	0
	1	5	Untreated	(28.4)	100	50.0
72	1	5	SLICE	43.8	80.0	0
	1	5	Untreated	(14.6)	100	67.0
77	4	10	SLICE	16.7	100	20.2
	4	10	Untreated	(27.0)	100	55.0

( ) Numbers in parentheses are the mean number of sea lice/fish.
* Percent efficacy was calculated by Abbott's formula, based on the geometric mean number of sea lice/fish.

Table 11. Efficacy of SLICE administered daily in feed for 7 days (Day 0 to Day 6) at a dose rate of 50 µg/kg/day against sea lice (*Caligus elongatus)* on Atlantic salmon (*Salmo salar*) naturally infested with chalimus, pre-adult and adult stages

Treatment	% Efficacy*		
	Day 4	Day 13	Day 27
Untreated control	(3.3)	(2.8)	(0.7)
SLICE	(4.8)	100	100

Efficacy: Commercial Field Trials (Norway)

SLICE (emamectin) vs. Ektobann (teflubenzuron)

In these trials conducted at four different sites in western Norway, a total of 1,170,543 Atlantic salmon were treated with either SLICE (emamectin benzoate) or Ektobann (teflubenzuron; also marketed as Calicide) at their recommended therapeutic dose rates.[49]

The 561,266 salmon in the groups treated with SLICE received a target dose rate of 50 µg/kg bodyweight/day for 7 consecutive days. Salmon in the Ektobann-treatment groups, numbering 609,277, were administered a target dose rate of 10 mg/kg bodyweight/day, also for 7 consecutive days.

Salmon in these Norwegian trials ranged in size from 92 g to 347 g. All were held under commercial rearing conditions and fed a diet medicated with SLICE or an Ektobann-medicated diet at the rate of 0.5% biomass/day throughout the treatment period.

Housing: Six commercial rearing pens

Treatment design: Allocated randomly into 3 x 2 replicates (each replicate consisted of a pen treated with SLICE and an Ektobann-treated pen)

Seawater temperature: 12.8° C-15.8° C

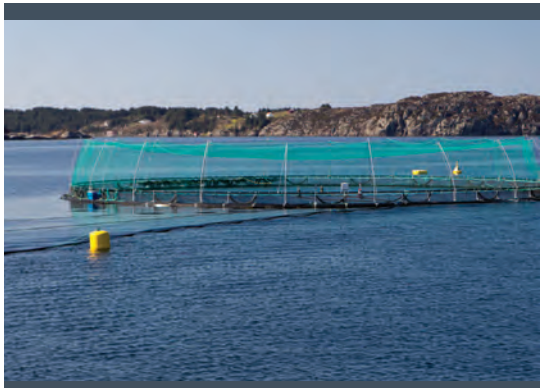
Salinity range: 13.0–31.5 ppt (at the surface)

Infestation: Naturally infested with primarily *L. salmonis* and secondarily *C. elongatus*

Data-gathering criteria: Salmon (20) were randomly selected from each cage and killed on Study Days -2, 1, 7, 14, 21, 36, 51 (Study Day 0 was the first day of treatment). Sea lice in all stages (chalimus, pre-adult, adult) were counted.

Results: In all four of these Norwegian studies, there was an excellent response to SLICE therapy within 7 days of beginning treatment (Table 12). After 21 days, the mean number of sea lice on fish that received the diet medicated with SLICE was significantly lower than those treated with Ektobann. This efficacy differential continued, following observations on Day 36 and again on Day 51. At Site 2, the mean number of sea lice 51 days after treatment on salmon treated with SLICE was 0.23 lice/fish, while fish administered Ektobann presented increased reinfestation levels with an average of 13.9 sea lice per fish.

Separate analysis (data not shown) of the efficacy against individual stages of sea lice showed that no chalimus were found on fish treated with SLICE by Day 36 (Site 1). At Site 2, chalimus numbers on treated fish increased by Day 51 to 0.23 chalimus/fish treated with SLICE and 10.5 chalimus/Ektobann-treated fish. In addition, no pre-adults or adults were found on fish treated with SLICE on Day 51 (Site 2).



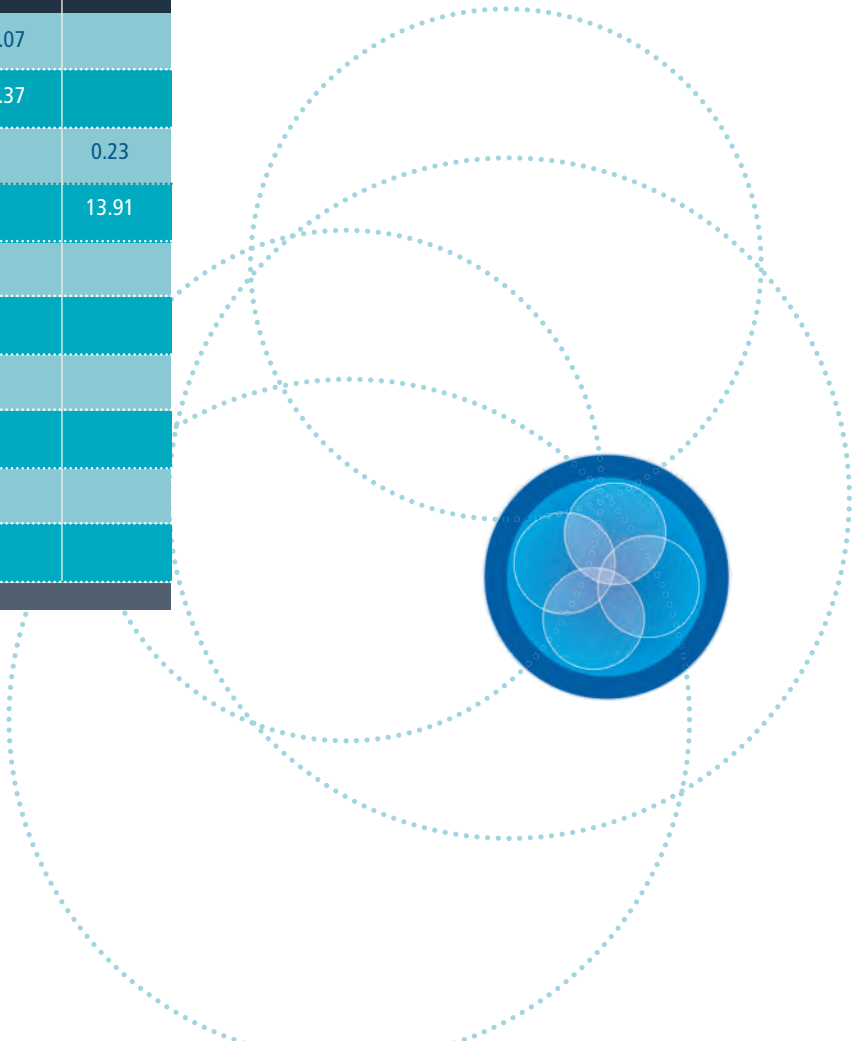
Within 7 days after the start of treatment, the response to SLICE therapy was excellent.

Efficacy: Commerical Field Trials (Norway)

Table 12. Efficacy of SLICE (emamectin benzoate) and Ektobann (teflubenzuron) for control of sea lice on salmon, Norway, 1998

Site	Treatment	No. of fish treated	Mean number of lice (all stages) per fish						
			Day -2	Day 1	Day 7	Day 14	Day 21	Day 36	Day 51
1	SLICE	100,369	3.00*	3.45	0.79**	0.38**	0.03**	0.07	
	Ektobann	111,203	3.02*	3.00	1.05**	1.13**	1.30**	0.37	
2	SLICE	129,469	3.70	1.63	0.25	0.05	0.07		0.23
	Ektobann	130,945	4.43	2.80	0.00	0.15	0.07		13.91
3	SLICE	176,550	4.11	1.73	0.67	0.55	0.48		
	Ektobann	193,166	2.53	1.50	0.58	0.53	0.93		
4	SLICE	154,608	9.70	7.83	2.07	0.63	0.68		
	Ektobann	173,963	9.28	8.01	1.90	1.32	2.28		
Summary (all sites)	SLICE	561,266	5.13	3.66	0.94	0.40	0.32		
	Ektobann	609,277	4.82	3.83	0.88	0.78	1.18		

* Indicates sea lice were counted 1 day earlier than shown in the column heading.
** Indicates sea lice were counted 1 day later than shown in the column heading.



Efficacy: Commercial Field Trials (US)

An extensive field efficacy study conducted in Maine (US) from 2001-2009 involved nearly 35 million Atlantic salmon ranging in size from smolts (newly transferred to sea) to market-weight fish.[50-51] All fish were administered feed medicated with SLICE for treatment of sea lice infestations (*Lepeophtheirus salmonis* and *Caligus* spp.).

Sea lice populations were monitored when water temperatures exceeded 4° C, which usually occurred between April and December in typical years. Monitoring was initially accomplished monthly by sampling five fish in each of five pens when water temperature was between 4° C–8° C, and biweekly when water temperature exceeded 8° C. Later, sea lice populations were monitored biweekly when water temperature was between 4° C–8° C and weekly when water temperature exceeded 8° C. Sea lice were counted on a total of 49,656 fish. When lice counts achieved a threshold of 0.2 gravid female *L. salmonis* per fish, all fish were treated with SLICE at the recommended dose of 50 µg emamectin/kg/day for 7 days.

Parameters and results of this study are detailed below.

Housing: Commercial rearing pens

Water temperature: 9.8° C–14.0° C

Infestation: All fish naturally infested with *L. salmonis* and *C. elongatus* with continual reinfestation pressure during the entire study

Study design: Approximately 34,898,122 Atlantic salmon treated with feed medicated with SLICE from 2001 to 2009 (treated biomass = 39,934,721 kg). Treatment initiated when monitoring indicated infestation. SLICE administered at 50 µg emamectin/kg/day for 7 days, with a total of 94 marine treatments administered.

Data collection criteria: Salmon monitored weekly post-treatment for sea lice infestation. At each sampling time, five fish were sampled from each cage and from five net pens per site. The *L. salmonis* counts were the sum of larvae, pre-adults, males and gravid females; the *C. elongatus* counts were analyzed separately from *L. salmonis* counts.

Of 94 marine treatments, 36 were administered for precisely 7 days but three of these treatments lacked pre-treatment sea lice counts. Therefore, statistical analysis was based on the remaining 33 treatments, which were administered for exactly 7 days from 2001-2006. SLICE efficacy was determined by comparing the mean number of lice observed on fish prior to treatment with the mean number of lice observed on fish at weekly intervals after the 7-day treatment period.

Results: Averaged across 6 years of the study (2001-2006), the median percent efficacy of SLICE against *L. salmonis* increased from 32.7% at 1 week after treatment to 92.5% by 4 weeks after treatment (Table 13). The median percent efficacy against *C. elongatus* increased from 65.0% at 1 week after treatment to 100.0% at 4 weeks after treatment, and exceeded 90% from 2 to 6 weeks after treatment.

Administration of feed medicated with SLICE to salmon infested with sea lice (*L. salmonis* and *C. elongatus*) was highly effective in reducing infestation levels and preventing reinfestations.

Table 13. Percent efficacy of SLICE during weeks 1 to 7 post-treatment based on 33 treatments administered for exactly 7 days

Weeks after treatment	1	2	3	4	5	6	7
Efficacy against *L. salmonis* (%)	32.7	82.7	90.1	92.5	87.8	71.8	54.0
Efficacy against *C. elongatus* (%)	65.0	96.1	89.1	100.0	97.5	98.6	88.9

5

Efficacy: Commercial Field Trials (Canada)

In a Canadian efficacy study, salmon fed readily on feed medicated with SLICE and no adverse events were observed.

An efficacy study with Atlantic salmon was conducted at two commercial sea farm sites in eastern Canada.[52] Four cages at each site were selected for the study, i.e., two cages received feed medicated with SLICE and two cages were non-treated. A total of 76,210 fish were treated with SLICE, and 75,141 fish started the study as non-treated controls. Fish weighed approximately 470 g at the start of the study and received a target dose rate of 50 µg/kg biomass/day for 7 consecutive days, Day 0 to Day 6. Feeding rates for the sites were 2.7% and 3.1% biomass/day, respectively.

Parameters and results of this study are detailed below.

Housing: Eight commercial rearing pens

Treatment design: Two replicates (one treated pen and one non-treated pen/replicate) per site

Infestation: Fish were naturally infested with sea lice, primarily *Lepeophtheirus salmonis* and secondarily with *Caligus elongatus*.

Data collection criteria: Ten salmon were randomly selected from each cage by hand net at each sea farm site on Study Days -5/-6, 7/8, 14/16, 22, 28/29 and 43/44. Additionally, at one site, 10 fish were sampled on Study Days 57 and 73 and five fish were sampled on Study Days 92 and 115. Fish were anesthetized and the number of sea lice (chalimus, pre-adult/adult, gravid females) were counted on each fish.

Results: Data were analyzed for each sea lice stage: non-motile, motile and gravid female. The percent efficacy based on the total number of sea lice/fish is shown in Table 14. The non-treated control pens had to be treated with Salmosan®, (azamethiphos, Fish Vet Group) on three occasions during the study: Site 1 on Study Days 9, 26 and 34, and Site 2 on Study Days 10, 33 and 58. As a result of the control group treatments, the calculated efficacy was lower than would otherwise have been observed. The duration of the clinical effect of SLICE on sea lice populations was confirmed statistically through 44 days, and despite considerable reinfestation pressure, nearly complete sea lice control was observed through 67 days after treatment (Study Day 73). Efficacy declined from 79% on Study Day 92 to 63% on Study Day 115 when deteriorating weather conditions prevented further monitoring of lice populations. Pre-adult, adult and gravid female sea lice were virtually eliminated on fish treated with SLICE. Salmon fed readily on feed medicated with SLICE and no adverse events were observed during the study.

Table 14. Efficacy (%) of SLICE against sea lice (*Lepeophtheirus salmonis)* on Atlantic salmon (*Salmo salar*) in a multicentered field trial, New Brunswick, Canada, 1995

Study Day	C/T	N	Copepodites and chalimus	Pre-adults and adults	Gravid females	Total # lice
-12	C	2	(2.0 ± 0.4)	(0.3 ± 0.2)	(0.1 ± 0.1)	(2.4 ± 0.5)
	T	2	(1.7 ± 0.1)	(0.7 ± 0.2)	(0.0 ± 0.0)	(2.3 ± 0.1)
-5 or -6	C	4	(3.6 ± 2.6)	(1.9 ± 0.7)	(0.0 ± 0.0)	(5.5 ± 2.3)
	T	4	(7.4 ± 7.3)	(2.2 ± 1.6)	(0.0 ± 0.0)	(9.5 ± 5.9)
7 or 8	C	4	(3.6 ± 3.3)	(15.7 ± 10.4)	(0.1 ± 0.2)	(19.3 ± 13.4)
	T	4	16.7%	82.8%	100%	70.5%
14 or 16	C	4	(4.3 ± 4.6)	(2.2 ± 2.5)	(0.1 ± 0.1)	(6.5 ± 7.2)
	T	4	58.1%	63.6%	100%	58.5%
22	C	4	(15.7 ± 17.2)	(10.7 ± 11.7)	(0.1 ± 0.1)	(26.5 ± 28.8)
	T	4	84.1%	94.4%	100%	88.3%
28 or 29	C	4	(17.7 ± 20.9)	(8.7 ± 8.1)	(0.2 ± 0.2)	(26.3 ± 28.8)
	T	4	96.1%	94.3%	100%	95.4%
35	C	2	(34.9 ± 12.3)	(14.6 ± 7.6)	(0.3 ± 0.2)	(49.7 ± 20.1)
	T	2	80.0%	93.2%	100%	84.1%
43 or 44	C	4	(1.9 ± 1.4)	(2.6 ± 3.0)	(0.5 ± 0.6)	(5.1 ± 5.0)
	T	4	0%	96.2%	100%	60.8%
57	C	2	(7.1 ± 1.2)	(4.4 ± 2.4)	(0.1 ± 0.1)	(11.5 ± 3.5)
	T	2	92.3%	100%	100%	95.6%
73	C	2	(6.6 ± 1.3)	(16.6 ± 1.5)	(0.4 ± 0.4)	(23.6 ± 3.2)
	T	2	71.2%	98.2%	100%	90.7%

Treatment (T) or Control (C)
( ) Indicates the mean number of sea lice/per fish ± standard deviation
Note: As one size, the efficacy was 79.2% on Study Day 92 and 62.9% on Study Day 115.

Efficacy: Commercial Field Trials (Chile)

An efficacy study with Atlantic salmon was conducted at a commercial sea farm site in the vicinity of Puerto Montt, Chile.[53] Six cages were selected for the study, i.e., three cages received feed medicated with SLICE and three cages were non-treated. Fish received a target dose rate of 50 µg/kg biomass/day for 7 consecutive days, Day 0 to Day 6.

Parameters and results of this study are detailed below.

Housing: Six commercial rearing pens

Treatment design: Three treated and three untreated pens

Infestation: Fish were naturally infested with sea lice, primarily *Caligus flexispina*.

Data collection criteria: Salmon (n = 10) were randomly selected from each cage by hand net on Study Days -1, 26, 46 and 102. Fish were anesthetized and the number of sea lice (chalimus, pre-adult/adult, gravid females) were counted on each fish.

Results: Data were analyzed for each stage: non-motile, motile and gravid female. The percent efficacy based on the total number of sea lice/fish is shown in Table 15. The efficacy of SLICE exceeded 90% against all parasitic stages of *Caligus*. Infestations were not monitored between Study Days 46 and 102 because of an algal bloom. Efficacy against non-motile stages and pre-adults declined to zero during this period. However, efficacy against gravid females exceeded 80% 95 days after treatment was concluded. The suppression, induced by SLICE, of maturing reproductive females would be expected to cause eventual reductions in reinfestation pressure. Hence, fewer sea lice treatments would be required per growing season. Salmon fed readily on feed medicated with SLICE and no adverse events were observed during the study.

Table 15. Efficacy of SLICE against sea lice (*Caligus flexispina)* on Atlantic salmon (*Salmo salar*) when administered as a dose rate of 50 µg/kg/day for 7 consecutive days, Chile, Region X, 1998

Study Day	C/T	N	Copepodites and chalimus (1-4)	Pre-adults and adults	Gravid females	Total # lice
-1	C	3	(38.6)	(34.8)	(18.1)	(91.5)
	T	3	(15.9)	(26.3)	(12.7)	(54.8)
26	C	3	(62.2)	(11.5)	(11.3)	(84.9)
	T	3	79.3% (12.9)	84.3% (1.8)	88.2% (1.3)	81.2% (16.0)
46	C	3	(114.9)	(26.3)	(30.2)	(171.4)
	T	3	92.1% (8.8)	90.3% (2.5)	96.3% (1.1)	92.6% (12.4)
102	C	3	(79.8)	(14.3)	(23.5)	(117.6)
	T	3	0% (61.1)	0% (7.5)	83.7% (3.8)	48.0% (61.1)

Treatment (T) or Control (C)
( ) Indicates the mean number of sea lice/per fish ± standard deviation

Efficacy: Duration of Efficacy Trial (Scotland)

A study was conducted in Scotland to determine the duration of efficacy of SLICE following periodic experimental sea lice reinfestation challenges.[54] A total of 612 Atlantic salmon seawater-adapted smolts (mean weight 92.9 g) were allocated to each of two experimental tanks. Fish were marked with a dye to identify control (untreated) and treated fish. One tank of fish was treated with feed medicated with SLICE at the recommended rate of 50 µg emamectin benzoate/kg/day for 7 consecutive days (Study Days 0-6), and the other tank received an unmedicated ration. On Study Day 11, the two groups were re-allocated to 18 tanks (1 m^3) so that each tank held 17 control fish and 16 or 17 treated fish. At weekly intervals, the control and treated fish in two replicate tanks were challenged with larval copepodites of *Lepeophtheirus salmonis*. Challenges were carried out on Study Days 27, 34, 41, 48, 55, 62, 69, 76 and 83. Each tank was subjected to only one copepodite challenge. Thus, nine pairs of tanks were challenged at post-treatment intervals of 3 to 11 weeks.

Parameters and results of this study are detailed below.

Housing: 18 experimental holding tanks

Challenge: Experimental challenge infestations of 76 to 200 *L. salmonis* copepodites/fish

Treatment design: 306 Atlantic salmon smolts treated for 7 days with SLICE, 306 smolts untreated (controls). Five days after treatment, groups commingled across nine pairs of tanks (16-17 treated fish and 17 untreated control fish per tank). Pairs of tanks received a single challenge at weekly intervals, 3 to 11 weeks following treatment.

Data collection criteria: Developing lice on each fish were enumerated 8 to 15 days after each challenge when the majority of the lice were at chalimus stage IV. Control and treated fish in each of the two replicate tanks were anesthetized for enumeration. Once chalimus started to develop on treated fish, the control and treated fish were divided into separate tanks to prevent cross-infestation of motile lice and held over for further evaluation of lice development.

Results: Treatment with SLICE prevented the development of settled copepodites for at least 41 days from the start of treatment, and for subsequent challenges up to 69 days from the start of treatment, the number of chalimus present on treated fish remained low (Table 16). Treated fish challenged from Study Days 27-69 had significantly ($p < 0.005$) lower numbers of lice than control fish. In the two tanks challenged at Study Day 83, both treated groups still had a lower mean number of lice than the two control groups, and some treated fish had fewer lice than any control fish. Treatment also reduced the numbers of newly recruited chalimus maturing to adults on fish challenged as late as Study Day 83. Survival of female lice on treated fish was lower than that of male lice when compared to the control groups. There did not appear to be any effects on sea lice fecundity, although there may be some delay in egg production and hatching where lice are exposed to sub-lethal doses of SLICE.

Conclusions: Treatment of fish with a diet medicated with SLICE resulted in a duration of efficacy of > 90% for at least 65 days post-treatment. Efficacy remained high (> 70%) for at least 81 days post-treatment. Treated fish had a lower average number of lice per fish than did the controls through Day 98 of the study.

Efficacy: Duration of Efficacy Trial (Scotland)

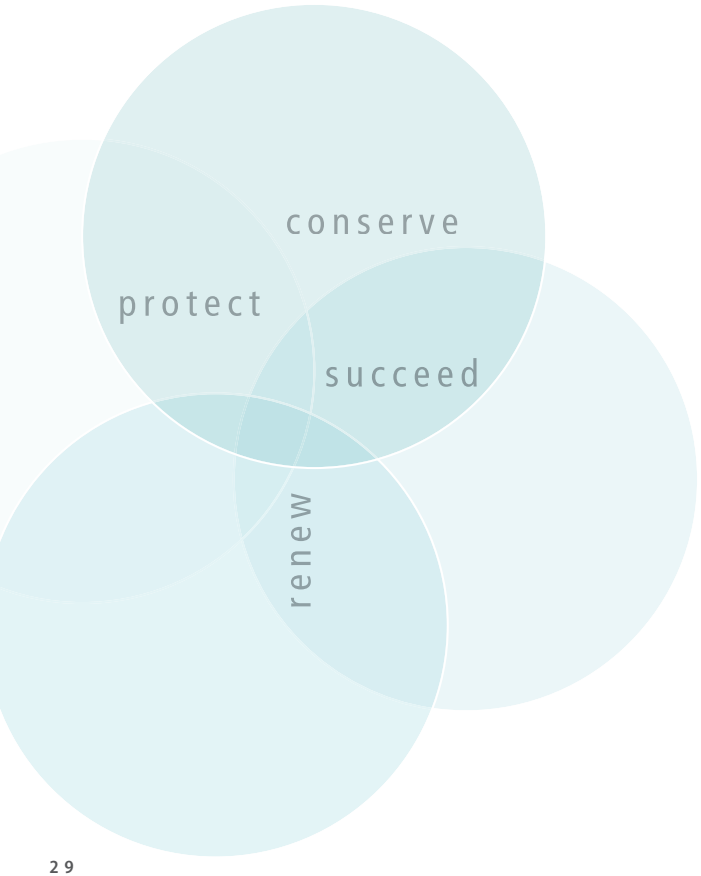


Table 16. SLICE duration of efficacy against sea lice (*L. salmonis)* on Atlantic salmon (SLICE administered Days 0-6)

Study Day challenged (Study Day sampled)	Treatment group	Mean number of lice/fish	SLICE efficacy
27 (41)	SLICE	10.5*	85.0%
	Untreated	69.3	
34 (43)	SLICE	1.9*	97.3%
	Untreated	71.0	
41 (49)	SLICE	22.0*	85.4%
	Untreated	151.2	
48 (58)	SLICE	5.3*	94.8%
	Untreated	101.9	
55 (65)	SLICE	6.6*	91.5%
	Untreated	77.4	
62 (73)	SLICE	7.1*	87.8%
	Untreated	58.1	
69 (81)	SLICE	22.7*	74.2%
	Untreated	88.1	
76 (89)	SLICE	47.6	36.0%
	Untreated	74.3	
83 (98)	SLICE	30.2	35.4%
	Untreated	46.9	

* $p < 0.005$ SLICE vs. untreated
Percent efficacy was calculated by Abbott's formula, based on the mean number of sea lice/fish.

Efficacy: Prevention of Infestation Trial (Scotland)

A study was conducted in Scotland to determine the efficacy of SLICE in preventing sea lice infestation in Atlantic salmon treated at the end of the freshwater phase, and then challenged with sea lice following transfer to seawater.[55] The study involved 840 Atlantic salmon smolts (40-85 g) that were allocated to two freshwater tanks and marked with dye for identification. One tank of fish was treated with SLICE at the recommended rate of 50 µg emamectin benzoate/kg/day for 7 days (Study Days 0-6). On Study Day 9, fish were transferred to eight seawater tanks (30 treated, 30 control fish per tank). Ten additional control indicator fish, marked with a colored tag, were added to each tank to determine the success of sea lice challenges.

On Study Day 28, 3 weeks after treatment, two tanks were challenged with copepodites of *Lepeophtheirus salmonis*. The remaining pairs of tanks were challenged on Study Day 56 (7 weeks post-treatment), Study Day 77 (10 weeks post-treatment), and Study Day 109/113 (15 weeks post-treatment).

Housing: Two freshwater tanks, then transfer to eight seawater tanks

Water temperature: 6° C–11° C

Challenge: Experimental challenge with copepodites of *L. salmonis*

Treatment design: 420 Atlantic salmon smolts treated for 7 days with SLICE, 420 smolts untreated (controls). Treatment administered in freshwater tank, then fish allocated to four pairs of seawater fish tanks with treatment groups commingled (30 treated, 30 untreated control, 10 challenge-indicator fish per tank). Pairs of tanks received copepodite challenge at 3, 7, 10 or 15 weeks following treatment.

Data collection criteria: When lice in each group reached chalimus stage III or IV, control and treated fish from each tank were sacrificed and the number of lice recorded. Once chalimus appeared on treated fish, following challenge at Study Day 109, control and treated fish were retained in separate tanks to avoid transfer of motile lice between groups and sampled again when lice were at pre-adult and adult stages.

Results: In fish challenged at Study Days 28, 56 and 77, SLICE efficacy was 83.33% to 99.8% (Table 17). Treated fish in both replicates had significantly ($p < 0.001$) fewer lice than control fish. Most lice on control fish were at chalimus stage III or IV when sampled 16 to 19 days after each challenge, whereas most of the lice present on treated fish were still copepodites.

When fish challenged at day 109 from the start of treatment were sampled at Study Day 128, efficacy had declined to 44.3% although 7% to 20% of treated fish still had no chalimus or motile lice present. When these fish were sampled again at Study Day 159, subsequent survival of chalimus to adult lice was lower on treated fish than on control fish, and efficacy increased to 73.0%. Treated fish challenged at Day 109 had significantly ($p < 0.05$) higher mean weights than control fish at Day 159.

Conclusions: SLICE treatment of salmon smolts in freshwater, prior to transfer to seawater, was highly successful, and development of lice to chalimus was prevented for at least 77 days from the start of treatment.

Efficacy: Prevention of Infestation (Scotland)

Table 17. Efficacy of SLICE against all stages of sea lice (*L. salmonis*: copepodite/chalimus, pre-adult/adult, gravid female) on Atlantic salmon (SLICE administered Days 0-6)

Study Day challenged (Study Day sampled)	Treatment group	Mean number of lice/fish	SLICE efficacy
28 (35)	SLICE*	6.2	83.3%
	Untreated	37.2	
28 (44)	SLICE	7.3	85.0%
	Untreated	48.5	
56 (75)	SLICE	0.03	99.8%
	Untreated	21.9	
77 (96)	SLICE	2.1	93.6%
	Untreated	33.7	
109/113 (128)	SLICE	7.3	44.3%
	Untreated	13.1	
109/113 (159)	SLICE	1.4	73.1%
	Untreated	5.2	

* $p < 0.001$ SLICE vs. untreated
Percent efficacy was calculated by Abbott's formula, based on the mean number of sea lice/fish.

Conclusions

The high-order efficacy of feed medicated with SLICE demonstrated against all stages of sea lice, for up to 10 weeks post-treatment, should lead to improved sea lice control as well as fewer overall treatments. And since a diet treated with SLICE can eliminate pre-adult and adult sea lice, administering SLICE treatments early in the year could significantly reduce or delay the development of economically significant parasite populations, where wild fish are not a major source of reinfestation.

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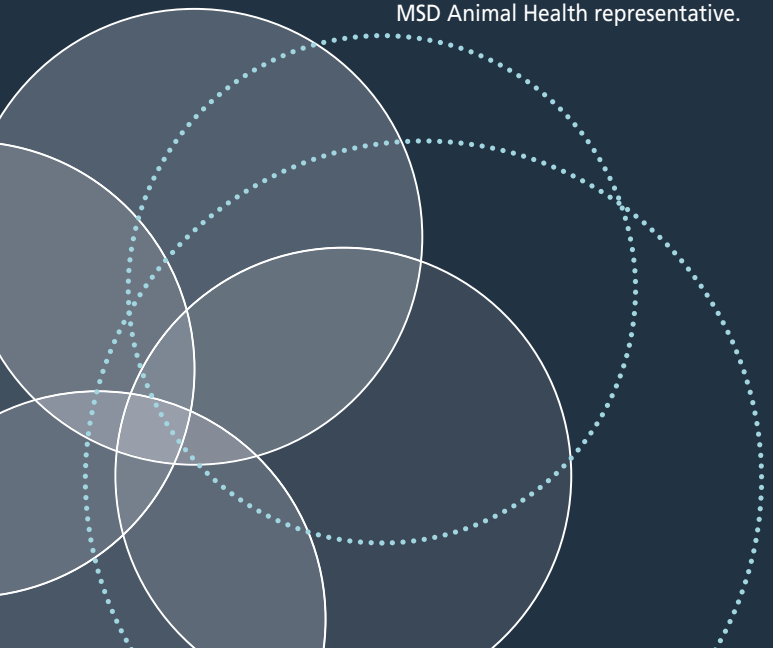

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