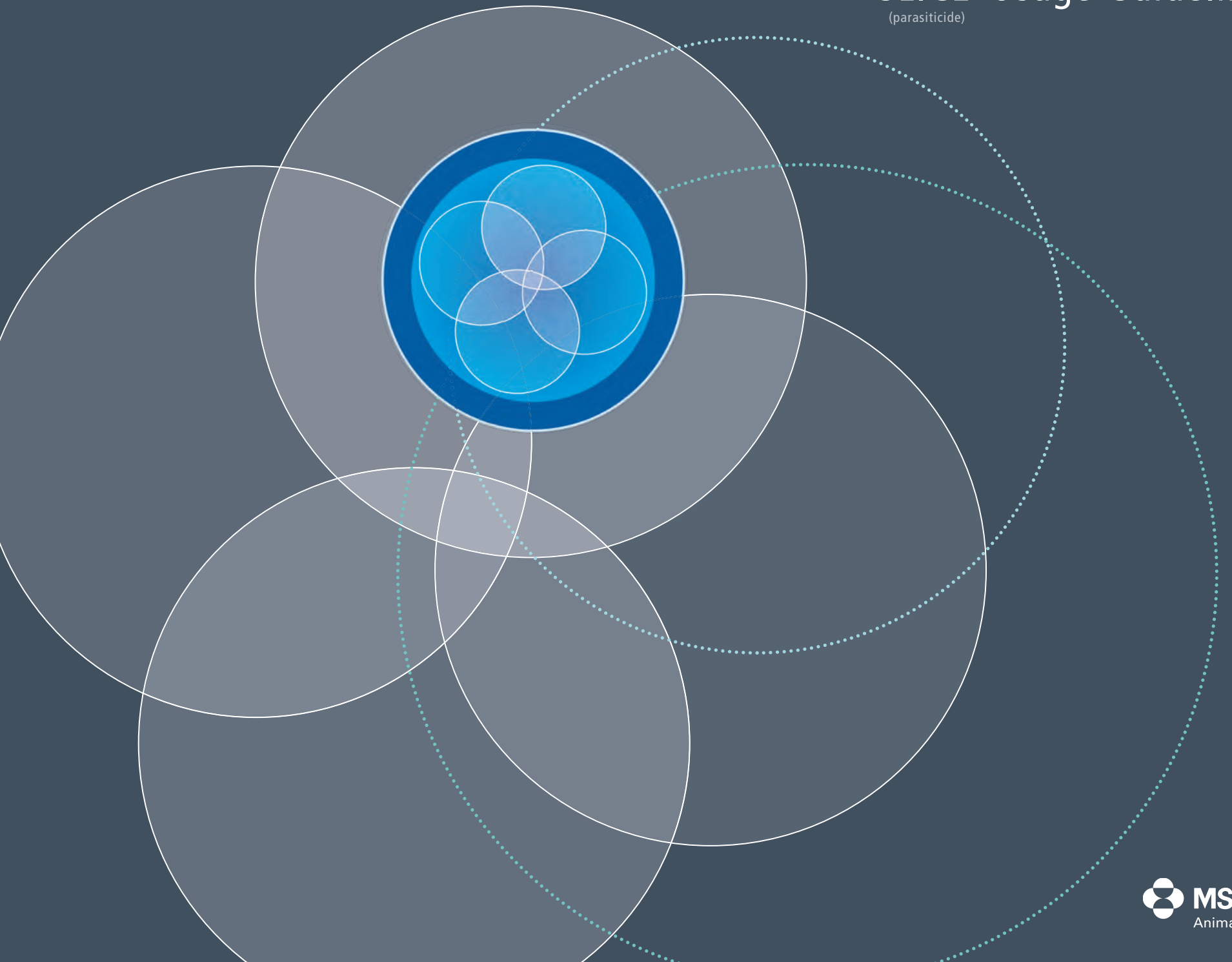
SLICE® Usage Guidelines

(parasiticide)



MSD Animal Health

The usage guidelines for SLICE presented in this booklet are based on MSD Animal Health's global experience with the product under a wide range of conditions and management practices. Consult with your local MSD Animal Health representative for any additional guidelines that may apply to your area.

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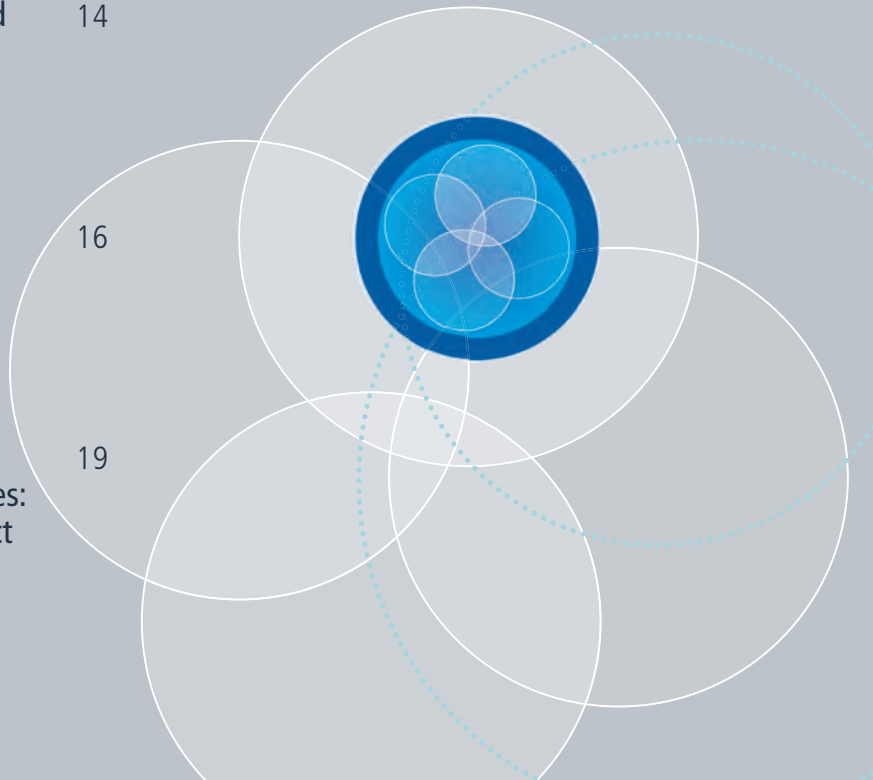

Introduction

Sea lice are naturally occurring, external parasites of farmed fish. They have been recognized as long as man has fished for salmon. However, sea lice infestation was not a serious concern until salmonids were reared in large, commercial production facilities.

The consequences of sea lice infestation for the commercial salmonid industry are serious and costly. In fact, sea lice infestations represent the most significant disease problem currently affecting sea-farmed salmon and trout around the world.

Sea lice feed on fish skin, mucus and blood, especially on the head, back and perianal region. If untreated, the infestations can lead to severe erosion and exposure of subcutaneous tissues, secondary bacterial and viral infections, osmotic imbalance and extreme stress. Infested fish may have poor growth and feed efficiency or they may die, causing substantial economic losses for fish farmers.

Because sea lice control is imperative to the sustainability of the commercial salmonid industry, MSD Animal Health developed SLICE®, which is an in-feed premix introduced in 2000 for the control of sea lice on farmed salmon and trout. Preformed fish pellets are top-coated with SLICE, then an overcoat of fish oil is applied to ensure even distribution of the premix on pellets.

SLICE contains emamectin benzoate in a 0.2% formulation. SLICE, administered to salmonids in feed at a dose rate of 50 µg active/kg/day for 7 consecutive days, kills all parasitic stages of sea lice.

Originally, SLICE was developed for use against the two main species of lice affecting farmed salmonids, which are *Lepeophtheirus salmonis* and *Caligus elongatus*, but it is also used effectively against *Caligus teres* and *Caligus rogercressyi*.

Today, SLICE is used extensively in the Northern and Southern hemispheres and has made significant contributions to improved sea lice control. Despite substantial progress, the risk for sea lice infestation and resulting losses remains high because some strains of sea lice have become less sensitive to available therapeutics. Finding new, effective, safe and environmentally friendly active ingredients to combat parasitic infestations is becoming far more costly and difficult. Consequently, it is imperative that the effectiveness of current treatments, such as SLICE, be preserved through proper and judicious use.

Preservation of effectiveness requires careful monitoring of sea lice sensitivity, the appropriate use of available treatments, such as SLICE, and identification of management practices that yield the best efficacy with available therapeutics.

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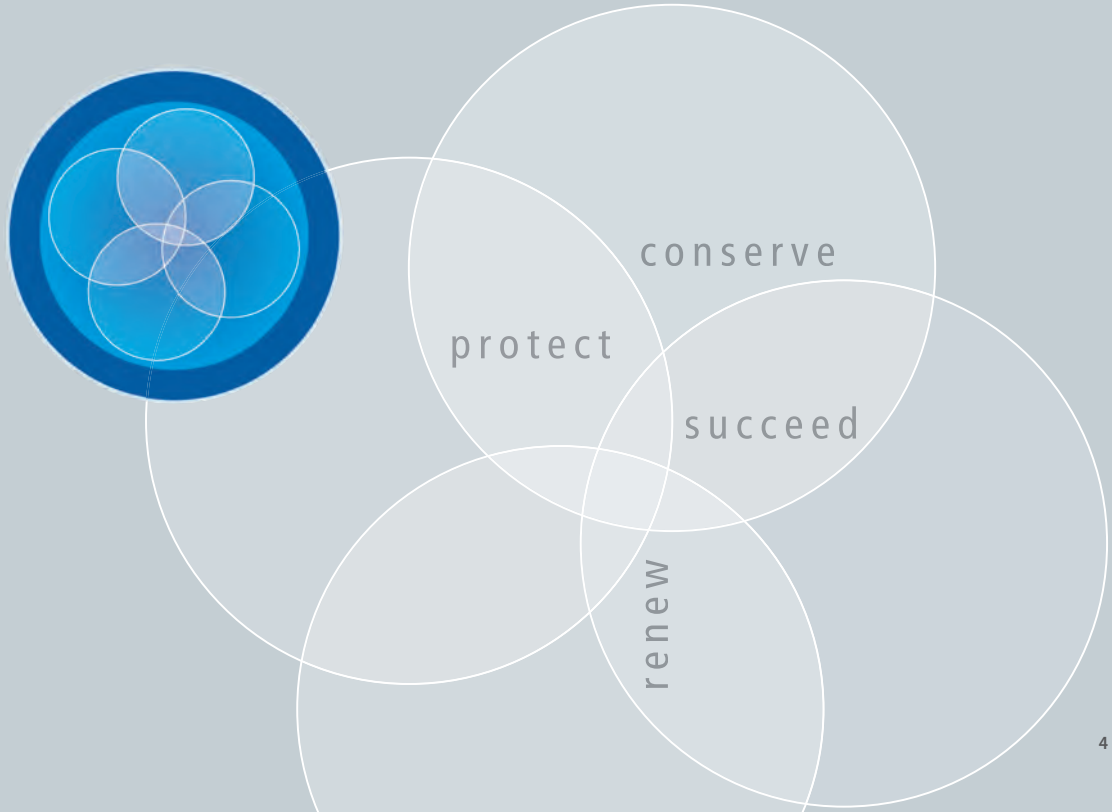
Toward this end, MSD Animal Health has developed considerable expertise in integrated sea lice management. We have promoted and participated in collaborative initiatives involving veterinarians, health professionals, farmers, regulatory authorities, feed companies and other pharmaceutical companies in both the Northern and Southern hemispheres. We also have run workshops on integrated lice management and medicine-use reduction.

These collaborative efforts have led to vast improvements in the responsible use of SLICE, which remains one of the most effective tools in the management of sea lice. Despite the reports of apparent resistance or increased tolerance to emamectin benzoate in some countries and geographic areas, SLICE still serves as a valuable tool in the control of sea lice.

The SLICE Sustainability Project was initiated by MSD Animal Health in 2010. It is a global initiative that provides monitoring for sea lice sensitivity, training of fish farmers and their personnel in how to get the best results with SLICE, and testing for emamectin benzoate concentrations in feed and fish. Its aim is to help fish farmers **protect, renew, conserve** and **succeed** in their battle against sea lice.

This document is a general reference guide about the use of SLICE in Atlantic salmon and trout. It is intended for fish farmers and prescribing veterinarians and has been updated to take into account more than a decade's worth of field experience with SLICE. Recommendations made herein are based on the known best SLICE treatment practices. The guidance provided in this document is designed to help the salmon industry achieve optimum results under commercial farming conditions, while avoiding potential problems associated with tolerance and the development of sea lice resistance.



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SLICE Aquaculture Premix

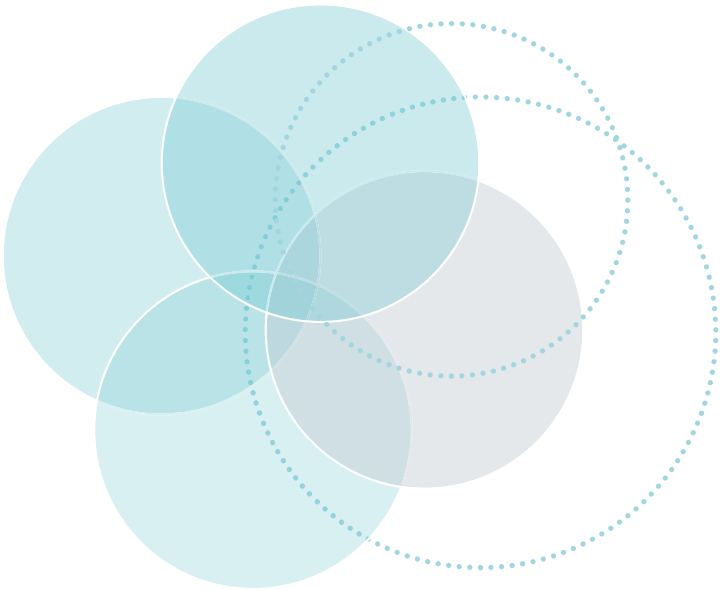
SLICE Premix has been formulated specifically for preparation of medicated fish feed for the treatment and prevention at group level of infestations of all parasitic stages of sea lice (*Lepeophtheirus* spp. and *Caligus* spp.) on Atlantic salmon (*Salmo salar*) ranging in size from smolts in freshwater (just prior to transfer to seawater) to market-weight fish in seawater. It consists of 0.2% emamectin benzoate in an inert carrier consisting of corn starch, maltodextrin, antioxidant and solvent.The corn starch is from plants that have not been genetically modified.

Use of a premix helps ensure homogenous dispersion of the active ingredient on the feed.

SLICE is supplied in 2.5-kg sachets which should be top-dressed onto feed using a dry-coating method. Storage and withdrawal information appear in Table 1.

Table 1. Storage and withdrawal of SLICE Premix

Shelf life of SLICE Premix	24 months (unopened)
Shelf life of medicated feed	6 months
Storage conditions	Dry conditions between 2° C and 30° C
Withdrawal period	UK, Europe, Canada and Chile: zero days. No pre-slaughter withdrawal period required. Norway: 175 degree days USA: not established To ensure that tissue residues do not exceed the maximum residue limit (MRL), fish must not be treated more than once in the 60 days prior to harvest.
Maximum residue limit	MRL is 100 µg/kg for finfish edible tissue (Europe). http://www.ema.europa.eu/ema/index.jsp?curl=pages/home/Home_Page.jsp&mid=



Emamectin Benzoate: The Active Ingredient in SLICE

Emamectin is a member of the avermectin family of compounds that are produced by fermentation of the soil organism *Streptomyces avermitilis*. The avermectins include ivermectin, which is a widely used parasiticide in humans and animals, and emamectin, which was developed initially as a food-crop pesticide. After years of extensive research, the benzoate salt of emamectin was shown to be safer in fish and more effective against sea lice than other avermectins, and as a result, it was developed for use in aquaculture as a treatment for parasitic sea lice.

Emamectin benzoate is a mixture of two homologues: ≥ 90% 4″-epimethlyamino-4″-deoxyavermectin B_{1a} benzoate and ≤ 10% 4″-epimethlyamino-4″-deoxyavermectin B_{1b} benzoate.

After years of extensive research, the benzoate salt of emamectin was shown to be safer in fish and more effective against sea lice than other avermectins.

Mode of action: the effect of emamectin benzoate on sea lice

When emamectin benzoate is administered to fish in feed, it is absorbed from the gut and distributed into fish tissues, particularly the skin and mucus. When sea lice feed on the skin, mucus, blood and muscle of the host fish, emamectin benzoate is taken up into the tissues of the louse. It then binds to the glutamate-gated chloride channels of nerve cells and disrupts chloride ion movement and, hence, transmission of nerve impulses. The parasite stops feeding, becomes paralyzed and dies.

Emamectin benzoate is slowly metabolized and/or excreted from the fish, resulting in an extended period of protection from lice, long after treatment has been completed.

SLICE Advantages

1. Kills all stages of sea lice (motile and non-motile), including gravid adult females, and can protect up to 60 days.
2. Protects fish from new infestations, thereby allowing fish to recover from existing damage.
3. Effective under a wide range of water temperatures in both freshwater and seawater.
4. Well tolerated by fish. In field trials, salmon receiving more than 3x the recommended dose rate showed no mortality or significant reductions in feeding associated with treatment.
5. SLICE is also well tolerated and effective when administered to smolts prior to transfer to sea.
6. Unique and proprietary in-feed formulation minimizes labor, stress and handling of fish.
7. Can be top-coated on feed for a wide range of feeding rates (0.25% to 5.0%).
8. SLICE is highly palatable and does not affect feed intake when administered according to label directions.
9. Proven safe to handlers and the environment when used according to label directions.
10. Made according to Good Manufacturing Practices recognized by regulatory authorities in the US, Europe and other key markets.

For information about SLICE efficacy and safety, see the SLICE Technical Monograph (MSD Animal Health, 2012, MSD-SSP-12).

Requirements for SLICE use

- Follow package insert and/or recommendations of your prescribing veterinarian.
- Veterinary prescription — SLICE is a prescription-only medicine, available only through a registered veterinary practitioner.
- Local environmental and use/discharge regulations may affect the use of medicines on fish farms. In the UK, a "Discharge Consent" or other permit must be obtained from the competent authority before any medicated feed or other medicine can be used.
- Be sure to accurately estimate the biomass to be treated and account for fish growth during the treatment period.
- Where local regulations dictate, feed medicated with SLICE may only be prepared and supplied by designated feed mills. Contact your feed supplier to ensure it can supply feed medicated with SLICE.
- Observe the following warnings and contraindications:
  - **Warning:** SLICE should not be used in salmonids intended for broodstock. (Note: Safety studies have not been conducted in broodstock.)
  - **Contraindications:** When used at the recommended dose, SLICE (emamectin benzoate) produced no undesirable effects in clinical trials apart from a slight reduction in appetite during the medication period in two trials only. A change in the source and pellet size of the medicated diet may have contributed to this effect.
  - Safety studies conducted in salmon showed that at 7x the recommended dose, administration of emamectin benzoate produced lethargy, dark skin coloration and loss of coordination commencing on Day 5 of medication and reduced appetite 2 days after treatment. Recovery was not evident in the week following treatment.
- Report any adverse events or unexpected treatment outcomes to the prescribing veterinarian and your local MSD Animal Health representative.

Indications, withdrawal times, etc. may vary by market. Users should refer to their local package insert for details or contact their local MSD Animal Health representative.

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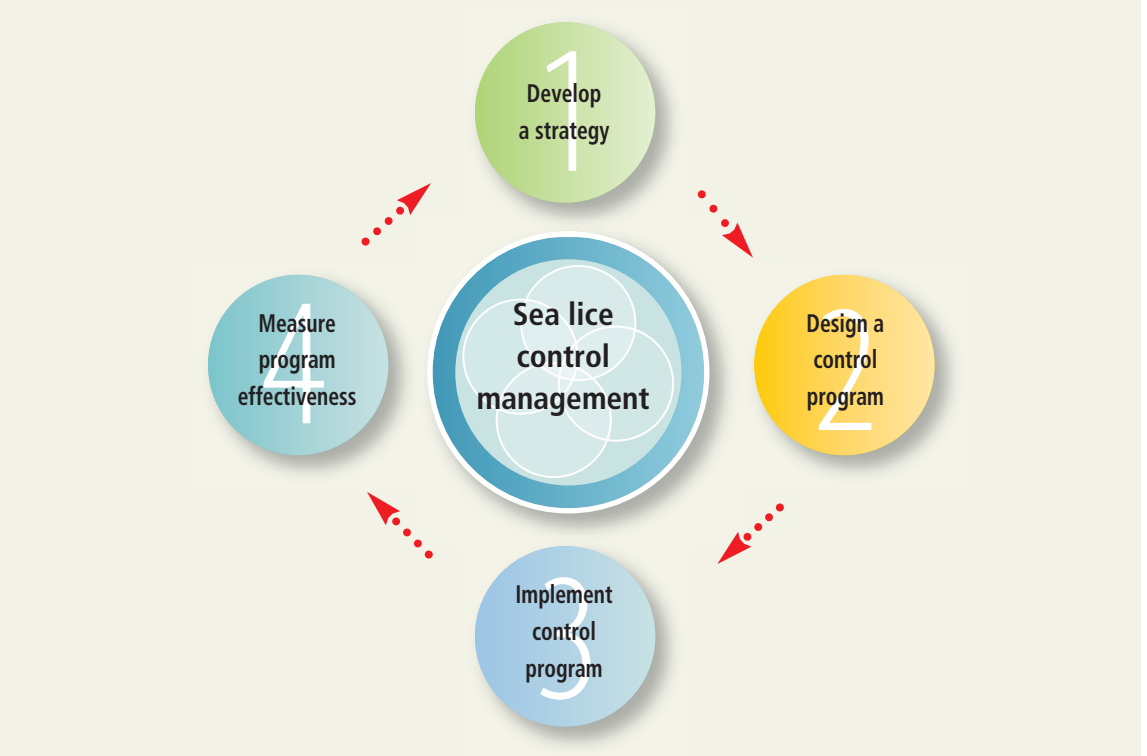
Sea Lice Resistance Management/Integrated Sea Lice Management Principles

Due to the adaptive nature of organisms, use of all chemotherapeutants in human and veterinary medicine has the potential for inducing resistance in the organisms intended for control. Development of resistance in sea lice has been found with organophosphates and pyrethroids, while populations of lice with reduced sensitivity to hydrogen peroxide have also been identified. Sea lice also have the potential for developing resistance to SLICE following continued use or use of related compounds (avermectins, such as ivermectin).

Once resistance has developed, the usefulness of these and related compounds is reduced. This is why it is vitally important to protect the treatment options still available to the industry through best treatment practices, product rotation and other measures.

Integrated pest management (IPM) practices employ strategic treatments and rotation of therapeutics in combination with other management and biological control measures. These should be adopted for optimal lice control, to limit the use and discharge of medicines and delay the development of resistance.



Best-practice Treatment Principles — The Basics

The SLICE Sustainability Project can help fish farmers develop treatment and management protocols that will help combat reduced sensitivity and resistance.

The SLICE Sustainability Project is available to help fish farmers implement and maintain their sea lice control program and provides invaluable services such as monitoring sea lice sensitivity, flesh and feed analysis and treatment efficacy assessment.

1

Develop a strategy

- Form area management agreement
- Decide on your stocking policy
- Coordinate fallowing
- Agree on your lice-monitoring protocol
- Adjust your strategy if needed, based on data gathered

2

Design a control program

- Embed your control program into your veterinary health plan
- Obtain permission for all available licensed medicines
- Be sure to include non-medicinal measures
- Determine sensitivity of sea lice to available medicines and parameters of use (lice treatment thresholds for the number of lice and bioassay breakpoints)
- Write down treatment regimen to be used for the entire cycle
- Prepare a rotation plan

3

Implement control program

- Write standard operating procedure (SOP) for program management
- Fish sampling and lice counts
- Feeding regimen before and during treatment
- Treatment triggers
- Provide training and implement your SOP
- Carry out bioassays in all areas to be farmed
- Coordinate treatments
- Carry out treatments in accordance with plan
- Incorporate medication at an appropriate feed ration to ensure complete feed consumption in all fish
- Carry out simultaneous treatment of all fish on a site
- Coordinate treatments of all farms in a loch, bay site or hydrographic area to reduce cross-infestation
- Employ careful feeding practices and observe fish to monitor the feed response
- Maintain feed and flesh samples for analysis

4

Measure program effectiveness

- Record all bioassay results
- Analyze feed and record the findings
- Analyze flesh and record the findings
- Monitor and record every treatment according to plan
- Record any deviation from plan and the reasons
- Monitor lice numbers
- Create a database of all information
- Regularly review the information and adjust the strategy as needed
- Suspected adverse reactions, such as a lack of efficacy that might be attributed to the development of resistance, should be reported to the appropriate veterinary medicines authority and to MSD Animal Health

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Treatment Options

Treatment with SLICE in seawater

SLICE is intended for use as a therapeutic treatment of existing sea lice populations in fish that are on-grown at sea. However, therapeutic treatment may also prevent subsequent reinfestation of new sea lice populations.

Preventative treatment with SLICE

Preventative treatment should only be used when considered appropriate by the prescribing veterinary surgeon, (i.e., where there is likely to be a louse-associated welfare risk to fish following transfer to sea and where therapeutic treatment at sea is not an option due to lack of appetite or adverse environmental conditions). In some countries, consent to discharge in freshwater may be required from the appropriate environmental authority.

Treatment of smolts with SLICE in freshwater

At sites where there is a history of high lice infestation, fish may be treated in freshwater prior to their transfer to sea. This was the subject of an intensive study in Scotland and the procedure is now regularly used in Norway, Chile, Canada and the UK.

In one study, treatment of 40- to 60-g smolts with SLICE was carried out in the autumn, 1 week before they were transferred to seawater. Treatment was well tolerated and highly effective and prevented infestation by sea lice for up to 11 weeks from the start of treatment. However, the duration of efficacy in each case may vary, according to temperature and other factors.

When to Treat with SLICE

The formation of local area management groups is encouraged so treatments can be carried out according to agreements drawn up between neighboring farms. In the UK, a National Treatment Strategy for control of sea lice was adopted as a Code of Practice in 1999, then updated in 2006. This promotes the use of strategically timed, coordinated treatments.

Area management agreements encourage farmers to develop treatment strategies with other farms in the area and to liaise with wild fishery interests. (See *A Code of Good Practice for Scottish Finfish Aquaculture.*) Responsibility for ensuring the correct use of medication, however, lies with prescribing veterinary surgeons, and they should always be consulted as the main source of treatment advice.

The use of SLICE should be incorporated into a sea lice management system that is appropriate for the site and the environment. The timing of sea lice treatments should take into consideration IPM practices that include routine monitoring of lice numbers and agreed treatment thresholds as well as the susceptibility of lice to available treatments.

Bioassays are recommended as part of best-practice principles. They are not an entirely accurate predictor of resistance but are among the best tools currently available. Bioassays should be used to decide when a particular medicine may or may not be effective.

Treatment thresholds based on ranges of bioassay values may be developed to determine which lice treatment should be used. These, again, may vary by country or region.

MSD Animal Health recommends the routine use of bioassays before treatment to help determine if SLICE treatment will be effective on a particular farm or area. In some cases, the attending veterinarian may choose to follow up SLICE administration with bath treatments.

Control of sea lice at different life stages

Treatment thresholds may be set by local authorities in different countries and/or regions. These thresholds may also vary by season, taking into account the movement of wild salmon populations, for example. Specific treatment thresholds are not provided here, as these recommendations change or evolve with changes in local rearing conditions and increasing experience in sea lice population management.

The decision when to treat may also be influenced by records of previous sea lice infestations and treatments. While large fish may be able to cope with higher numbers of lice than small fish, lice numbers should not be allowed to build up.

Young smolts are very susceptible and may be damaged by as few as five mobile lice. Ideally, treatment should be administered when lice are still at the chalimus stage. This prevents sea lice from reaching the more damaging pre-adult stage and the reproductive adult stage. Therefore, early treatment is important since fish that are damaged by sea lice may show a reduced feed response — and a good feed response is necessary to ensure better uptake of medicated feeds.

Fish should only be treated when they are feeding uniformly, even if this is at a low percentage of bodyweight per day.

To help maximize treatment outcomes and evaluate results, work with your local MSD Animal Health representative and participating laboratories on SLICE Sustainability Project program implementation in order to get the most out of your SLICE treatment.

General management practices to control sea lice and reduce the potential for resistance

While SLICE is highly effective in the control of sea lice, it is also recommended that it should be used in conjunction with other recognized measures to ensure optimum control of lice and reduce the potential for resistance.

1: Stock defined areas with a single-year class of fish only, to reduce potential transmission from existing stocks to newly introduced, uninfected fish.

2: Use an all-in/all-out stocking policy, i.e., where each site is completely harvested out, and ideally fallowed, before being stocked with new fish.

3: Synchronize fallowing with neighboring farms; leave whole sites unstocked for a minimum of 6 weeks prior to restocking to break the reproductive cycle of lice.

4: Practice good husbandry, such as minimal handling of fish, which helps reduce stress, decrease the susceptibility to sea lice and reduce the possibility of secondary infections.

5: Routinely monitor sea lice populations. This ensures that any increase in sea lice numbers will be detected early on and allow treatment to be carried out before lice reach the more damaging mobile stages. Counts should be conducted using a standard protocol to ensure useful results.

6: Use efficient feeding practices to ensure effective administration of feed medicated with SLICE.

7: Control other diseases. It has been shown that fish affected by intercurrent disease such as furunculosis or pancreas disease are more susceptible to sea lice.

8: Remove moribund fish. Sick fish should be removed on a regular basis since they may fail to respond to medicated feeds and can harbor high lice numbers.

9: Nets should be kept clean. This helps to ensure good water flow through the pens, thereby reducing the potential for new lice infestation from larval stages that remain in the pens.

10: Grade fish to remove grilse. Maturing fish may stop feeding and, therefore, continue to harbor lice when medicated feeds are employed.

11: Use cleaner fish (wrasse). Stocking pens with wrasse may prove beneficial in summer months. Wrasse, however, must be carefully managed to ensure their survival and effective lice control. Supplementary feeding may have to be supplied following sea lice treatments.

Preparation of Feed Medicated with SLICE

SLICE is administered to deliver 50 µg emamectin benzoate/kg fish biomass/day for 7 consecutive days. The suggested feeding rate is 0.5% fish biomass per day. If the feeding rate differs from 0.5% biomass/day, then the concentration of SLICE in feed must be adjusted accordingly (Table 2).

Feed medicated with SLICE should be fed as the sole ration and feeding rates/feed concentrations should be selected to achieve this.

Feed medicated with SLICE should be manufactured according to label directions and prepared only at commercial feed mills and not on the farm. In accordance with label directions, medicated feed should be top-coated as the active ingredient, emamectin benzoate, is not stable at high extrusion temperatures.

Table 2. This table illustrates typical emamectin benzoate (EB) concentrations in feed based on varying levels of feed intake and expressed as a percentage of live weight.

Fish feeding rate (as % of bodyweight)	**Target EB concentration in feed** (mg/kg)
0.25	20.0
0.5	10.0
1.0	5.0
2.0	2.5
3.0	1.67
4.0	1.25

Dose calculations

To calculate the correct dosages the prescribing veterinarian needs to know:

1. The mean weight of the fish biomass at the start of treatment — Some veterinarians may prefer to use end-treatment biomass to ensure sufficient dose throughout the dosing period. Always check the accuracy of the biomass to ensure that the correct dose is calculated.

2. The number of fish in each pen

3. The current feed rate

Sample weighing should be carried out as close to treatment as possible, ideally no more than 2 weeks before, to confirm the mean weight of fish and the data used to predict the biomass at the time of treatment.

Ideally, set up a spreadsheet to determine quantities of SLICE Premix and feed required for medication. (Example shown in Table 3.)

Table 3. Calculation of SLICE Premix incorporation rate (sample farm, all pens)

Total biomass (tons)	56.103
Amount of feed required per day (tons)	0.4208
Number of medication days	7
Total feed required	2.945 tons — rounded up to 3 tons
Number of 1-ton bags required	3
Premix incorporation rate (kg SLICE/ton of feed), e.g., 0.75% feed rate	3.33
Total amount of premix required (kg)	9.99
Number of 2.5-kg bags of premix required	4

SLICE Premix is coated onto the surface of non-medicated fish feed pellets which may be of any size appropriate to the size of fish.

Medicated feed should only be prepared by appropriately authorized facilities. Although the method presented here has been shown to be effective at a number of mills, individual companies may wish to validate their coating procedures to ensure that the method and equipment employed result in target concentrations.

8

Preparation of Feed Medicated with SLICE

This method relies on the top-coating of oil, which adheres SLICE to the feed, but if SLICE is prescribed to fish that are currently on a high oil diet, the oil content in the feed should be reduced to ensure the applied oil is able to penetrate and adhere SLICE to the pellets.

- Standard feed should be fractionated to remove dust and fragments.

- Sorted pellets should be transferred to an intensive mixer.

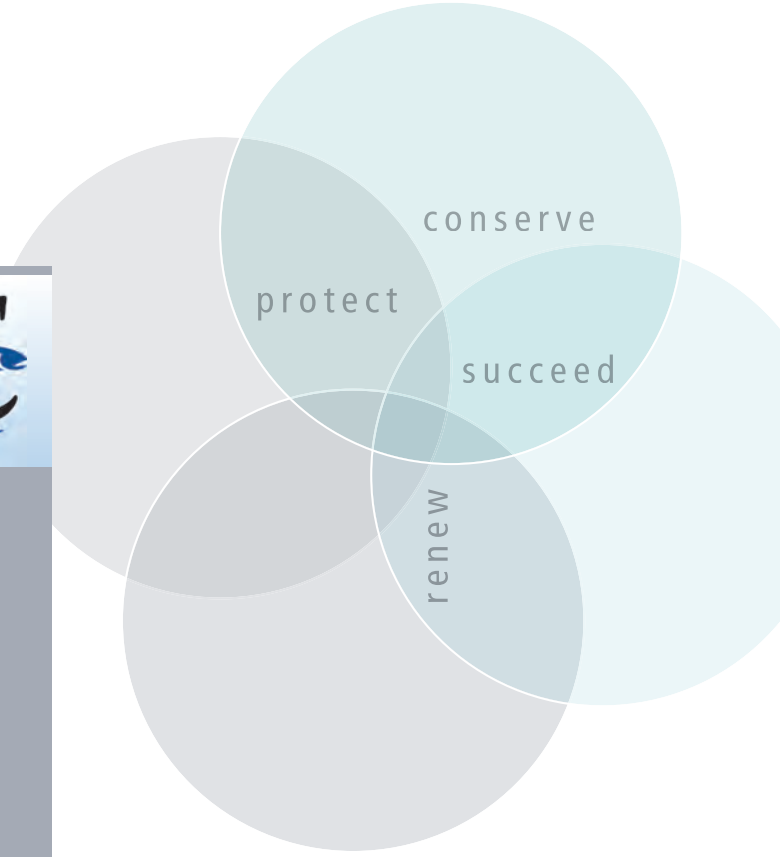
- The pellets are dry-mixed with the pre-determined amount of SLICE for up to 2 minutes. 0.5% to 1% fish or vegetable oil is added and mixing is continued for up to 5 minutes. The added oil seals the premix powder to the feed pellet.

Emamectin benzoate is temperature sensitive. Therefore, it is recommended that SLICE not be exposed to temperatures above 30° C for long periods of time during the top-coating process.

- At the completion of mixing, the product is transferred to a feeder tank for packaging into bags.

SLICE

Feed medicated with SLICE should be manufactured according to label directions and prepared only at commercial feed mills and not on the farm.



- Bags should be clearly labeled as in the following example:
MEDICATED FISH FEED.
CONTAINS 0.2% EMAMECTIN BENZOATE.
FOR ANIMAL TREATMENT ONLY.
KEEP OUT OF REACH OF CHILDREN.

On-farm storage of feed medicated with SLICE

Medicated feeds can be stored for 6 months. They should be stored in a secure place and should not be left on pens for extended periods. When stored on farms, medicated feed should be covered and secured to prevent attack by sea birds or other wildlife.

Guidelines for Feeding Feed Medicated with SLICE

1 **Handling feed medicated with SLICE.** As with all medicinal products, wear gloves and overalls when handling medicated feed and products. Do not smoke, eat or drink while handling medicated feed and products. Wash hands thoroughly after use.

2 **Do not administer medicated feeds when fish are not feeding well.** This will prove wasteful and ineffective and may promote the development of resistance. First, correct the underlying cause for poor feeding. If necessary, the feeding rate used for medication can be reduced to as low as 0.25%.

3 **Check the accuracy of the biomass** to ensure that the correct dose is calculated.

4 **Remove non-feeders** within the population being treated as they can harbor sea lice after treatment.

5 **Withhold feed** from the population for 24 hours prior to treatment.

6 Feed medicated with SLICE should be the **sole source of feed** for the 7-day treatment period.

7 **Ensure dose rate is calculated using the expected mean weight at the end of treatment.** This must be determined from biomass predictions, based on the most recent sample weighing carried out ideally no more than 2 weeks before treatment. Remember, if there is a delay in starting medication, the fish will have grown and the estimated weight may need to be recalculated.

8 **Avoid making changes to regular feeding practices.** Changing feed type or pellet size, for example, may negatively affect intake and absorption of SLICE. If a change is unavoidable, introduce the new feed type prior to medication and ensure the new feed is being consumed before starting medication.

9 **Ensure administration of medicated feed is carried out by experienced operators;** they will be better able to assess the feed response and adjust accordingly.

10 **Distribute feed evenly across the entire surface of each pen.** This will give all fish a chance to feed.

11 **Distribute feed at a rate equivalent to the rate of consumption.** If pellets are all consumed in the top few inches of water, increase the rate of feeding. If pellets are seen to be sinking toward the bottom of the pen, reduce the rate at which pellets are fed.

12 **Monitor feeding response in each pen.** Feed consumption should be observed closely during feeding or monitored using underwater cameras where available. Ideally, daily records should be kept of feed response in each pen to identify any problems and relate to subsequent efficacy.

13 **Administer medicated diets in accordance with regular feeding schedules.** The number of meals per day is determined by feeding conditions and size of fish to be treated.

14 **Do not carry out any other procedures or treatments just prior to, or during, the medication period,** e.g., movement of cages and fish, grading, net changing or swim-through procedures as this may reduce the feeding response. No other medication should be carried out during treatment with SLICE.

15 **Adhere to the recommended treatment period.** By spreading treatment over a 7-day period, this gives

all fish a greater chance to receive an adequate dose. Even if fish appear to be free of lice, continue to feed for the full 7 days. This will reduce the potential for resistance and will provide a longer period of protection from lice after completion of medication. If treatment is interrupted, e.g., owing to bad weather, resume treatment as soon as possible and complete the 7-day medication period. In all cases, follow the recommendations of the prescribing veterinarian.

16 **Follow label dosing instructions and instructions from the attending veterinarian.** It is very important that fish not be under-dosed as this could result in treatment failure and eventual build-up of resistance.

17 **Conduct sea lice counts** for post-treatment. If efficacy is not as desired, consider immediate use of a bath treatment (i.e., a treatment with a different mode of action on the same cohort of sea lice).

18 **Collect feed and flesh samples** as recommended by your veterinarian or local MSD Animal Health representative. (See SLICE Sustainability Project section.)

Table 4. *Lepeophtheirus salmonis* – Typical Efficacy: Ranges shown below are based on data from therapeutic trials conducted in experimental tanks and from field and commercial trials at sea. Results are expressed as percentage efficacy (the reduction in the total lice count on fish treated with SLICE relative to lice counts on unmedicated control fish). All fish were infested with chalimus and motile lice at the start of treatment on Day 0.

Temperature	Day 7	Day 14	Day 21	other
> 13° C	40-60%	80-90%	90-99%	96% (Day 64)
10-13° C	45%	71-77%	>90%	44% (Day 72)
7-10° C	20-37%	36-74%	59-95%	80% (Day 55)
< 7.5° C	20%	36%	59%	89% (Day 35)

Table 5. *Lepeophtheirus salmonis* – Duration of Efficacy: Results of trials showing percentage reduction in lice relative to control groups when fish were experimentally challenged with copepodids at Days 55-109, following treatment with emamectin benzoate on Days 0-6. All fish were sampled when lice were at chalimus stage III or IV, and fish challenged at Days 76 and 109 were sampled again when lice were at the adult stage.

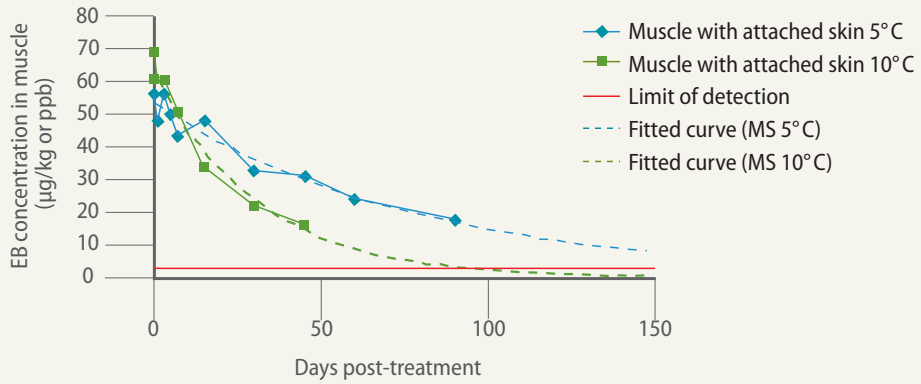
Temperature	Lice sampled at:	Fish challenged with copepodids at:		
		Day 55	Day 76	Day 109
8-15° C	Chalimus III/IV	92%	36%	/
Fish treated in seawater	Adult stage	/	55%	/
6-11° C	Chalimus III/IV	100%	94%	44%
Fish treated in freshwater	Adult stage	/	/	73%

Efficacy — What to expect

Lice numbers may not start to decline until at least Day 5 or 6 of treatment because SLICE is an in-feed treatment, and it takes a few days for emamectin benzoate to reach the required concentration in the fish tissues. Radiolabeled depletion studies have shown that tissue concentrations of emamectin benzoate are higher in the skin than in the muscle and are depleted more slowly from the skin than muscle.

Reductions in lice numbers are temperature dependent. At low temperatures, it takes longer to achieve good efficacy, although the duration of efficacy is greater. This is backed up by depletion studies, which have shown that emamectin benzoate levels decline more rapidly in fish tissues at 10° C than at 5° C (Tables 4, 5 and Figure 1).

Figure 1. Depletion curve for emamectin benzoate (EB) in flesh at 5° C and 10° C



Source: Kim-Kang H. *et al.* 2004

Other factors affecting efficacy

- Fish with significant lice damage may not feed well, and there is a risk of under-dosing and survival of lice on these individuals. Early treatment — before lice damage occurs — will give the best results. Any improvement in fish condition will depend on water temperatures and the rate at which lice numbers are reduced. However, once the fish are free of lice, skin lesions tend to heal rapidly, providing there are no other concurrent diseases.

- Differences in hierarchical feeding behavior can result in greater variation in efficacy.

- There may be a small number of fish with lice of any stage present. These are usually poor feed responders which have not benefited from treatment. Ideally, any runts or moribund fish, which are unlikely to feed, should be removed prior to treatment as they may represent a source of re-infection.

In the event of poor efficacy or any changes in fish appearance or behavior, the prescribing veterinarian should always be informed as soon as possible.

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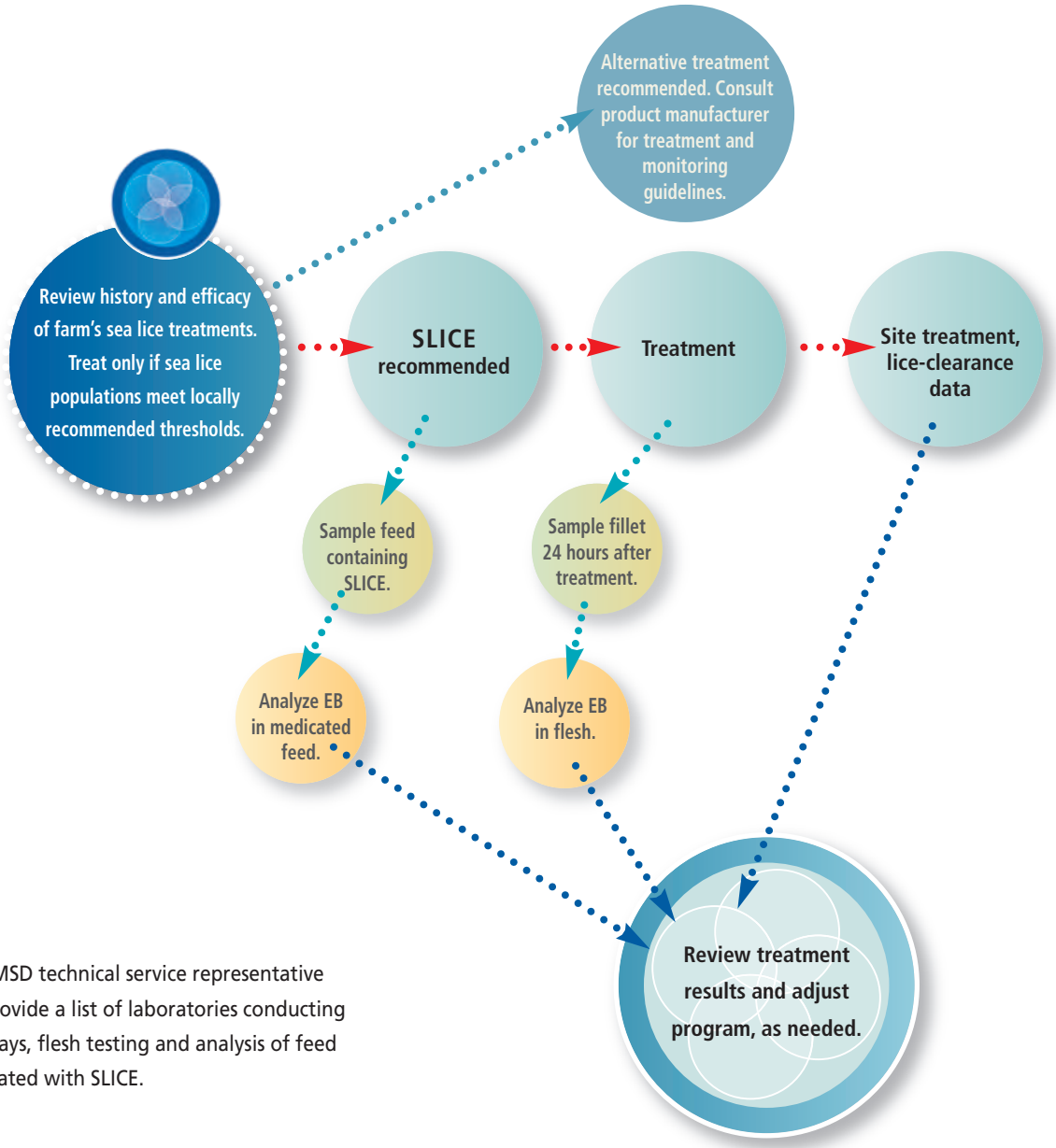
Maximizing Efficacy and Evaluating Treatment Outcomes: The SLICE Sustainability Project

Integrated and sustainable sea lice programs involving SLICE have proved to be highly effective in major salmon-producing countries — not only for controlling sea lice, but for conserving the effectiveness of SLICE.

In 2010, MSD Animal Health introduced the SLICE Sustainability Project (SSP) to maximize and further conserve the effectiveness of SLICE. Under this initiative, MSD Animal Health technical and sales teams work with salmon producers and veterinarians to develop site-specific strategies for long-term control of sea lice. This also includes comprehensive reviews of sea lice control programs and treatment outcomes to ensure that these strategies are providing optimum results.

The SSP Program focuses on:

- Bioassays to determine sea lice susceptibility
- Tissue analysis to evaluate intake of feed medicated with SLICE and absorption/distribution of the active ingredient in the fish
- Feed analysis to ensure that medicated feed contains the correct concentration of active ingredient as determined by the prescribing veterinarian



Your MSD technical service representative can provide a list of laboratories conducting bioassays, flesh testing and analysis of feed medicated with SLICE.

Key SSP elements

Monitoring

- Record the results of the bioassay to check the sensitivity of sea lice in the area to be treated. Record sea lice numbers weekly and, particularly, prior to each treatment.

- Analyze feed that was administered to fish to ensure the target dose was included in the diet.

- Take samples of flesh 24 hours post-treatment and freeze. Then, if required, analyze the samples to check for therapeutic levels of emamectin.

- Record sea lice numbers post-treatment and compare against the pre-treatment number and bioassay results.

- Analyze the results and make adjustments, if necessary, to the strategy and medication employed.

Bioassays — Predicting sea lice susceptibility

Bioassays can be used as an *in vitro* tool to evaluate or monitor sea lice susceptibility to parasiticides.

The following are MSD-recommended guidelines for conducting bioassays:

Bioassays are recommended as part of best-practice principles, but they are not a definitive tool to be used when making treatment decisions.

Field experience has made it clear that the LC_{50} or EC_{50} values determined from bioassays on sea lice are not an entirely accurate predictor of resistance, but bioassay values are among the best tools currently available.

Bioassays should be viewed as one tool among several that veterinarians should use to decide when a particular medicine may or may not be effective and when it is time to consider changing to a treatment with a different mode of action.

The routine use of bioassays, coupled with treatment monitoring as described in the next section, should make it possible to produce records that can be used to correlate treatment success with bioassay results.

How to use bioassays

- Ideally, bioassays should be conducted according to a published protocol. Further information on protocols can be found within the *Sea Lice Resistance to Chemotherapeutants — A handbook in resistance management, Search Project* (QKK2-CT-00809) or within the paper entitled "Optimization and field use of a bioassay to monitor sea lice *Lepeophtheirus salmonis* sensitivity to emamectin benzoate" by Jillian D. Westcott, Henrik Stryhn, John F. Burka and K. Larry Hammell in *Diseases of Aquatic Organisms*, Vol. 79:119–131, 2008.

- Many farm companies have their own in-house bioassay facilities and there may be subtle differences in the protocols they use, which may or may not affect end results. With so many variables, it may be difficult to compare results among laboratories using different protocols, but the fundamental rationale for using bioassays remains.

Tissue analysis

Flesh sampling after SLICE treatment is important since the levels of emamectin benzoate in flesh can be indicative of dosing success; this, together with lice monitoring, enables a scientific determination of SLICE efficacy.

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Maximizing Efficacy and Evaluating Treatment Outcomes: The SLICE Sustainability Project

The following are directions for collection of flesh samples for analysis. These are general guidelines applicable in all markets where SLICE is sold. For additional information on flesh testing, sample submission and reporting forms, please contact your local MSD Animal Health representative.

Flesh sampling

- Take samples **24 hours** after the last administration of SLICE. If flesh samples are taken more than 24 hours after treatment, water temperatures and dates must be recorded.

- Sample apparently **healthy fish**; avoid non-feeders unless they are required for specific sampling programs.

- Catch **five "normal" fish** at random, e.g., by sweep-net or shallowing cages, where possible, and without using feed as a lure.

- Any runts or "poor-doers" that are sampled should be in addition to the "normal" samples and identified as such on the **Emamectin Analysis Request Form (EARF)** provided by your local MSD Animal Health representative. See Figure 2 for a sample form.

Figure 2. Example of a sample data and authorization form, which should be obtained from your MSD Animal Health representative and returned to that representative when completed. A copy of the completed form must also be submitted with your samples.

The SLICE® Sustainability Project

Emamectin Analysis Request Form (EARF):
Fish/Feed

DESC 1 — Farm company	
DESC 2 — Farm name and location	
Biomass and number of fish	
Total number of cages on site	
Smolt input date	
Smolt type and source	
SLICE treatment dates (this cycle)	
Feeding regimen and dose (µg/kg/day)	

Feed supplier		
Feed type and size		
SLICE batch number(s)		
SLICE fortification rate		
Feed sample date		
Feed sample point (bag, pipe, etc.)		
Farm feed administration system		
Water temperature		

Fish sample details							
Fish sample date							
Pen numbers							
Sample number							
Fish weight							

Enter other relevant info in the box below:

PO number

MSD authorization number (required for analysis)

MSD Animal Health

- Sacrifice fish by an approved, appropriate method.

- Record the **length and weight** of each fish whenever possible.

- **Wipe or rinse the surface of the fish** to remove extraneous matter such as feces or feed.

- **Remove a fillet consisting of skin and fish.** A 250-g sample is ideal; if fish are small and this sample size isn't possible, take the whole fillet from one side. See Figure 3 to ensure proper sampling. Check to make sure the sample is not contaminated with feces or feed, and place in a labeled plastic bag.

- **FREEZE** each bagged fillet sample as soon as is practicable.

- **Pack frozen samples** in an insulating box (e.g., polystyrene) and send by overnight delivery to the designated analytical laboratory. Remember to include a copy of the EARF with your samples and send a separate EARF copy to your MSD Animal Health representative.

As a general guideline, a standard dose of 50 µg/kg/day of emamectin benzoate for 7 days should achieve a target flesh level of 60 µg/kg emamectin benzoate, which should deliver a satisfactory reduction in numbers of sensitive lice (Table 6, Figure 4).

Figure 3. This diagram shows the area where the sample for flesh analysis should be taken 24 hours after SLICE treatment.

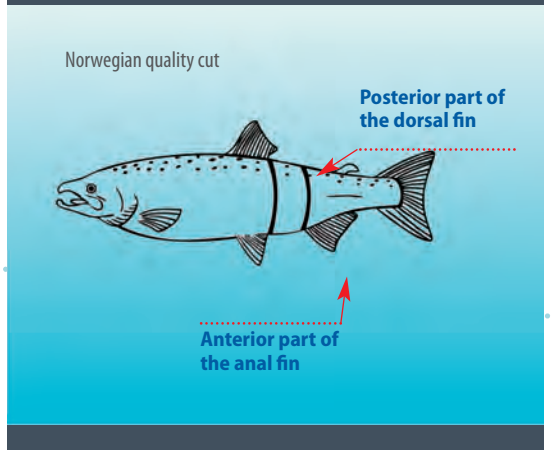


Table 6. Concentration (ppb) of emamectin benzoate in blood, mucus and muscle of Atlantic salmon (SLICE administered Days 1-7)

	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	

Maximizing Efficacy and Evaluating Treatment Outcomes: The SLICE Sustainability Project

Feed analysis

Feed samples from each batch of feed medicated with SLICE should be retained for quality control purposes and treatment follow-up.

Sampling guidelines are as follows:

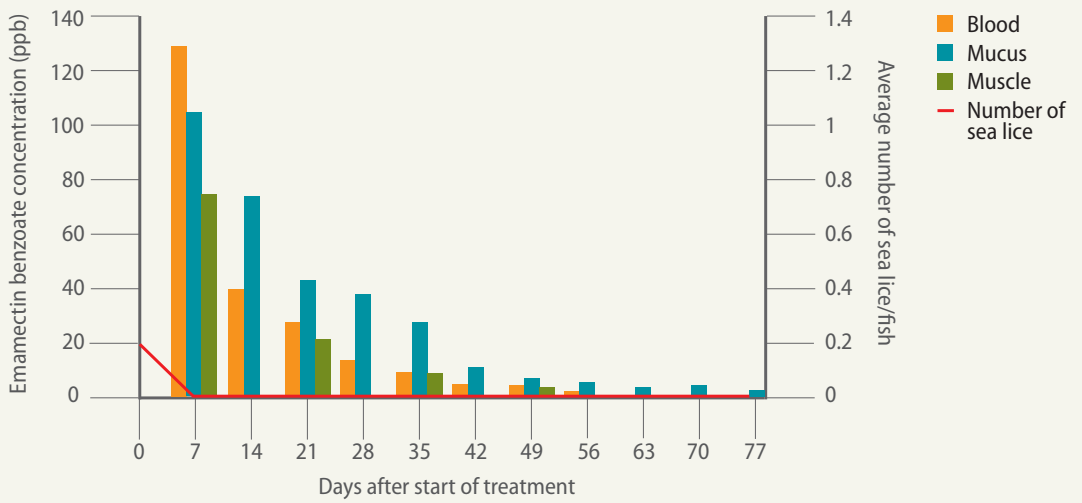
Feed sampling (on farm)

To determine feed dosage via feed assays, approximately 250 g of medicated feed, as delivered to the farm and to fish, should be placed in labeled containers with identifying details and sent for analysis.

- Ship the sample to the analytical lab
- Label containers with the following information:
  - Type of feed
  - Date of manufacture
  - Batch number
  - Location where sample was taken
  - Name of the person performing sampling

Submit samples as instructed by MSD Animal Health, and be sure to submit samples with the EARF, available from your local MSD Animal Health representative. A sample form appears on page 21 in this document.

Figure 4. 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)



Source: Sevatdal S. *et al.* 2005

Samples of feed medicated with SLICE should be stored or shipped to the analytical laboratory cold or frozen.

One composite sample should be submitted for testing, and the laboratory should be requested to test the sample in duplicate and report both the individual and average assay values.

If an atypical result is obtained and follow-up testing is necessary, retained samples from the batch should be submitted and each tested once. Again, submit samples with the EARF.

Note: Feed samples will be analyzed in duplicate for EB content by standard high-performance liquid chromatography methods. Results for each duplicate sample will be reported to MSD Animal Health and to the submitting feed mill or farm. If batch results are within the approved regulatory target*, then no further analysis of samples from the batch will be performed.

SLICE efficacy — Case study

Field case

A leading salmon-farming company in Scotland was due to stock one of its sites.

The site was stocked in April 2010 and SLICE treatment was initiated in May 2010, when the fish weighed an average of 80 g.

Twenty-four hours after SLICE treatment, samples were taken for flesh and feed testing, to gather data for the SLICE database, and farm employees were taught how to perform future sampling.

Fish were sampled by a SOP; the samples were frozen, packaged and sent for analysis at the Eclipse Scientific Group, an analytical testing service.

Variability within flesh samples is normal, given the variable feed rates of fish in a pen holding 50,000 to 60,000 fish and considering that only five fish are sampled to minimize the number of fish producers must sacrifice for testing. To achieve a more statistically significant result, more fish would need to be sampled.

To determine feed dosage via feed assays, approximately 250 g of medicated feed, as delivered to the farm and to fish, were placed in labeled containers with identifying details and sent for analysis.

Note that the target feed concentration is rarely achieved via analysis. The goal is to have as few instances as possible below the target level. A new testing method has been developed that will improve sample recovery and feed analysis.

The target feed concentration is rarely achieved via analysis. The goal is to have as few instances as possible below the target level.

*Regulatory targets may vary by market.

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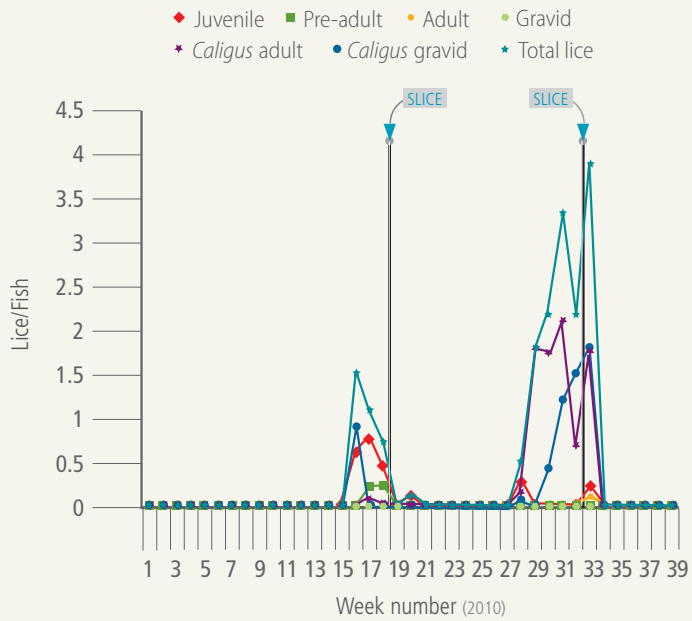
Maximizing Efficacy and Evaluating Treatment Outcomes: The SLICE Sustainability Project

Efficacy after first SLICE treatment

The efficacy of SLICE treatment is reflected by lice data, which were supplied by the salmon-farming company. The first SLICE treatment proved to be efficacious, with lice numbers remaining at or near zero for 8 weeks (Figure 5).

The results gave the producer confidence in SLICE, and when the farm required a second lice treatment, SLICE was chosen as the most appropriate treatment option. Again, lice counts remained at or near zero for another 6 weeks. In this instance, farm staff handled the sampling of fish and feed (Figure 5).

Figure 5. Numbers of lice at all stages after treatment with SLICE on weeks 18 and 32



Discussion and conclusions

Although flesh samples, feed targets and lice counts are important aspects of SLICE monitoring, it is imperative that *all* aspects of monitoring be adopted and considered in order to enable a full assessment of SLICE treatment efficacy.

For instance, *in vitro* bioassays conducted with sea lice are recommended to help determine the sensitivity of lice in a producer's region. Bioassays, as in this case, are not always available, due to a variety of reasons, but can be particularly helpful; they can be used routinely throughout the growing cycle to help predict the sensitivity of sea lice to treatment or to detect changes in sensitivity within a population of sea lice.

The experience at this salmon-farming company demonstrates the effectiveness of SLICE treatment and corroborates other extensive field experience with SLICE Premix. It also underscores the benefits of animal health companies and producers sharing data that can be used to help make future treatment decisions. Cooperation and the exchange of information are central to the SLICE Sustainability Project.

Frequently Asked Questions and Answers

General

Q What advantages does SLICE have over other treatments?

A SLICE is highly effective against all developmental stages of susceptible sea lice and should protect fish for an extended period after the completion of medication. It has a very good safety margin for fish and the advantage that it is applied as an in-feed treatment. Other treatments are only effective against certain stages of lice or show limited protection beyond the medication period.

Q What are the advantages of using in-feed treatments?

A In-feed treatments are less stressful for fish since the treatment is incorporated into the normal feed ration. This reduces the risk of fish mortality that may be associated with bath treatments caused by crowding, low oxygen levels and stress.

In-feed treatments are easy to apply and there is no disruption to the normal feeding routine. They can also be used to treat whole sites and management areas at the same time, enabling control of local sea lice populations.

Q Are there any disadvantages to in-feed treatments?

A Due to variable feed consumption in individual fish, there is a chance that some fish may receive a higher or lower dose than intended. However, SLICE has a broad safety margin and there is little risk of toxicity, even if some fish consume a higher dose than intended.

Extensive trials using SLICE at commercial farm sites resulted in good efficacy in every case and no mortality was associated with treatment, despite wide variations in fish weights of up to 1.5 kg in individual pens. In some cases, there may be a few fish which do not consume an adequate dose and therefore fail to benefit from treatment.

Q At what temperature range is SLICE effective and safe to use?

A Field trials and experience have shown that SLICE can be used safely and effectively at temperatures of 5.5° C to 15° C without any adverse effects. It is possible that this temperature range will increase, as further information is collected during commercial use.

At low temperatures it may take longer for lice numbers to decline. In one trial conducted at 7° C, efficacy did not reach 89% until Day 35 from start of medication. There are no data available at present on efficacy at temperatures below 7° C, although there are some accounts of success at low temperatures from recent commercial use. However, if feed consumption is poor at low temperature, in-feed medication may be inappropriate.

Q What tissue levels should be expected following SLICE treatment according to label recommendations?

A If sampled 24 hours after the end of treatment, then the levels should be an average of greater than/equal to 60 ppb, the therapeutic dose, across the population. There are, however, a number of variables, such as temperature or feed intake, that could affect the levels obtained.

Q How long before the fish will need to be treated again?

A The duration of efficacy will depend on sea temperatures, re-infestation pressure, treatment history on the farm as well as the susceptibility of the lice. Re-infestation may also occur due to the presence of wild fish in the area, which may serve as new sources of infection.

Trials indicate that fish may not need to be treated again for up to 10 weeks from the start of treatment, but monitoring of lice numbers should still be conducted on a regular basis. At warmer temperatures (>15° C), fish may metabolize emamectin faster, reducing the duration of efficacy.

Q How often can treatments be carried out?

A Fish must not be treated more than once in the 60 days prior to harvest. However, due to the high efficacy of SLICE and the duration of protection, the need for frequent treatments should be reduced. In some countries, the relevant environmental authority may limit the frequency of treatments.

Q If SLICE is so effective, why do you recommend alternating with other available treatments?

A Reliance on a single method of treatment encourages the development of resistance. Use of other treatment alternatives can help remove any lice populations that may be developing some tolerance to emamectin; this will reduce the frequency of exposure to emamectin and help prevent sea lice from developing resistance. The use of rotation programs and well-planned treatments will help to ensure that SLICE remains effective for longer.

Q Pens at one end of the site have very high lice counts, but pens at the other end have only a few lice per fish. Can we just treat the worst-affected pens?

A This is not unusual since hydrographic characteristics may affect lice burdens in different pens or sites. However, you must treat all pens in a site since there are likely to be many infective larvae around that may, in turn, affect fish with low lice counts.

In addition, even with relatively few lice present, untreated fish will provide a reservoir of infection that will re-infect treated fish once levels of emamectin have declined. By treating all pens on a site, future infection pressure will be reduced, thereby allowing more effective long-term control.

Q Medicated feeds were administered for 7 days and fish were returned to normal feeds 2 days ago. They are now taking very little feed. Is this a result of medication?

A When SLICE is used at the correct dose rate under normal conditions, no reduction in feed consumption should be observed during or after medication. In fact, most fish show an improved feed response because lice numbers are reduced.

In safety trials, there was only one occasion when a reduction in feed consumption was seen, but this was attributed to a change in the type of feed pellet used. Loss of appetite has only been observed in experimental tolerance trials where fish were fed at 7x the therapeutic dose, and there were no effects on appetite when fish were fed at 3.5x the therapeutic dose.

Unless fish are accidentally fed a massive overdose, the reduction in feed uptake is very unlikely to be a result of SLICE treatment and you should consider other possible causes.

Q When feed uptake is so variable between individual fish, is there a risk that some fish will eat more and receive an overdose?

A SLICE has a broad safety margin. At the normal dose rate, individual fish would have to eat around 5x to 7x the intended ration before any problems might occur. Only minor signs of toxicity were observed in tolerance studies, and this was only achieved by incorporating a very high dose of emamectin onto the normal feed ration.

Q I am worried about overuse of SLICE on my operation. Can you recommend a standard treatment rotation program?

A As a general rule, it is a good idea to rotate treatment between different classes of compounds,

but the options for rotation vary among countries, depending on other products that have a marketing authorization where treatments are required for sea lice control. In any case, IPM practices such as fallowing and cleaner fish should be employed before chemical control agents are used.

Medication management

Q The instructions on SLICE packaging are to incorporate SLICE on a 0.5% feed ration, but fish are only taking a 0.3% ration at present. Can we still carry out treatment?

A Yes. SLICE can be used at rates down to 0.25% without any problems. (See Table 2 in the Preparation of Feed Medicated with SLICE section). The 0.5% ration is just a guide. However, although lower feed rates will not cause any palatability problems, fewer feed pellets make it more difficult to ensure that all fish receive a similar ration.

Q How can I be sure fish are receiving the right ration?

A Medicated-feed administration should be monitored by experienced farm personnel. If most pellets are being taken at, or near, the surface, continue feeding at the same rate. If the response slows down and pellets are seen to sink toward the bottom of the net, then slow down the rate of feeding or stop and come back later in the day.

Such observations are not always possible in bad weather conditions, but experience will help judge the feed response under such conditions. Feed consumption may be monitored using underwater cameras where these are available.

Q How should we calculate the emamectin concentration in medicated feed if we expect decreasing temperatures and decreased appetite during SLICE treatment?

A The target concentration in the feed should be calculated on the basis of the lowest level of feeding expected over the course of the 7-day treatment period. However, with so many variables to consider, it is best to consult the prescribing veterinarian.

Q Although fish were feeding well at the start of medication, the feed response has declined due to a fall in temperature and not all the ration has been fed at the end of the day. Can we extend the medicated-feed period to ensure they get the right dose?

A Yes. You can reduce the daily ration and extend the medication period. Keep a record of the amount of medicated feed not administered, and this should be fed on additional days after the recommended 7-day period until the whole target ration has been used.*

Q Due to severe weather conditions, we were unable to continue administering medicated feeds for 2 days. Can we resume feeding now?

A A short break in medication of up to 2 days should not cause any problems. Providing the fish are still feeding, just continue to feed until you have completed the 7-day course.*

Q We need to change the type and size of pellet fed to our fish. Can we just order medicated feed at the new pellet size?

A This is not the time to introduce a change of pellet. Always use the same size of pellet and feed type for medicated feeds. If this is unavoidable, introduce the new pellet in unmedicated form a few days before starting medication and ensure there is no reduction in feed uptake. This will give fish time to adapt and there will be no wastage of medicated feed or a reduction in efficacy.

*Different dosage administration could result in greater variation in feed intake and subsequent efficacy.

Frequently Asked Questions and Answers

Q I just received an analytical report back from the lab showing that emamectin benzoate levels in the feed were 10% lower than what should be expected. Should I be concerned?

A No. Due to expected loss in recovery from sample preparation, one could reasonably expect sample results to be plus or minus 10% of the theoretical value. In fact, many regulatory agencies set an even wider range of acceptable limits or specifications, given the variability inherent in manufacturing, sampling and the complexity of feed-testing methods.

Q Does the type of oil used in the manufacture of feed medicated with SLICE affect the absorption and distribution of emamectin benzoate in treated populations?

A Limited data has been generated on use of different oils in the manufacture of feed medicated with SLICE. These data suggest that there is no major difference on emamectin benzoate uptake based on dietary oil type/source, but further study is warranted.

Q Can we store bags of medicated feed out on the sea pens overnight?

A No. There is always a risk that feed bags may be lost in bad weather and medicated feeds should always be stored in a secure location.

Efficacy

Q Can SLICE be used to prevent outbreaks of sea lice as well as killing existing lice?

A SLICE is highly effective in preventing the development of newly settled copepod. However, preventative treatment should only be used where there is clear indication that heavy infestation is likely to occur and the risk cannot be reduced by other means.

Unlike other currently available treatments, trials have shown that SLICE prevents the development of copepod on treated fish for up to 10 weeks, depending on temperature.

Q How long after starting treatment will it take before lice numbers are reduced?

A While treatment starts working straight away, it may take a few days before lice numbers start to decline. This depends very much on temperature and how well fish are feeding. At temperatures above 13° C, lice numbers may start to fall as early as Day 5 of medication, and at temperatures above 10° C, maximum efficacy will be seen within 14 to 21 days from the start of treatment. At lower temperatures, it may take longer to reach maximum efficacy.

Recent (2011) studies conducted at the University of Stirling have also shown that the timing of response is also dependent on the sensitivity of the lice population being treated.

Q Why does it take so long compared to bath treatments?

A Because SLICE is administered in feed, it takes a few days for the drug to reach its maximum concentration in the skin of fish. Once this level has been reached, however, efficacy against susceptible lice should be high and the duration of protection will extend far beyond the medication period.

Q I have just treated my fish with SLICE, and although free of lice, I can still see a small number of fish with chalimus and mobile lice. Why?

A These fish are probably poor feed responders or those that have been unable to compete with bigger fish. This could be avoided by ensuring fish are well graded to reduce competition.

Ideally, remove any runts or moribund fish, as these are often heavily infected, and if they do not take medicated feed, they may harbor a source of re-infection. Fish with significant lice damage will tend not to feed as well and there is a risk of under-dosing and survival of lice on these individuals. Early treatment, before lice damage occurs, will give the best results. Monitoring feed response is important and should be carried out by experienced operators so they can adjust the rate of feeding according to appetite.

Q Following completion of SLICE treatment 4 days ago, we carried out routine lice counts and found no mobile lice on fish, but why are there still a few chalimus stages left when SLICE is claimed to be effective against chalimus and mobile stages?

A SLICE is very effective against chalimus stages of lice. However, the frontal filament that attaches chalimus to the fish may take some time to break down; although the chalimus are unable to develop further, it may take a while for them to be shed from the skin.

Q Bioassays conducted using sea lice collected on my farm are showing tolerance to SLICE (emamectin benzoate). How long will it be before lice will become susceptible so that I may use SLICE on my farm again?

A There is no firm evidence to indicate how long it would take, although reversion to susceptibility has been observed with other parasites and classes of control agents. This is a function of many factors that include: (1) frequency of resistance genes in the population, (2) rate of gene mixing between susceptible and non-susceptible or reduced-susceptibility populations and (3) selection pressure on the sea lice populations such as the number of SLICE treatments per year, which is influenced by factors such as fallowing practices, the use of wrasse and use of treatments from a different chemical class. Bioassays should be conducted to determine lice sensitivity before considering the next treatment with SLICE.

Q Two weeks ago we treated with SLICE. The numbers of *Lepeophtheirus salmonis* and *Caligus elongatus* were dramatically reduced and there are no chalimus stages present, but now a small number of mobile *Caligus* have started to re-appear. Why is this?

A These may have originated from wild fish passing through the area. *Caligus* are more mobile than *Lepeophtheirus* and more likely to transfer between hosts. They are often present on wild fish, particularly on gadoids and clupeoids such as saithe and herring, especially in the summer months. These *Caligus* will probably not survive long on fish treated with SLICE or build up in great numbers, although they may remain in low numbers as a result of new recruitment from wild fish.

Q It is now 30 days since I started treatment and there is no reduction in lice numbers. Is this what I should expect?

A **SSP**, launched by MSD Animal Health in 2010 (known as SLICE Monitor in the UK), is designed to help the global salmon industry develop sustainable sea lice control programs. It provides tools to answer this question with the aid of bioassays and feed and flesh data.

After more than 10 years of commercial use and reliance on SLICE for control of sea lice infestation in farm-raised salmon, some resistance has been reported (in select areas of Scotland, Norway and Canada). This is not an unexpected occurrence, given the extensive use of SLICE and the limited availability of other medicines to control sea lice. That is why it is, first, important to check sea lice susceptibility through the use of bioassays and to conduct post-treatment feed and tissue analysis to ensure that the correct dose has been delivered to the fish.

Assuming that the lice being treated are susceptible to emamectin benzoate, the most likely explanations are poor feed consumption, inaccurate dose calculations, incorrect incorporation of medication into the

feed or underestimation of biomass at point of treatment. The prescribing veterinarian should always check the dose calculations and medicated rations. It may also be necessary to carry out further sampling and weigh the fish again to check biomass.

Always check to be sure that there was a good feed response in the treated pens. This should be done by an experienced operator who can usually assess whether all the feed is being consumed. Post-treatment tissue analysis can be conducted to determine whether sufficient levels of drug were delivered to the fish.

Retain samples of feed in the freezer, which can be sent for analysis of emamectin benzoate to ensure the drug was mixed with the feed at the correct rate. Check to make sure that medicated feeds were actually delivered and administered at the site.

Q For 10 weeks after completing SLICE treatment, lice numbers remained very low in all the pens on the site and we are really pleased with the results. Then 3 weeks ago, we started to find high numbers of chalimus on the fish, but even now there are still very few adult lice present. Why is this?

A Following treatment with SLICE, copepodid stages will settle on host fish initially, but these fail to develop to chalimus. Depending on water temperatures, the efficacy of SLICE will start to decline post-treatment as tissue concentrations start to decline in the fish. At this time, chalimus may start to re-appear on the fish but, in the early stages, their development may be affected and they will fail to reach maturity. This may continue for some weeks, but eventually, as concentrations of SLICE decline further, lice may develop to maturity normally.

Q Can I expect differences in efficacy between male and female sea lice?

A Yes. Male sea lice are typically less susceptible than female lice. Recent bioassay data suggests that both sexes of *L. salmonis* are equally sensitive to emamectin benzoate when the lice are at pre-adult stage. When the lice moult to adults, sensitivity in both sexes decreases but females are generally more sensitive than males. This is also seen in *in vivo* studies where females appear to be affected earlier than males following SLICE treatment.

Q I recently treated with SLICE and the duration of activity is not what it used to be. What should I do?

A First, always report any unexpected treatment outcomes to your veterinarian and local MSD Animal Health representative. Be prepared to discuss details such as bioassay results and prescription and feeding records.

It is very important for future lice management decisions to try and establish if unexpected results have been obtained because the treated lice population is demonstrating an increased tolerance to SLICE — or if it is because only a suboptimal treatment has been achieved (i.e., therapeutic dose has not been achieved in the whole population). This can be determined through the use of bioassays, by analyzing retained feed samples to check if fortification rates were as required and by analysis of retained fillet samples taken 24 hours after completion of treatment to ensure that therapeutic flesh levels of emamectin have been achieved. This sample retention and analysis procedure is all part of the SSP process.

Safety

Q We completed medication with SLICE 2 weeks ago. Now some of the fish are dark and show a poor feed response and still have many lice present. We have also had a few mortalities. Is this a result of toxicity?

A It is very unlikely. If fish had received a high dose, susceptible lice would have been removed, so if the fish still have high numbers of lice present this would not be a result of toxicity.

It is more likely these were poor feed responders that succumbed to sea lice damage. If there is a significant number of affected fish, contact the prescribing veterinarian. Such signs could also be a result of any number of causes and environmental conditions, and fish should be examined and pathology samples collected for examination.

Q On Day 5 of medication, we had a sudden increase in fish mortality. Is this a result of toxicity?

A Unless there was a gross error in calculation or preparation of medicated feeds, there should be no problems associated with treatment. Other possible causes of mortality should be investigated and the prescribing veterinarian should be informed. A sample of medicated feed can be sent for analysis of emamectin benzoate to check if there has been a mistake in the feed preparation. Contact your MSD Animal Health representative or your feed supplier for details regarding analysis of feed content. The amount of medicated feed administered to each pen should also be checked again.

Q The farm cat has just broken into the feed bags in our shed and eaten medicated feed. Will he be alright?

A Unless he has consumed a whole bag of medicated feed or does this on a regular basis, there should be no problems. Medicated feeds, however, should always be stored in a secure place. While many farms keep cats in feed sheds to control vermin, bags should be protected with tarpaulins; this is a good husbandry practice that not only prevents damage, especially by rodents, but prevents spoilage and other contamination of feeds.

Q The pens are stocked with cleaner fish (wrasse) as an additional measure to control lice. Will they be affected by treatment?

A Trials have not been conducted to evaluate the tolerance of wrasse (*Labridae*) to SLICE. However, its use has been found to be safe with a variety of fish species, including salmon, trout, bluegill sunfish and sheepshead minnow. Field experience has not indicated any risk to cleaner fish.

A How long after treatment can we harvest the fish?

Q This depends on the withdrawal period set by the regulatory authority in each country. As the withdrawal period in Europe, Canada and Chile is zero, you can harvest fish any time after treatment. In Norway, the withdrawal period of 175 degree days must be adhered to. Fish should not be treated with SLICE more than once in the 60-day period prior to harvest to avoid exceeding the MRL of 100 µg/kg.

In cases where tissue levels above the MRL are suspected or observed, fish should be sampled before harvest to confirm that tissue levels are less than 100 ppb (100 µg/kg).

Q How can there be a zero withdrawal period when the duration of efficacy is so long?

A Sea lice are far more sensitive to emamectin benzoate than fish or other animals. This is why there is such a good safety margin in fish and why the product was chosen for development in aquaculture.

Even at very low concentrations several weeks after the end of treatment, when levels cannot be detected in the tissues, emamectin can still prevent the development of newly recruited copepodids.

The withdrawal period is set on the basis of the MRL (100 µg/kg).

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List of Relevant Publications

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

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SLICE Technical Monograph. MSD-SSP-12. (2012)

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Stone J, Sutherland IH, Sommerville C, Richards RH, Varma KJ. Field trials to evaluate the efficacy of emamectin benzoate as an oral treatment of sea lice, *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* Nordman, infestations in Atlantic salmon, *Salmo salar* L. Aquaculture. 2000;186:205-219.

Stone J, Sutherland IH, Sommerville C, Richards RH, Varma KJ. Commercial trials using emamectin benzoate to control sea lice, *Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* Nordman, infestations in Atlantic salmon, *Salmo salar* L. Diseases of Aquatic Organisms. 2000;41:141-149.

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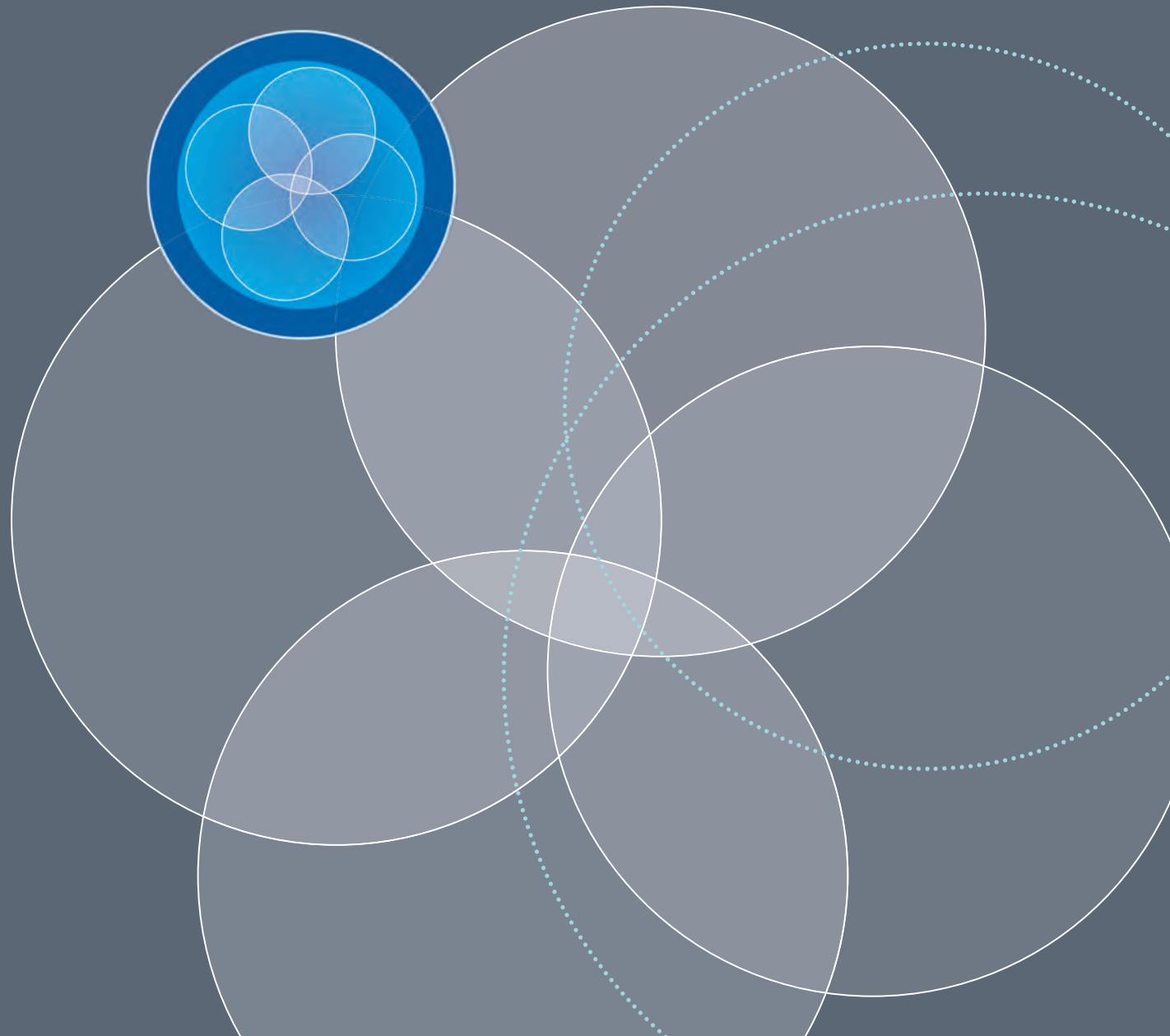
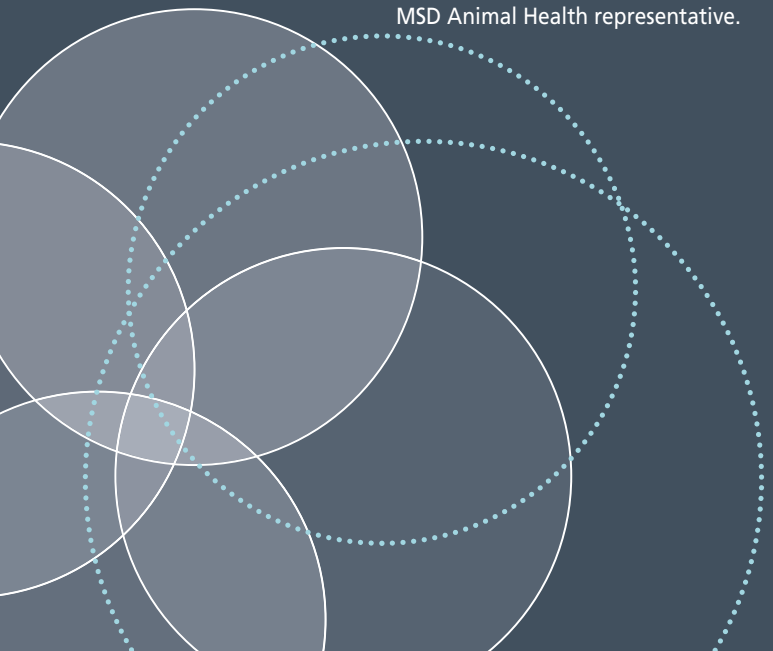


Specific product details such as indications, withdrawal time, legal category, etc. may vary by country. Please refer to your local package insert for details or contact your local MSD Animal Health representative.



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