



## Bioassay protocol for *in vitro* determination of sea lice sensitivity to emamectin benzoate

Bioassays are an important tool for determining the sensitivity of sea lice to treatments. They can be used routinely throughout the growing cycle to help predict the sensitivity of sea lice to available treatments or to detect changes in sensitivity within a population of sea lice.

The bioassay protocol for emamectin benzoate used by the Fish Vet Group (FVG) of Inverness, Scotland, is reproduced in this bulletin with permission from FVG.

Emamectin benzoate is the active ingredient in SLICE®, a highly effective, medicated feed premix manufactured by Intervet/Schering-Plough Animal Health. SLICE is indicated for the treatment and prevention of all parasitic stages of sea lice on salmon.\* Since its introduction over a decade ago, SLICE has become the leading product for control of sea lice due to its track record of proven field performance.

When performing bioassays, consider the following:

➤ Ideally, bioassays should be conducted in accordance with a published protocol. Please see the suggested reading list at the end of this bulletin.

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

➤ Bioassays can, however, provide the veterinarian with one additional tool among a suite of considerations to help make decisions about when a particular medicine may or may not be effective or when it may be time to consider changing to a treatment with a different mode of action.

The FVG utilizes propylene glycol as the solvent for preparation of emamectin benzoate stock solution. Other published methods recommend using methanol as the solvent. Either solvent can be used; propylene glycol and methanol are not thought to produce significantly different end results.

A protocol for utilizing methanol as the solvent is available in the handbook *Seallice Resistance to Chemotherapeutants — A handbook in resistance management*. Please see the suggested reading list at the end of this bulletin.

### THE FVG BIOASSAY PROTOCOL

#### ➤ Equipment required (sufficient for seven dilutions, including a seawater control)

2 L seawater	Curved forceps
50 and 100 ml measuring cylinders	5 ml pipette & tips
1 x 500 ml flask	5 to 10 ml pipette (& tips)
1 x 250 ml flask	1 x 50 ml syringe
1 x 50 ml flask	Large petri dishes (sort lice)
14 petri dishes	Sieve
7 x 0.5 L beakers	Magnifying glass
Salinometer	

\*Check your local package insert for details.



THE FVG BIOASSAY PROTOCOL *continued*

**Preparation of stock solution (100 ppm)**

- Start with 5 mg emamectin benzoate in 50 ml propylene glycol. Prepare up to 24 hours in advance, as it can take time to fully dissolve. Store in an amber bottle in the refrigerator at 4° C (40° F).
- Program the incubator for 12° C (53.6° F).
- Measure and note the salinity of seawater supplied with lice.
- Using the stock solution, prepare the Work Solution (1.0 ppm): 10.0 ml stock solution + 990 ml seawater

**Prepare dilutions, starting with weakest first, in 500 ml volumetric flasks**

1. Control, seawater only (0 ppb)
2. 31.3 ml Work + 468.7 ml seawater (62.5 ppb)
3. 62.5 ml Work + 437.5 ml seawater (125 ppb)
4. 125 ml Work + 373 ml seawater (250 ppb)
5. 250 ml Work + 250 ml seawater (500 ppb)
6. 350 ml Work + 150 ml seawater (700 ppb)
7. 500 ml Work Solution (1000 ppb)

**Carrying out the bioassay**

- Sea lice are collected from fish, transported to the laboratory and left overnight in seawater (with gentle aeration) to ensure viability before starting the bioassay. Poor survival following overnight holding may suggest sub-viable sea lice, which should not be used for a bioassay.
- Work from dilute solutions to concentrated solutions at all times.
- Using large petri dishes, separate male and female lice. Use only pre-adult II lice. Do not select adult

**Carrying out the bioassay *continued***

females for testing. Discard any lice that do not swim. Generally, only use lice that are attached to the side of the collection vessel. Lice that are caught in the sieve when seawater is filtered off are usually compromised.

- Run the test in duplicate; dispense 50 ml of each solution into each deep-dish petri dish, using a 50 ml syringe, working from weakest to strongest concentrations.
- Place five males and five females into each petri dish. Discard any lice that do not swim off when dropped into the dish.
- Place lid on dish. Note time that all plates are stocked with lice as the starting point of the bioassay. Place dishes in incubator at 12° C (53.6° F).
- Read the test after 24 hours. Grade lice as follows, noting whether affected lice are male or female:  
**Live:** Swims off when picked up by forceps  
**Moribund:** No swimming response when picked up; twitching seen under dissecting microscope  
**Dead:** Absence of any movement
- Acid wash all glassware. Autoclave at 123° C (253.4° F).

**Suggested reading**

*Seallice Resistance to Chemotherapeutants — A handbook in resistance management, Search Project (QKK2-CT-00809)*, which can be downloaded at: <http://www.rothamsted.bbsrc.ac.uk/pie/search-EU/index.php>.

Optimization and field use of a bioassay to monitor sea lice *Lepeophtheirus salmonis* sensitivity to emamectin benzoate, *Diseases of Aquatic Organisms*, available at: <http://www.int-res.com/abstracts/dao/v79/n2/p119-131/>.

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