



ASSESSMENT OF THE FATE OF EMAMECTIN BENZOATE, THE ACTIVE INGREDIENT IN SLICE[®], NEAR AQUACULTURE FACILITIES IN BRITISH COLUMBIA AND ITS EFFECT ON THE PACIFIC SPOT PRAWN (*PANDALUS PLATYCEROS*)



Pacific Spot Prawn (*Pandalus platyceros*)
Photo: Phillip Colla, OceanLight.com

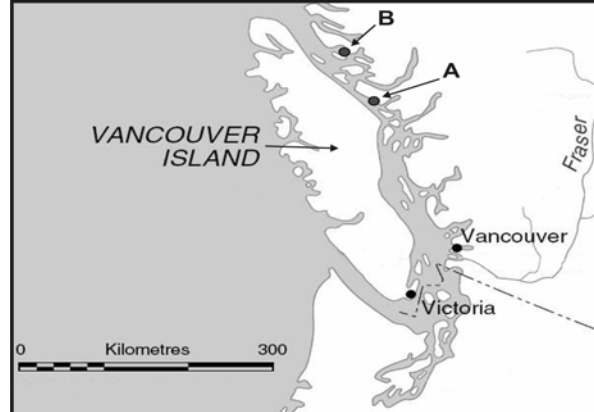


Figure 1. Map showing the locations of the two salmon farm sites A and B where water, sediment and spot prawns were collected for EB analysis before, during and after the application of SLICE[®].

Context

Sea lice are naturally occurring parasites found on salmon and other fish in Canadian and international waters. Farmed salmon are subject to infestation with sea lice, and there exists the potential for transfer of sea lice between farmed salmon and wild fish populations. Sea lice levels are monitored and controlled on British Columbia's salmon farms to minimize impacts on farmed fish and reduce the risk of infecting fish that live outside the farm. Current sea lice management strategies include harvesting, fallowing, and preventative treatment using anti-parasitic chemotherapeutants. The only chemotherapeutant used to control sea lice at salmon farm sites in British Columbia is SLICE[®], an in-feed treatment in which the active ingredient is emamectin benzoate (EB). The application of such treatments can lead to the release of EB into the broader environment through a variety of pathways, including solubilization, transport and sedimentation of particles containing EB from uneaten feed and fish excreta. This creates the potential for chemical presence in the water column, accumulation in benthic ecosystems, and exposure of non-target organisms to EB. DFO Science has carried out initial research to investigate the environmental concentrations of EB and its main conversion product 4'-deoxy-4'-epi-amino avermectin B1a (AB) following the application of SLICE[®] at salmon farms in B.C., and the uptake and potential toxicological impacts of EB/AB in the Pacific spot prawn (*Pandalus platyceros*) under laboratory and field conditions. DFO Pacific Region's Fisheries and Aquaculture Management (FAM) Division has requested advice regarding the spatial and temporal distribution of EB near salmon farms and the biological effects on non-target organisms. This report documents the findings of a Regional Science Advisory Process based on the related DFO research.

This Science Advisory Report has resulted from a Fisheries and Oceans Canada, Canadian Science Advisory Secretariat (CSAS) Regional Advisory Process (RAP). Additional publications resulting from this process will be posted as they become available on the DFO Science Advisory Schedule at <http://www.dfo-mpo.gc.ca/csas-sccs/index-eng.htm>.

SUMMARY

- Sea lice infections at salmon farms in British Columbia are monitored and controlled to minimize impacts on farmed fish and reduce the risk of infecting fish that live outside the farm. The chemotherapeutant used to control sea lice on B.C. salmon farms is SLICE®, an in-feed treatment in which the active ingredient is emamectin benzoate (EB).
- A Regional Science Advisory Process was undertaken to review the results of DFO research to determine the environmental concentrations of EB near two B.C. salmon farms following the application of SLICE®, and to assess the uptake and potential toxicological impacts of EB in the Pacific spot prawn (*Pandalus platyceros*) under laboratory and field conditions.
- Using a new and very sensitive analytical method, concentrations of EB and its main conversion product 4'-deoxy-4'-epi-amino avermectin B1a (AB) were measured in water, sediment and spot prawns collected at two salmon farm sites with differing hydrodynamic and biophysical characteristics before, during, and after the application of SLICE®.
- Low levels of EB were detected in the sub-surface water beneath each farm during SLICE® treatment. EB released into the water column dissipated quickly and was undetectable 4 to 5 weeks after treatment.
- The amount of EB measured in surface sediments varied between the two sites. At one site EB in sediment remained close to the limit of quantification (LOQ) of the analytical method. Most of the EB reaching the sediment at the other site remained localized, with levels falling to below the LOQ within 150 m of the farm. EB was detected in sediment from this site over 1.5 years after SLICE® treatment. AB concentrations were less than 30% of the concentration of EB in all sediments.
- The amount of EB estimated in sediment accounts for a small fraction of the EB applied during a full-cycle SLICE® treatment. It was not possible to generate a complete mass balance for EB with the available data.
- EB measured in the tissues of spot prawns collected within 150 m of each salmon farm increased over a period of 100 days following the application of SLICE®. The level and duration of exposure in the field differed from those used in the laboratory exposure experiments that formed part of the same study, so it was not possible to correlate directly the results of the lab experiments with field measurements of EB.
- Laboratory experiments suggest that short-term (8-day) exposure of spot prawns to sediment containing EB at concentrations significantly greater than those measured in the field can alter the expression of certain genes in muscle tissue. No clear dose-response relationship could be established for the mortality or differential gene expression observed during these experiments. Additional studies involving standard toxicological measurements, different life-cycle stages, and environmentally relevant EB concentrations are recommended, along with further gene expression studies under both laboratory and field conditions.
- Studies that include a broader range of salmon farm sites in B.C. are also recommended. Information regarding SLICE® usage, site conditions, and local prawn fisheries is available from a number of government and industry sources and can be used when planning and interpreting the results of future studies.
- Current research shows that the spatial and temporal distribution of EB near salmon farms varies from site to site and that, under certain conditions, EB can remain and so potentially

build up in sediments close to salmon farms, depending on the extent and frequency of SLICE® usage. EB is also bioavailable and can be measured in the muscle tissues of spot prawns near salmon farms treated with SLICE®. More research is needed to assess the potential biological impacts of low concentrations of EB and its metabolites on spot prawns and other non-target organisms.

INTRODUCTION

Sea lice are naturally occurring parasites found on salmon and other fish in Canadian and international waters. Farmed salmon are subject to infestation with sea lice, and there exists the potential for transfer of sea lice between farmed salmon and wild fish populations. Fisheries and Oceans Canada (DFO) recognizes the possibility that a heavy sea lice burden can affect the survivability of young salmon. Consequently sea lice levels are controlled on British Columbia's salmon farms to minimize impacts on farmed fish and reduce the risk of infecting fish that live outside the farm.

Current sea lice management strategies include harvesting, fallowing (modifying production cycles to minimize the presence of farmed fish during key periods), and preventative treatment of farms using anti-parasitic chemotherapeutants, as prescribed by attending veterinarians. The only chemical used to treat sea lice on salmon farms in British Columbia is SLICE®, an in-feed treatment in which the active ingredient is emamectin benzoate (EB).

The application of such treatments to manage sea lice levels on B.C. salmon farms can lead to the release of EB into the broader environment through a variety of pathways, including solubilization, transport and sedimentation of particles containing EB from uneaten feed and fish excreta. This creates the potential for chemical presence in the water column, accumulation in benthic ecosystems, and exposure of non-target organisms to EB. Concerns regarding the uptake and potential effects of EB on non-target organisms near B.C. salmon farms have been raised by several stakeholder groups, including the Pacific Prawn Fishermen's Association, and First Nations.

Understanding the extent to which aquatic organisms are exposed to EB and other chemicals represents an important aspect of DFO research and provides a critical knowledge base for a variety of end-users including Environmental Managers, Aquaculture Regulators and the aquaculture industry. Science advice is needed to support environmental regulation and decision-making with regard to aquaculture.

The Program for Aquaculture Regulatory Research (PARR) supports research carried out by DFO scientists to increase the science knowledge base used to inform ecosystem-based environmental regulation and decision-making with regard to the aquaculture sector. In recent years DFO Science has been conducting research supported by PARR and other programs into the fate of EB near B.C. salmon farms using SLICE® and its potential impact on the Pacific spot prawn (*Pandalus platyceros*), a commercially important non-target species.

This DFO research was carried out in partnership with the B.C. Pacific Salmon Forum, the B.C. Ministry of Environment, Environment Canada, the University of Victoria, the Pacific Prawn Fishermen's Association, Marine Harvest Canada, and Mainstream Canada.

Rationale for Assessment

DFO Pacific Region's Fisheries and Aquaculture Management (FAM) Division has requested advice regarding the fate and distribution of emamectin benzoate (EB), an in-feed treatment for

controlling sea lice, near salmon farms in British Columbia and its potential biological effects on non-target organisms. This advice will be used by Environmental Managers, Aquaculture Regulators and the aquaculture industry, and may form the basis for elements of future consultation processes on aquaculture activities through Integrated Management of Aquaculture Plans (IMAPs).

ASSESSMENT

The goal of this review was to provide a detailed assessment of (i) the fate of EB and its main conversion product 4'-deoxy-4'-epi-amino avermectin B1a (AB) at marine cage salmon farms following the application of SLICE®, and (ii) the uptake and potential biological effects of EB/AB in spot prawns under laboratory and field conditions, using a genomics-based approach.

This dual approach was intended to provide a standardized framework for assessing the potential biological effects of EB/AB, with the outcomes of the laboratory study providing a context for evaluating field measurements of EB and AB in water, sediment and biota. Environmental measurements can also be used to test, calibrate and implement the DEPOMOD particle tracking model to predict the behaviour of EB in relevant aquatic ecosystems and contribute to developing policy for the regulation of SLICE® usage.

Methods

Sample Collection

Sampling locations at Sites A and B (Fig. 1) and at the Reference sites were established and water, sediment and biota (Pacific spot prawn) samples were collected before, during and after the application of SLICE® at Sites A and B. Hydrodynamic and biophysical data for the two salmon farm sites are shown in Table 1.

Table 1. Available biophysical and hydrodynamic data for salmon farm sites A and B.

Site	Maximum allowable peak biomass (Tonnes)	Depth (m)	Mean near-surface current flow (cm/s)	Mean near-bottom current flow (cm/s)
A	2550	70	6.7	3.9
B	650	50-80	9.1	5.1

For logistical reasons the number of samples collected was greater, and the resulting dataset more comprehensive, for Site A than for Site B (where benthic sediment was relatively sparse).

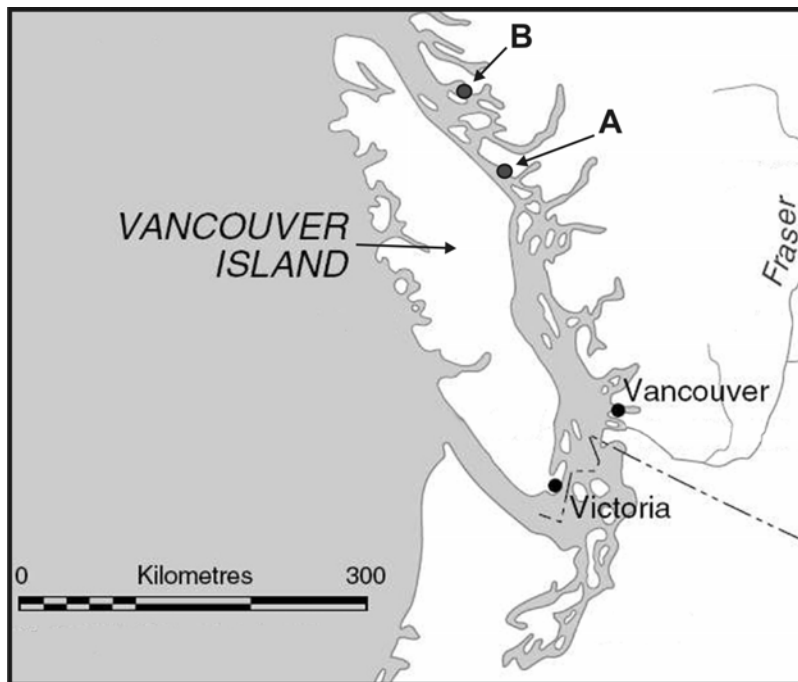


Figure 1. Map showing the locations, in the Broughton Archipelago, of the two salmon farms (Sites A and B) where water, sediment and spot prawns were collected for EB and AB analysis before, during and after the application of SLICE®.

Water samples were collected at a depth of 30 to 60 cm using 4-L amber glass bottles. Water sampling at Site A was carried out along two transects extending East and West at distances of 0, 30, 60, 100 and 150 m from the net-pens, and at a Reference station. Triplicate samples were collected at the Reference station first, then from lowest to highest expected concentration along each transect. At Site B water was only collected at 0 m from the South-West corner of the farm. The 4-L bottles were transported in sealed totes, frozen at -20 °C on arrival at the Institute of Ocean Sciences (IOS) in Sidney, B.C. and stored until analysis.

Sediment samples were collected using a Van Veen grab. Again, sampling at Site A was carried out along transects extending East and West from the net-pens at distances of 0, 30, 60, 100 and 150 m, and at a Reference station. Replicate samples were prepared by removing the top 1 cm of sediment and storing in 100-mL trace clean amber jars. Samples were collected at the Reference station first, then from lowest to highest expected concentration along each transect. At Site B, sediment samples were collected at 0, 100 and 300 m South-West of the net-pens. Samples in the amber jars were transported in coolers, frozen (-20 °C) on arrival at IOS, and stored until analysis.

Wild Pacific spot prawns were collected in standard prawn traps baited with salmon diet pellets. Prawns were collected from Site A at distances 0, 100 and 300 m West of the net pen, in order to parallel the East-West current directions at this site. Control prawns were collected at a reference site distant from the farm. Live captured prawns were immediately sampled for genomic analysis by removing the first abdominal segment and excising a small dorsal section of muscle tissue, which was immediately stored in RNA-Later solution and kept on ice for 24 hr prior to freezing. The remaining prawn tissue was stored in a zip lock bags and frozen for EB analysis.

Containment traps holding live prawns collected at the reference site were deployed at a distance of 0 m (at the corner of the net pens) at Sites A and B prior to SLICE® treatment, and at the Reference site. The latter acted as a control for both the wild and contained prawns sampled at Sites A and B. Contained prawns were fed with squid at variable times during deployment, and were sampled for genomic and EB analysis in the same manner as the wild prawns. Due to unforeseen problems, however, not all of the planned samples from the containment traps were obtained, and there were insufficient data to interpret the results of genomic analysis for wild and contained spot prawns.

Chemical Analysis

Measurements of EB and its main conversion product (AB) in water, sediment and biological samples were made using a new and highly sensitive analytical method based on high-performance liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS). This method allowed precise and accurate quantification of EB and AB at parts-per-billion (ppb) levels in sediment and tissue extracts, and at parts-per-trillion (ppt) levels in water samples. Limits of quantification (LOQs) for EB were 0.006 ng/L (ppt), 0.12 ng/g (ppb) and 0.09 ng/g (ppb), respectively, in water, sediment and prawn muscle tissue (determined on a wet weight basis). Further details of the method can be found in Ikonomou and Surridge (2012).

Genomic Analysis

Wild spot prawns collected near IOS were exposed for 8 days to sediment containing 0.1 to 4.8 mg/kg (parts-per-million) EB under static aquarium conditions, then sampled for EB and genomic analysis as described above. A subtracted cDNA library containing expressed gene sequences that increased in the muscle transcriptome following EB exposure was constructed. Partial sequences (mRNA transcripts) from 12 distinct expressed genes were then selected for qPCR assay development, and screening for biological effects resulting from EB exposure. Further details of the methodology used can be found in Veldhoen *et al.* (2012).

Results

The Terms of Reference for this review included the general question:

Does the current use of SLICE® in B.C. salmon farms result in the exposure of non-target organisms to levels of emamectin benzoate (EB) that could result in biological effects?

The findings of this study show that (i) EB can remain and so potentially build up in benthic sediments close to salmon farms, depending on the frequency and extent of SLICE® usage and the local site conditions; and (ii) EB is bioavailable and can be measured in the muscle tissues of spot prawns collected near salmon farms treated with SLICE®. However, more research is needed to assess the bioaccumulation and potential biological effects of EB and its metabolites in spot prawns and other non-target organisms.

The following summarizes how the results of the study address the specific questions posed in the Terms of Reference.

1. *Is emamectin benzoate (EB) detectable in water and sediment surrounding Salmon Farms and at distant Reference Sites in B.C. coastal waters?*

EB was detected at parts-per-trillion (ppt) levels in sub-surface water samples collected in the immediate vicinity of two salmon aquaculture sites in B.C. following SLICE® treatment. EB was also measured at parts-per-billion (ppb) levels in surface sediment samples collected within 150

m of the net pens at each site. The limits of quantification (LOQs) for water and sediment were 0.006 ppt and 0.12 ppb, respectively.

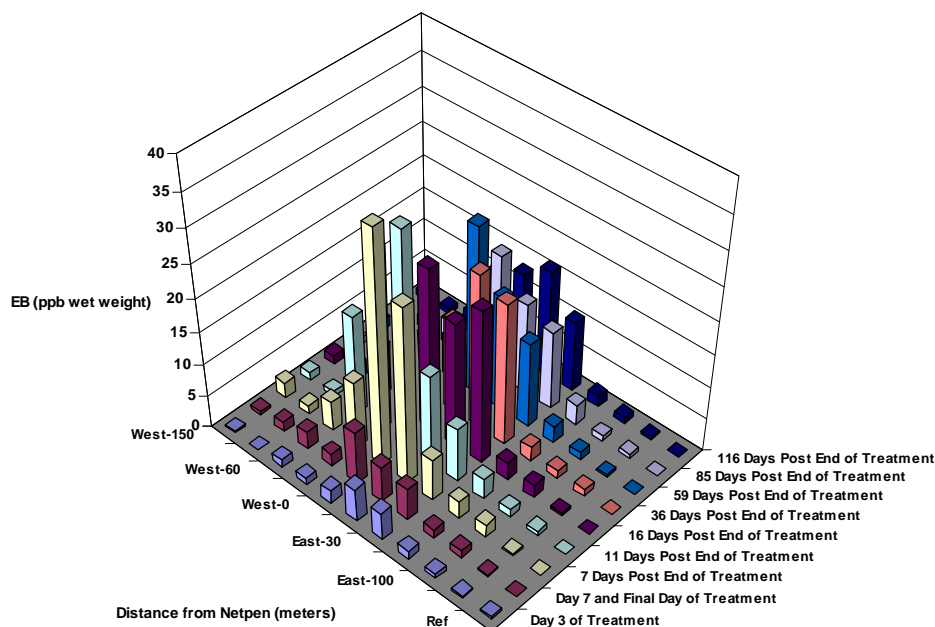


Figure 2. Emamectin benzoate (EB) concentrations (ppb wet weight) measured in surface sediment samples collected at Site A up to 4 months following the application of SLICE® on January 12, 2009. Samples were collected at distances of 0, 30, 60, 100 and 150 m due East and West of the farm, and at a distant Reference site. The average moisture content of the sediment samples was 69.7% ± 11.4%.

2. What is the spatial and temporal distribution of EB in the vicinity of Salmon Farms following the application of SLICE®, and how does this compare with the Reference Sites?

The highest EB levels measured at Site A (35 ppb) and Site B (0.33 ppb) were found in surface sediment collected at a distance of 0 m (i.e. at the edge of the net pens) about 2 to 3 weeks following the application of SLICE® (Fig. 2 shows sediment data for Site A). EB levels in sediment collected at a distance of 100 to 150 m from Sites A and B were close to the limit of quantification for sediment (0.12 ppb). EB was close to or below the limit of detection (0.06 ppb) in sediments collected from the two Reference sites

EB levels in water were highest (0.6 ppt) within 50 m of the net pens 1 day after SLICE® treatment, falling to below the method limit of quantification for water (0.006 ppt) at a distance of 150 m from Sites A and B, and at the Reference sites. EB and its main conversion product AB were undetectable in water samples 4 to 5 weeks after SLICE® treatment.

3. How does the EB footprint relate to local conditions (e.g. depositional vs. dispersive sites, pH) and can the distribution and decomposition of EB be effectively modelled?

EB released into the water column during SLICE® treatment dissipates quickly, whereas most of the EB reaching the sediment remains localized within 150 m of the farm. EB was measured at ppb levels in sediment from Site A but at less than ppb levels in sediment at Site B, reflecting the different hydrodynamic and biophysical characteristic of the two sites (Table 1).

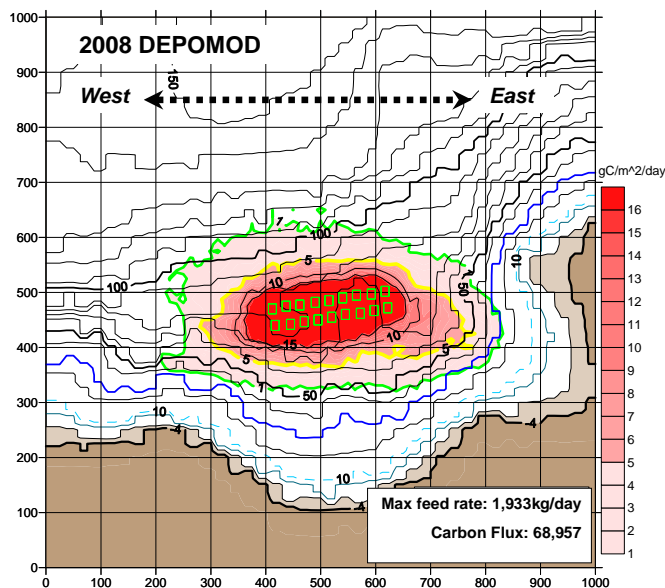


Figure 3. DEPOMOD modeling of solids accumulation at Site A in 2008. The orientation of the salmon pens remained the same in 2009 when the present study was undertaken.

Although the distribution of EB in sediments at Site A shows some correlation with the pattern of sediment accumulation predicted by the particle tracking program DEPOMOD (Fig. 3) the amount of EB estimated in sediment accounts for only a small fraction of the EB applied during a full-cycle SLICE® treatment. More information (such as the amount of EB bound to suspended particles) is needed before the distribution and fate of EB can be effectively modeled, or a complete and accurate mass balance obtained.

EB was present at low ppb levels in sediments at Site A over 1.5 years after SLICE® treatment. This shows that EB can remain and so potentially build up in sediments near salmon farms. It was also noted that AB levels remained less than 30% of the EB concentration measured in the same sample. The relatively low concentrations of EB and AB measured at Site B highlight the need for further studies involving additional B.C. salmon farm sites representing a broader range of hydrodynamic conditions, and incorporating detailed information about the extent of SLICE® usage at each site.

4. How do the concentrations of EB measured in water and sediment at Reference Sites and within 300 metres of Salmon Farms compare with those measured in non-target organisms (e.g. shrimp) and with those found, through toxicological studies, to result in biological effects?

EB measured in the muscle tissues of spot prawns collected within 150 m of Sites A and B reached 3.1 ppb over a period of 100 days following the application of SLICE®. However, the level and duration of exposure differed from those used in the laboratory experiments that formed part of the same study. Hence, it was not possible to correlate the results of those experiments with field measurements of EB.

The results of the genomics-based laboratory exposure experiments, conducted under static aquarium conditions, suggest that short term exposure to EB at concentrations significantly greater than those measured in the field can alter gene expression in spot prawns. However, no

clear dose-response relationship could be established for the mortality or differential gene expression observed during these experiments. Standard toxicological measurements such as Lowest or No Observed Adverse Effect Concentrations (LOAEC, NOAEC) and Lethal Concentration (LC50) were not included in the study, and such information for spot prawn is currently unavailable in the literature for comparison with genomics results or field measurements of EB. Additional studies including standard toxicological measurements, different life-cycle stages, and environmentally relevant EB concentrations are therefore recommended, along with further gene expression studies under both laboratory and field conditions.

Sources of Uncertainty

Study sites A and B represent a small sub-set of marine cage salmon farm sites in B.C., although different hydrodynamic and benthic conditions (e.g. less flushing and greater sedimentation at Site A) provide some context for interpreting EB measurements at each site. Further studies involving additional aquaculture sites, DEPOMOD predictions of the distribution of suspended particles originating from those sites, and analysis of EB in such particles through suspended sediment sampling are recommended. Detailed information regarding SLICE® usage and local conditions will also be required in order to effectively model and predict the environmental fate and potential impacts of EB arising from SLICE® treatments.

It is not possible at this point to draw linkages between the results of genomics-based laboratory studies of the potential biological effects of EB on spots prawns and field measurements of EB in water, sediment and biota, due to differences in the level and duration of exposure to EB. Further studies involving standard toxicology measurement (e.g. LOAEC, NOAEC, LC50), different life-cycle stages, and environmentally relevant EB concentrations are needed to provide a toxicological assessment of the effects of EB on spot prawns, and to interpret field measurements of EB in terms of their potential impact on wild prawns, fisheries and ecosystems.

Pacific spot prawns are commercially important, but other species may be more susceptible to biological effects caused by EB. Relevant studies of these effects in other species (e.g. crab, shrimp, planktonic crustaceans) are therefore warranted.

Ecosystem Considerations

Although the results provide information about the distribution and fate of EB near salmon farms and its potential effects on spot prawns, the current study does not provide sufficient information for assessing the potential impacts of EB at the ecosystem level. Further studies addressing the cumulative impacts of EB, including bioaccumulation of EB and AB in spot prawns, other resident organisms and predators, and the wider dispersion, persistence and possible build-up of EB in sediments are required to evaluate these potential ecosystem effects.

CONCLUSIONS AND ADVICE

The following are the specific findings and recommendations arising from this review.

- Following SLICE® treatment at the two study sites, EB concentrations in surface sediment were found to range from 0.12 ppb (the limit of quantification, or LOQ, of the analytical method) to 35 ppb within a radius of 150 m from the farm site. The EB sediment concentrations were substantially lower at Site B than at Site A, which may be attributed to different hydrodynamic and/or biophysical conditions at the two sites.

Pacific Region Fate of Emamectin Benzoate from SLICE and its Effect on Spot Prawn

- EB was found in surface sediments where SLICE® has been used. EB residues on the order of 3 ppb remain in the sediment around study Site A for an extended period (>1.5 years). Site B and four neighboring sites were also sampled at the same time with levels of EB measured between 0.12 ppb (method LOQ) and 6.5 ppb. It is recommended that future work links the history of SLICE® usage with sediment profiles at these and other salmon farm sites in B.C.
- Conversion of EB to its main conversion product AB was observed in sediment collected at Site A. The concentration of AB was less than 30% of the EB concentration measured in the same sample over a 115 day period following SLICE® treatment. This ratio did not change in samples collected up to 1.5 years later at Site A, or at the other sites examined.
- EB was detected in sub-surface water samples at levels between 0.006 ppt (method LOQ) and 0.635 ppt at both study sites during SLICE® treatment. EB appeared to dissipate quickly over time and was not detected in sub-surface water 4 to 5 weeks after treatment. AB was not detected in water samples.
- EB was measured at between 0.09 ppb (method LOQ) and 3.1 ppb in the muscle tissue of spot prawns collected in the vicinity of salmon farms treated with SLICE® over a period of 100 days post-treatment. AB was also detected, at approximately 30% of the EB concentration.
- The laboratory-based examination of spot prawn using static aquarium conditions indicates that short-term (8 day) exposure to sediment containing >100 ppb EB can alter the expression of specific genes (mRNA abundance patterns) in muscle tissue. It is not possible to draw direct linkages between these studies and field measurements of EB in water, sediment or biota. Additionally, research involving standard toxicological measurements (LOAEC, NOAEC, LC50), different (e.g. pre-molt) life-cycle stages, and environmentally relevant EB concentrations is needed to assess the impact of EB on prawns and other susceptible organisms, fisheries and ecosystems.
- Gene expression analysis was performed on wild and contained prawns collected near salmon farm sites. However, there was not enough information to interpret the results of these analyses. It is recommended that additional gene expression work be undertaken in the future.
- The available sampling techniques were adequate for an initial study to assess the fate of EB. Recommendations for future studies include greater use of the particle tracking model DEPOMOD and sampling strategies that provide additional information (including EB levels in suspended particles and bottom waters) for determining the environmental concentrations and fate of EB.
- Liquid chromatography-tandem mass spectrometry provides the sensitivity, specificity and accuracy required to measure EB, AB and other chemicals of concern in environmental and biological samples. This technology is available to DFO researchers through the Laboratory of Expertise in Aquatic Chemical Analysis (LEACA) at the Institute of Ocean Sciences in Sidney, B.C.

The results of the study show that (i) EB can remain and so potentially build up in benthic sediments close to salmon farms, depending on the frequency and extent of SLICE® usage and local site conditions; and that (ii) EB is bioavailable and can be measured in the muscle tissues of spot prawns near salmon farms treated with SLICE®. EB released into the environment following the application of SLICE® dissipates quickly, and most of the EB reaching benthic sediments remains localized within a short distance (150 m) of the farm site. However, it was not possible to extrapolate measurements made at the two study sites to other aquaculture sites

in B.C., as there are insufficient data to determine a relationship between site conditions and the environmental fate of EB or its potential impact on fisheries and ecosystems. Further research is needed to assess the persistence of EB in aquatic ecosystems, as well as the bioaccumulation and potential biological effects of EB and its metabolites in spot prawns and other non-target organisms.

SOURCES OF INFORMATION

This Science Advisory Report has resulted from a Fisheries and Oceans Canada, Canadian Science Advisory Secretariat (CSAS) regional advisory process (RAP) meeting held on October 18-19, 2011 on the Assessment of the environmental impact of the treatment of sea lice with the pesticide SLICE® at aquaculture facilities in British Columbia. Additional publications from this process will be posted as they become available on the DFO Science Advisory Schedule at <http://www.dfo-mpo.gc.ca/csas-sccs/index-eng.htm>.

Ikonomou, M.G. and SurrIDGE, B.D. (in press). Ultra-Trace Determination of Aquaculture Chemotherapeutants and Degradation Products in Environmental Matrices by LC/MS/MS. *Intern. J. Environ. Anal. Chem.*

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FOR MORE INFORMATION

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