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Proceedings of the Pacific regional peer review on the 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*)

October 18-19, 2011
Sidney, BC

Chairperson: Dr. Andrew R.S. Ross
Editor: Dr. Andrew R.S. Ross

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Foreword

The purpose of these Proceedings is to document the activities and key discussions of the meeting. The Proceedings may include research recommendations, uncertainties, and the rationale for decisions made during the meeting. Proceedings may also document when data, analyses or interpretations were reviewed and rejected on scientific grounds, including the reason(s) for rejection. As such, interpretations and opinions presented in this report individually may be factually incorrect or misleading, but are included to record as faithfully as possible what was considered at the meeting. No statements are to be taken as reflecting the conclusions of the meeting unless they are clearly identified as such. Moreover, further review may result in a change of conclusions where additional information was identified as relevant to the topics being considered, but not available in the timeframe of the meeting. In the rare case when there are formal dissenting views, these are also archived as Annexes to the Proceedings.

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SUMMARY

These Proceedings summarize the relevant discussions and key conclusions arising from a Fisheries and Oceans Canada (DFO), Canadian Science Advisory Secretariat (CSAS) Regional Advisory Process (RAP) meeting held at the Institute of Ocean Sciences in Sidney, B.C. on October 18-19, 2011 during which a working paper (CSAP WP2011-P55) on the environmental fate and potential biological effects of the anti-parasitic chemotherapeutant emamectin benzoate (the active ingredient in SLICE®) was presented for peer review.

In-person and web-based participation included Fisheries and Oceans Canada (DFO) staff from Fisheries & Aquaculture Management and Science Branch, together with external participants from Health Canada, the BC Ministry of Agriculture, the Scottish Government and Association for Marine Sciences, the aquaculture, prawn fishing and chemotherapeutant manufacturing industries, a number of environmental non-governmental organizations, and academia.

The conclusions and advice resulting from this review will be presented in the form of a Science Advisory Report, which will provide advice to DFO Fisheries & Aquaculture Management regarding the spatial and temporal distribution of emamectin benzoate near salmon farms and its potential biological effects on Pacific spot prawn, a commercially important non-target organism. This advice will support ecosystem-based environmental regulation and decision-making with regard to the aquaculture sector, and may form the basis for elements of future consultation processes on aquaculture activities through Integrated Management of Aquaculture Plans (IMAPs).

The Science Advisory Report and supporting Research Document will be made publicly available on the Canadian Science Advisory Secretariat (CSAS) website.

Compte rendu de l'examen par les pairs de la Région du Pacifique sur l'évaluation de l'impact environnemental du traitement contre le pou du poisson avec le pesticide SLICE® dans des installations aquicoles de Colombie-Britannique

SOMMAIRE

Le présent compte rendu résume l'essentiel des discussions et les conclusions clés découlant de la réunion du processus de consultation régionale (PCR) de Pêches et Océans Canada (MPO) et du Secrétariat canadien de consultation scientifique (SCCS) qui a eu lieu à l'Institut des sciences de la mer à Sidney, en Colombie-Britannique, les 18 et 19 octobre 2011, au cours de laquelle un document de travail (CASP WP2011-P55) sur le devenir environnemental et les effets biologiques potentiels du benzoate d'émamectine (un agent chimiothérapeutique antiparasitaire qui est la matière active contenue dans le SLICE®) a été présenté aux fins d'examen par les pairs.

Au nombre des participants qui ont assisté à la réunion en personne ou par conférence Web, il y avait notamment des employés de la Gestion des pêches et de l'aquaculture et des Sciences de Pêches et Océans Canada (MPO), ainsi que des participants externes de Santé Canada, du ministère de l'Agriculture de la Colombie-Britannique, du gouvernement écossais et de la Scottish Association Marine Science, de l'industrie de l'aquaculture, de l'industrie de la pêche à la crevette et de l'industrie manufacturière d'agents chimiothérapeutiques, de plusieurs organisations non gouvernementales de l'environnement et du milieu universitaire.

Les conclusions et les avis découlant de cet examen seront présentés sous la forme d'un avis scientifique, lequel fournira des conseils à la Gestion des pêches et de l'aquaculture du MPO sur la répartition spatiotemporelle du benzoate d'émamectine près des exploitations salmonicoles et ses effets biologiques potentiels sur la crevette tachetée du Pacifique, un organisme non ciblé d'importance commerciale. Cet avis appuiera la réglementation environnementale écosystémique et la prise de décision concernant le secteur de l'aquaculture, et il pourrait constituer la base de certains éléments de futurs processus de consultation au sujet des activités aquacoles dans le cadre de plans de gestion intégrée de l'aquaculture (PGIA).

L'avis scientifique et le document de recherche à l'appui seront rendus publics sur le site Web du calendrier des avis scientifiques du Secrétariat canadien de consultation scientifique (SCCS).

INTRODUCTION

A Fisheries and Oceans Canada (DFO) Canadian Science Advisory Secretariat (CSAS) Regional Advisory Process (RAP) meeting was held at the Institute of Ocean Sciences in Sidney, B.C. on October 18-19, 2011 to review recent research concerning the fate of emamectin benzoate (the active ingredient in the chemical therapeutant SLICE®) near salmon farms in British Columbia and its potential biological effects on the Pacific spot prawn (*Pandalus platyceros*), a commercially important species in B.C. coastal waters.

The Terms of Reference (TOR) for the science review (Appendix A) were developed in response to a Request for Science Information and Advice (RSIA) from DFO Fisheries & Aquaculture Management (FAM). Notifications of the science review and conditions for participation were sent to representatives with relevant expertise from the aquaculture, prawn fishing and chemotherapeutant manufacturing industries, First Nations, Fisheries and Oceans Canada, other government Departments, a number of environmental non-governmental organizations, and academia.

The following working paper (WP) was prepared (Appendix B) and made available to the participants prior to the meeting:

Environmental Fate and Potential Biological Effects of the Anti-parasitic Chemo-therapeutant Emamectin Benzoate (active ingredient of SLICE®) in Canadian Waters by Michael G. Ikonomou. (CSAP WP2011-P55)

The meeting Chair Dr. Andrew Ross and the Coordinator of the Centre for Science Advice Pacific (CSAP) Marilyn Joyce (Hargreaves) welcomed the participants, reviewed the role of CSAS in the provision of peer-reviewed advice, and gave a general overview of the CSAS process. The CSAP Coordinator discussed the role of participants, the purpose of the various RAP publications (Science Advisory Report, Proceedings and Research Document), and the definition and process around achieving consensus decisions and advice. Everyone was invited to participate fully in the discussion and to contribute their knowledge to the process, with the goal of delivering scientifically defensible conclusions and advice.

The Chair reviewed the Agenda (Appendix C) and the Terms of Reference for the meeting and highlighted the objectives. The Chair then reviewed the ground rules and process for exchange, reminding participants that the meeting was a science review and not a consultation process. The room was equipped with microphones to allow remote participation by web-based attendees, and in-person attendees were reminded to address comments and questions so they could be heard by those on-line.

The attendees were reminded that everyone at the meeting has equal standing as a participant and is expected to contribute to the review process if they have information or questions relevant to the paper being discussed. In total, 31 people participated in the RAP (Appendix D). Marilyn Joyce was identified as the Rapporteur for the meeting.

Participants were informed that Dr. Les Burridge (Fisheries and Oceans Canada) and Dr. Kenny Black (Scottish Association for Marine Science) had been asked before the meeting to provide written reviews for the working paper to assist in the peer-review process. Participants were provided with copies of these reviews.

The conclusions and advice resulting from this review will be provided in the form of a Science Advisory Report to Fisheries & Aquaculture Management to inform ecosystem-based environmental regulation and decision-making with regard to the aquaculture sector. The

Science Advisory Report and supporting Research Document will be made publicly available on the CSAS Science Advisory Schedule.

REVIEW

Working Paper:"Environmental Fate and Potential Biological Effects of the Anti-Parasitic Chemo-therapeutant Emamectin Benzoate (active ingredient of SLICE®)" by Michael G. Ikonomou. WP2011-P55

Rappoteur: Marilyn Joyce

Presenters: Michael Ikonomou (DFO) and Nik Veldhoen (University of Victoria)

PRESENTATION OF WORKING PAPER

The Working Paper was presented in two parts. The first part, presented by Dr. Michael Ikonomou, focused on the measurement of emamectin benzoate (EB) and its main conversion product (AB) in water, sediment and spot prawns at two B.C. salmon farm sites and reference sites, and included a description of the sampling and analytical procedures used (Surridge and Ikonomou, 2013). The second part, presented by Dr. Nik Veldhoen, described the analysis of gene expression in muscle tissue from spot prawns exposed to sediment containing different concentrations of EB in laboratory aquaria (Veldhoen et al., 2012). Points of clarification included the positioning of reference sites (500 m from the farm sites) and the relative concentrations of EB in laboratory and field sediments (the former being significantly higher than the latter). An Abstract of the Working Paper can be found in Appendix D.

Internal Review

Dr. Les Burridge (St. Andrew's Biological Station)

An internal review of the Working Paper was provided by Dr. Les Burridge (Appendix E) and presented by him during the meeting. Most of the discussion surrounding this review concerned routes of exposure to EB and its broader environmental distribution. Specific issues included the dispersion and possible localization (e.g. at sheltered sites) of EB in the environment, and how this might be modeled using appropriate tools (DEPOMOD); the possibility that EB may still be present when below the analytical limit of detection (LOD); and the possible uptake of EB via ingestion of uneaten feed pellets by spot prawns and/or their avoidance of salmon farms treated with SLICE®. It was suggested that future studies would benefit from 'far-field' EB measurements at locations more remote from salmon farms, and from using records of SLICE® treatment at farm sites to inform data interpretation. It was also suggested that the presence of wild prawns close to salmon farms treated with SLICE® argues against avoidance, although there may still exist a threshold EB concentration above which prawns might relocate to other areas. It was noted that although prawns might be expected to show interest in eating feed pellets (prawns are known to eat other prawns) there was no evidence of this in the present study, or in previous studies in which prawns and crabs were offered but refused pellets. Regarding the results of mRNA (gene expression) analysis, these showed little or no change in expression levels for many of the candidate markers for EB exposure, including the gene NAG which is associated with exoskeleton molting. Previous studies involving lobsters showed that these had to be in the pre-molt stage for EB exposure to affect molting but that all lobsters fed (ground-up) pellets treated with EB proceeded to molt, though no toxicity was observed. Although hatching success was not included in these studies it was pointed out that, like lobsters, prawns are benthic organisms and that their eggs and brood-stock could be directly exposed to EB in sediment. It was also noted that juvenile lobsters do not move far during the

winter months whereas the adults tend to seek warmer waters (a citation for the lobster study was requested for inclusion in the Research Document). Regarding the dispersal of EB there was some discussion as to the amount of flushing that may result from tidal movement, although it was noted that flushing (and presumably dispersal) tends to be relatively high in B.C. waters.

External Review

Dr. Kenny Black (Scottish Association for Marine Science)

An external review of the Working Paper was provided by Dr. Kenny Black (Appendix E) and presented by the Chair on his behalf. The development during this study of a method for measuring EB and its principle metabolite AB at environmentally relevant concentrations was acknowledged in the review as a significant step forward. It was also noted that the present study differed from previous studies in using only two sites (albeit with more sensitive analytical methodology), although it was pointed out that sampling was actually carried out at a total of 6 sites, including the 4 additional sites (C to F) near Site B at which sediment samples were collected 1.5 years post-treatment. The fact that a previous study (Wells, 2007) appeared to find no evidence for the accumulation of EB in sediments near fish farm sites over time, nor any relationship between current speeds and sediment concentrations at these sites, was regarded as significant and requiring further discussion. The use of sediment traps to investigate temporal losses and distributions of particulate EB (both for modeling purposes and to assess the potential for continued excretion of EB from fish post-treatment) was recommended in future, although this type of mechanistic study would require a higher level of co-ordination and collaboration with industry than the current study, which was envisaged as more of a ‘snapshot’. Studies that have looked at lipophilic distributions appear to show that feed pellets do not ‘drive’ EB concentrations in sediments, and it was noted that EB is found (and therefore partially distributed) in organisms as well as sediment. It was also pointed out that sediment sampling with a van Veen grab during the present study is likely to have targeted the surface layer where EB tends to reside, whereas previous studies (e.g. Wells, 2007) used homogenized (5 cm) sediment cores. However, it was again argued that the aims of these studies were substantially different and that each made the best use the available sampling technology. It was suggested that redox and sulfide data (which are available) would be particularly useful in interpreting the sediment data, along with the use of DEPOMOD to normalize sediment data from the two study sites. It was agreed that these data should be included in the Research Document, along with the suggested references to previous studies and any available information on the history of SLICE® usage at each site. Regarding the aquarium-based gene expression experiments, it was agreed that the apparent decrease in toxicity with increasing sediment EB concentration requires verification/replication and that if there are parallels in the literature then these should be provided, along with more information on statistical significance (similar trends have apparently been noted with certain hormones but these do not involve toxicity based on mortality). Although the laboratory exposure study was a pilot aimed primarily at developing a toolbox for genomics assessments, it was based on established quality control principles and there were no obvious indications of any experimental problems. Nevertheless, the suggestion to carry out conventional toxicity testing to establish LC-50 values, ideally involving different uptake pathways and starting at lower EB concentrations, was widely supported, particularly since other studies have apparently shown dose-response relationships for EB. Regarding the observation that only about 1% of the EB used at Site A was found in sediment, the suggestion to attempt a mass balance to account for the remaining EB was not supported due to the fact that a full depth profile was not performed, and because the particle reactivity of EB would require the recovery and analysis of suspended sediments via filtration (achievable down to 0.5 microns) as well as the analysis of dissolved EB throughout the water column.

Following the presentation and discussion of the internal and external reviews the Chair once again reviewed the Terms of Reference prior to the General Discussion.

GENERAL DISCUSSION

WORKING PAPER

Terminology

The question of terminology had already been raised prior to the presentation of the Working Paper, with concerns expressed over use of the term 'pesticide' in the Terms of Reference. It was henceforth agreed by the participants that SLICE® would be referred to as a 'chemotherapeutant' in all documents arising from this Advisory Process.

Experimental Design

Regarding the choice of sites for the present study, and recommendations regarding site selection for future work, it was mentioned that prawn fisherman generally know when a particular farm has been treated with SLICE® and, although they do not see issues at every farm, they suspect that other sites where fishing problems have been encountered may also have been treated. Therefore, site selection should be expanded in the future.

In response to a question as to why data from all 6 sites were not included in the study, it was pointed out that the Working Paper does include data from the 4 additional sites (C to F) for sediment collected 1.5 years after treatment at neighbouring Site B. Questions were also raised as to why no comprehensive sampling regime had been adopted for these sites, and whether or not industry has been monitoring sites at large. The authors responded by saying that sediment sampling at the 4 additional sites had been opportunistic and that they did not have information on EB application or the history of those sites. To expand the present study and allow for predictions at other sites it would be necessary to have treatment history (as well as DEPOMOD data) for all study sites, and the authors were unaware of such information being available.

It was subsequently pointed out that both industry and DFO perform sampling at farm sites (one company had sampled at 10 sites), although much of this work is done by contract laboratories to meet regulatory requirements and is not in the public domain. Nevertheless, the opportunity may exist to analyse or otherwise obtain data from these samples, although the advice was given to make sure that 'apples are not compared with oranges' when bringing in new data, and to keep in mind that the present study was designed primarily to address the fate of EB.

Regarding the suggestion to use sediment traps, the comment was made that this doesn't really get at the fate of EB, and that a previous study that used homogenized sediment core samples (Wells, 2007) was not designed to look at EB residues. The authors stated that it might be useful to make comparisons and to reference other data, but not to include this in the current study. Although their new analytical method provides the opportunity to make high-resolution measurements, the authors decided to use the standard grab-sampling method for collecting surface sediment as this was the best tool available at the time. Knowing how sediment core data might complement the results of the present study, it was suggested that this might be a useful approach moving forward. However, it was argued that the design of the present study, which involved collecting water and sediment samples along transects (based on DEPOMOD modeling results), was appropriate for looking at the fate of EB and provides valid results.

It was again pointed out that bringing in other data would be complicated if different methods were used (though it was suggested that the top 2-5 cm of a sediment core would likely yield results similar to the surface sediment collected in the present study).

It was proposed by the Chair that reference should be made to other studies but that those data need not necessarily be included in the final Research Document.

As to how representative the chosen study sites are of BC farm sites in general it was pointed out that Site A, which has had 3 production cycles (peak biomass 2550 tonnes) with 3 applications of SLICE® and is now at third grow out (full grow out March 2007 to 2009), is a very low flow site in relatively shallow water (80m) with a soft substrate of small particle size. Of the 5 known farm sites with similar conditions this is one of the lowest in terms of flow, and so is not representative of farm sites in BC. Site B has a greater flow and different deposition properties than Site A, but is still not regarded as a high flush site (information on the characterization of these sites is in the public domain).

In response to these concerns, the Chair emphasised the importance of qualifying the results of this study in the broader context of BC farm sites and their characteristics.

It was noted that the farm sites included in this study were not sites at which fisherman had noticed problems. However, it was also noted that there is currently no data or analysis that indicates a reduction in the number of prawns near farm sites (the strategies used are not sufficient to draw such conclusions), and that uncertainty is compounded by the fact that some mortality was observed during the laboratory experiments. In addition, field sampling was carried out in October and November whereas fishing usually occurs in May. It was also mentioned that when catch around farm sites was looked at 3 years ago there was no indication of reduced prawn catch.

Apparent avoidance of fish farms by prawns has been described by fishermen, although this was initially due to anoxic conditions caused by the accumulation of fish feed when farms were first introduced. When feeding practices were modified the prawns started to return, but then in certain years fisherman would see a sudden reduction in number of prawns at a particular site compared with other neighbouring sites (e.g. within a 5-mile radius). This started happening in 2000 in the Broughton Archipelago, though it is now being seen in other areas, and appeared to coincide with SLICE® usage (though prawns would move back the following year). The effect seemed to be greatest downstream of the farms (and any changes in the locations where prawns can be caught can have a big impact on profitability). It was stressed that this is anectodal and not scientific information, but is based on 35 years of experience in the prawn fishing industry.

It was suggested that logbook data and (DFO) catch statistics might help to substantiate these anecdotes, although care should be taken when comparing these different data (e.g. logbook data of prawn fishermen does not appear to show the trend seen in catch percentages between 1994 and 2004). It was also noted that climate variability (e.g. El Niño and La Niña events) can affect prawn production, though it is not clear how. Nevertheless, it was acknowledged that scientists are listening to the concerns expressed by stakeholders and that these should be captured in the review process.

The authors pointed out that prawn assessments and Sites A and B were not within the scope of the original project, but that anecdotal concerns expressed by fishermen and other stakeholders regarding the potential effects of SLICE® on prawn populations, and other valued ecosystem components, are mentioned in the introductory Background section of the Working Paper. However, the request was made that no direct inferences are drawn from such statements in the SAR or Research Document.

With regard to water sampling, the question was raised as to why samples were not taken within 1m of the bottom, if that is where the prawns live. The authors chose to collect water samples at a depth of 2 feet for logistical reasons, and because one would expect to be able to measure EB near the surface (based on its chemistry), although depth profiles could be done. It was also suggested that all measurements be provided in the same units, and that information from Health Canada on the recommended limits for EB should be included.

The authors agreed to standardize units as appropriate and to clarify when dry or wet weight measurements are being reported. Regarding health limits, the Background section of the Working Paper mentions dose rates approved by Health Canada's Veterinary Drug Directorate for fish, but more discussion is needed as to how appropriate those values might be for other organisms, and whether limits for human consumption should be included in the SAR (see EB Limits and Exposure below).

Regarding the need for site-specific information, the concern was expressed that only limited conclusions can be drawn in the absence of site history and that unless there is sufficient information available concerning particles (whether it be from previous or current treatments) it may not be possible to estimate the environmental half-life of EB. The authors responded by saying that the study was designed to take a 'snapshot' of EB distribution, but that because sampling was carried out at sites where the history of treatment has been documented it should be possible to go back and link the conditions to the site. With respect to half-life, the intention was to use the DEPOMOD model (i.e. deposition and burial rates) together with data from Site A to look at dilution and degradation based on where the chemical has gone, whether new material has been added, and apparent conversion of EB to AB. The authors mentioned that they had performed some experiments in which sediment samples were stored in jars and sampled over time to see how EB changes, although these 'pseudo-'experiments were not carried out under a proper biodegradation study conditions.

Based on these discussions it was decided that inferences of half-life should be avoided as the current data are insufficient to make that calculation, although suggestions for future work could include measurements that would allow a proper mass balance for EB.

In response to a question as to the distance between Site A and the reference site (it was 500m) and whether more prawns were observed at the latter, the authors explained that they had trapped prawns at the reference site and deploying them as 'contained' prawns at Sites A and B in order to be able to compare them with wild prawns actually captured at those sites. They also confirmed that mortality data were determined for those traps but that this information was not included in the report, due to the fact that not all of the planned samples were obtained from the containment traps and so the results of genomic analysis for wild and contained prawns could not be interpreted.

Analytical Methodology

In response to questions regarding the apparent detection of EB at very low levels in samples collected before SLICE® treatment, the authors pointed out that even if EB can be detected and measured at such concentrations, its presence cannot be confirmed unless all the necessary quality control criteria are met. They also clarified the distinction between LOQ (Limit of Quantification, above which the measured concentration of EB can be reported with confidence), LOD (Limit of Detection, above which the presence of EB is implied), and NDR (Not Detected, meaning that the presence of EB cannot be confirmed because one or more quality control criteria have not been met).

The Chair suggested using LOQ rather than LOD in the SAR to indicate the limits above which both the concentration and presence of EB have been confirmed.

Brief comments were made to the effect that one pathway of uptake for EB could be food items; that it was difficult to draw interpretations from the bars on some of the figures in the Working Paper; and that, in the interest of study replication, it may be important to mention that some of the prawn traps were lost during the study.

Regarding bioavailability and uptake, it was suggested that EB is lipophilic and would tend to 'stick' to sediment, but that local chemistry (pH, redox conditions) may change over time resulting in hydrolysis and/or mobilization of EB (the example was also given of oxytetracycline binding to magnesium, which tends to reduce its bioavailability). The authors responded by suggesting that the binding of EB to sediment may not be that strong, based on its chemistry, and that they use solvents and surrogate compounds to extract, measure, and determine the recovery of EB in sediment. They also suggested that prawns may take up EB from water and/or sediment via their gills and food, and that the detection of EB in prawn tissues shows that it is bioavailable. The authors mentioned that they plan to carry our full-scale biodegradation experiments in the future. It was suggested that the need for such experiments to address questions about biological uptake, accumulation, and ecological impact of EB should be included in the SAR.

In response to various questions about the data presented in the Working Paper, the authors agreed to standardize moisture levels for sediment samples from different sites (by using average moisture content) and to specify their use of geometric as opposed to arithmetic means. Requests were also made to include EB and AB results for both study sites in Figure 4 (AB was not detected and so not plotted) and to add an Appendix with values and standard deviations for the data show in Figure 5, which are otherwise hard to visualize.

Following a brief summary from the Chair of the points raised so far, the discussion returned to the subject of water sampling with the comment that there was, in fact, a clear rationale for the strategy used (based on efforts to target particular pathways) and that this can be elaborated on in the Research Document.

In response to concerns about EB concentrating at the surface (due to its association with floating oil from fish feed) the authors pointed out that their sampling procedure involved sending a glass bottle down to a depth of 2 feet and then, on bringing it to the surface, disposing of the water in the mouth of the bottle. They have also conducted spiking experiments to validate this procedure.

The opinion was again expressed that sampling bottom waters would be more relevant in terms of studying uptake pathways. The lab experiments deal with potential uptake of EB from sediment, with the possibility of comparing inoculated sediment concentrations with those measured in the field, but having no information about the quality of the water that overlies the sediment limits what one can say about uptake pathways. Even if EB were to be measured in bottom water as well as sediment this still wouldn't indicate the route of uptake, especially since EB can penetrate the cuticle directly, as well as being taken up via gills and food.

Based on these discussions, it was acknowledged that if identifying mechanisms of uptake is an objective then further studies are required. It was also recognized that results from the lab-based gene expression studies cannot be extrapolated to what is occurring in the field study, based on the EB concentrations measured in water and sediment, and that plenty of work is still required to understand these associations.

It is clear that EB is getting into prawn tissues and, therefore, has the potential to affect the organism. However, managing these levels based on the concentration of EB in water and sediment will require characterisation of uptake pathways and mechanisms.

Following confirmation that the water samples were filtered (using 5-micron filters) the General Discussions on Day 1 wrapped up with the participants agreeing to endorse the Terminology, Experimental Design and Analytical Procedures described in the Working Paper, albeit with various caveats and requests for additional information.

Day 2

The Chair began by reviewing the Agenda for Day 2, summarizing the progress made during Day 1, and completing introductions. Progress to date included the review and endorsement of the experimental design and analytical methodology, and an agreement to use the term 'chemotherapeutant' when referring to SLICE® or EB in this Process.

EB Limits and Exposure

Regarding tissue concentrations vs. allowable levels of EB, it was pointed out that the former were measured at two sites (with the intention of providing a 'snapshot' of EB distribution) and that the results are not representative of the BC coast as a whole. Furthermore, toxicity to prawns and protecting human health are two separate issues; this study is concerned with the former (although mention of biological effects in the title could be interpreted as applying to humans too) but if an introductory statement were to be made regarding the latter it then it would need to be qualified with a Maximum Residue Limit (MRL). It was also stated that markets in e.g. Europe, Japan and the US are very sensitive and, although the fishing industry pushed for both issues to be considered, human health does not appear to be a concern at this point (and industry is very happy that the present study was carried out).

It was suggested that the relative timing of prawn fisheries and SLICE® treatment should be expanded upon in the Research Document. However, it was also pointed out that treatments are performed if and when needed, although they tend to occur between October and March in order to minimize impact on wild salmon, and that timings therefore vary with respect to fisheries (and the study was not designed to include surveys of prawn catches in BC). On the other hand, it was stated that both shrimp trawl and crab fisheries take place during this treatment period and that crustaceans also dig down into the mud, so there is a lot going on in the ecosystem.

Based on this discussion it was recommended that potential effects of EB on organisms other than prawns should also be considered in future studies.

It was also noted that prawns caught by fishermen are intended to be frozen live, and that control of oxygen and salinity generally results in a low (2%) mortality rate during transport, except for buried females, for which the mortality rate is much higher (50-60%). The present study involves the more robust stages of the prawn's life-cycle, but the timing of SLICE® usage often coincides with the period when female prawns are buried, during which sub-lethal effects of EB could be particularly important.

Regarding EB limits, it was noted that these may vary from one animal and/or tissue to another and that someone should contact the Health Canada Veterinary Drugs Unit or CFIA for more information. Some participants suggested that the Maximum Residue Limit (MRL) for EB might be 42 ppb. Others thought this to be an older value (since revised by HC) and that the MRL in Europe, Chile, Canada and (prospectively) the US is currently 100 ppb (Japan is also developing a MRL for EB), but that the adult daily intake is common across all jurisdictions. [NOTE: information subsequently obtained from the Association of Aquaculture Veterinarians of British Columbia (dated December 14, 2004) specified an administrative MRL of 50 ppb in fish and a withdrawal time of 25 days between harvest and the last date that the chemotherapeutant was administered].

Following the suggestion to include an introductory statement defining the goals of the study, and acknowledgment of the fact that although the title mentions biological effects the study looks more specifically at genomic responses to EB exposure, it was proposed that including MRL information would be outside the original scope of the study.

Biological Effects

Although some mortality was observed in the lab exposure experiments, it was argued that these observations do not constitute a toxicological study (to IOC standards) and that such a study would need to look at LD-50 and reproductive effects as well. It was also pointed out that there could be significant sub-lethal effects that could well have impacts at the population level, and that although standard toxicological tests should be done this does not negate the value of the laboratory study as a pilot for genomics-based monitoring of such effects. However, there was general agreement that the genomic experiments do not constitute a toxicological study (although it was noted that the expression of several gene markers did change over the course of the experiment).

The Chair subsequently confirmed with all participants that the genomics methodology did not provide sufficient information to meet the objectives of the Terms of Reference.

It was agreed that statistical information for tissue levels should be added in Section 2.4 of the Working Paper and that potentially misleading statements regarding mortality during the lab experiments should be revised in light of the preceding discussions.

To help put the lab observations from the present study into context, the group heard from one of the participants who had also conducted lab experiments in which prawns were exposed to EB. The experiments involved multiple exposures over 10-day and 20-day periods, with 12 tanks per treatment and 10 prawns per tank. The treatments did not appear to affect the rate of molting (which ranged from 10 to 20% of the population) but it was found that prawns tend to cannibalize each other when molting. After repeating the experiment while keeping the prawns separated it was noted that mortality increased with increasing EB concentrations, with 50% mortality above 1000 ppb, and although the rate of molting remained the same (10-20%) there were changes in the life stage at which molting occurred, with some prawns remaining stuck in their shells. There are plans to repeat these experiments at lower EB concentrations. Behavioural effects were also investigated, with motility apparently being affected at concentrations above 200 ppb (e.g. less ‘flicking’, reduced ability to move forward, and a tendency to walk in circles and swim to the surface). It was also noted that prawns are scavengers and, if starved, will consume SLICE® (they also eat nematodes, according to some literature sources).

Following this presentation it was pointed out that the dose-response relationship observed during these experiments contrasts with the gene expression results reported in the Working Paper, adding weight to the recommendation for further genomics work, and to refrain from drawing firm conclusions based on the current gene expression data.

The authors agreed to these recommendations, and to referencing dietary information along with other toxicological studies and results in the Working Paper. There was also general agreement to use approved wording from cited documents in the Working Paper to ensure clarity and consistency. It was acknowledged that different toxicological studies may provide different types of information (e.g. LC-50, LD-50) and that the sub-lethal effects identified through genomic analysis may also help to shed light on some of the mechanisms that result in lethal effects.

Further points made during these discussions included the need to standardize units, to recognize that the EB concentrations used in the genomics exposure experiments did not match the levels measured in the environment, and to recommend that both lethal and sub-lethal effects be investigated using environmentally relevant EB concentrations in future studies.

The Chair summarized the discussion on Biological Effects by restating that EB finds its way into prawn muscle tissue, but by an unknown pathway or pathways; that statistical information needs to be added to the Working Paper; that the lab exposure experiments were not performed in the context of standard toxicological assessments, and so no conclusions should be drawn from the observed mortality; that gene expression results are in contrast to other published studies that show dose-response relationships; and that appropriate wording from toxicological references should be included.

Toxicology References

The authors explained that it had not been their intention to review and reproduce all the relevant toxicological literature in the Working Paper, but to present their data and make reference to other studies as needed. It would have been useful to have a distilled version of the molecular biology; however, there are few (if any) other published studies on spot prawn. The authors suggested modifying the title of the study to focus on just the results obtained.

There was some concern that leaving out toxicological information from other studies (even a short table of published toxicity data) would make it difficult to put the results of the present study into context. However, it was also pointed out that this is a local study (of which there are relatively few) that is being reviewed through a Regional process, and that a National process (already planned) may be a more appropriate venue at which to review the broader literature and place the current work in a wider context. It was, however, agreed to include other references (e.g. Bright and Dione 2005) in the final Research Document, and to translate the technical language into something more understandable for the Science Advisory Report.

SCIENCE ADVISORY REPORT

The remainder of the Discussion period was spent working as a group on the draft SAR, reviewing the Terms of Reference and determining to what extent each question had been addressed:

Overall Question

Does the current use of SLICE® in B.C. finfish 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 showed that EB can potentially build up in benthic sediments close to salmon farms, 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 and ecological effects of EB and its metabolites in spot prawns and other non-target organisms.

Specific questions

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

This question was answered, with EB detected at parts-per-trillion (ppt) levels in sub-surface water samples and at parts-per-billion (ppb) levels in surface sediment samples collected within 150 m of the two farm study sites, but at less than the limit of quantification (LOQ) at reference sites.

2. *If so, what is the spatial and temporal distribution of EB in the vicinity of Finfish Farms following the application of SLICE®, and how does this compare with the Reference Sites?*

This question was also addressed, the highest sediment concentrations being measured directly below the net pens 2 to 3 weeks following the application of SLICE®, falling to levels at or below the LOQ between 100 and 150 m from each farm and at the reference sites. The highest concentrations in water were measured within 50 m of the net pens 1 day after SLICE® treatment, falling to below the LOQ at a distance of 150 m from each farm site and at the reference sites. EB and it's 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*?

The first part of this question was partially addressed, with EB levels in sediment reflecting the different hydrodynamic and biophysical characteristic of the two sites. However, 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, 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.

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

Again, the first part of the question was addressed, with EB in the muscle tissues of spot prawns collected within 150 m of Sites A and B reaching 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. Furthermore, standard toxicological measurements such as LC-50 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. Additional studies including standard toxicological measurements, different life-cycle stages, and environmentally relevant EB concentrations are therefore needed.

CONCLUSIONS

WORKING PAPER

As documented in the General Discussion, the Working Paper was accepted with the following revisions, which should be incorporated into the final Research Document:

- Include references (e.g. on p.2, para.2 where it says "..the physical and chemical properties and registration, history of use and application regimes…have also been reported in the literature…", and "…one can find several different methods…", etc.) and remove phrases such as …"not reproduced in this report."
- Include available redox and sulfide data for sediments.
- Remove any mention or inference of half-life.
- Use consistent/standardized units, including those for moisture content.

- Clarify use of geometric as opposed to arithmetic means.
- Mortality at lower EB concentrations in exposure studies needs to be further explained and/or the need for further experiments stated.
- Clarify the use/meaning of LOD, LOQ and NDR.
- Add references that address issues and/or concerns regarding the bioavailability and biodegredation of EB.
- Add Appendices that include tables of values and SDs for all data (including all plotted values/values below LOD).
- Indicate in the Figures where values were below the LOD and so not plotted, and provide additional clarification in such cases.
- Elaborate on the rational for water sampling strategy (i.e. why water was not collected immediately above sediments and/or at the surface, where EB measurements might have addressed points raised by RAP Participants).
- Indicate the need for further studies of uptake mechanisms (such information would be needed to manage EB exposure using sediment concentrations).
- Confirm that water was filtered (to 50 microns).
- Add actual measured concentrations of EB in tissues to Section 2.4.
- Update discussion on mortality in Sec. 2.4 based on what was discussed in the RAP Meeting; e.g. no apparent dose-response relationship (mortality apparently higher at lower levels of EB exposure) and other possible causes of mortality (e.g. trauma or possibly cannibalism). More work needed to substantiate the cause(s) of death/chemically induced mortality.
- Include published toxicological data and references to such data.
- Mention that other more conventional toxicological measurements (LC-50/LD-50, LOAEC, NOAEC) are important, but that genomics data may help to understand the mechanisms behind (sub-)lethal effects.
- Clarify that dose-response effects need to be re-examined, and that exposure experiments are not replicating levels in the environment.
- Include short table to summarize previously published toxicological results.
- Add Bright and Dione citation and update appropriate sections of text in the Working Paper to reflect the wording used in this publication.
- Make sure that Conclusions in the Working Paper/Research Document reflect those in the SAR.

SCIENCE ADVISORY REPORT

The SAR contains the following Conclusions:

- 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.

RECOMMENDATIONS & ADVICE

The following Information and Recommendations are included in the SAR:

- 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.
- 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.

Research involving standard toxicological measurements (LOAEC, NOAEC, LC-50), 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.

SUMMARY AND CLOSING

Before closing the meeting the Chair confirmed that all participants were in agreement concerning revisions to the Working Paper, and that the authors would produce a final Research Document incorporating those revisions. The Chair also reviewed the draft framework of the Science Advisory Report prepared during the closing stages of the meeting and confirmed that a complete draft of the SAR would be circulated amongst the participants for their review and comment prior to publication. Dr. Jon Chamberlain (FAM) then made some concluding statements in which he thanked all those present for participating fully and actively in the meeting and acknowledged the major contributions made by the two authors and reviewers, after which the RAP meeting was adjourned.

ACKNOWLEDGEMENTS

The Chair gratefully acknowledges the assistance of Marilyn Joyce (Hargreaves) (Coordinator, Centre for Science Advice Pacific) and Nic Dedeluk (Administrative Assistant, CSAP) in providing logistical support and acting as Rapporteurs. CSAP would also like to thank Dr. Michael Ikonomou and Dr. Nik Veldhoen for preparing and presenting the Working Paper, and Dr. Les Burridge and Dr. Kenny Black for providing timely expert reviews of the paper. The active participation of all those involved in this review process is greatly appreciated.

REFERENCES

Bright, D.A. and Dione, S. (2005). Use of Emamectin Benzoate in the Canadian Finfish Aquaculture Industry: A Review of Environmental Fate and Effects. *Environment Canada, Environmental Protection Branch. vi + 67 pp.*

Surridge, B.D.; Ikonoumou, M. G. (2013) . Ultra-Trace Determination of Aquaculture Chemotherapeutants and Degradation Products in Environmental Matrices by LC-MS/MS. Int. J. Environ. Anal. Chem. 93: 2, pp 183

Veldhoen, N., Ikonomou, M.G., Buday, C., Jordan, J., Rehaume, V., Cabecinha, M., Dubetz, C., Chamberlain, J., Pittroff, S., Vallee, K., van Aggelen, G., Helbing, C. (2012). Biological Effects of the Anti-parasitic Chemotherapeutant Emamectin Benzoate on a Non-target Crustacean, the Spot Prawn (Pandalus platyceros Brandt, 1851) under Laboratory Conditions. Aquatic Toxicol. 108:94-105Wells, A. (2007). Marine Fish Farm Data Review. *Scottish Environment Protection Agency*. *iv + 67 pp.*

APPENDIX A: AGENDA

Regional Advisory Process

Assessment of the environmental impact of the treatment of sea lice with the pesticide SLICE® at aquaculture facilities in British Columbia

October 18-19, 2011

DFO Institute of Ocean Sciences
Sidney, British Columbia

Chairperson: Andrew Ross

Day 1 - Tuesday, October 18, 2011

Time	Subject	Presenter
0930	Welcome & Introductions	Marilyn Joyce
0945	Review Agenda & Housekeeping	Andrew Ross
1000	CSAS Overview & Meeting Procedures	Marilyn Joyce
1015	Review of Terms of Reference	Andrew Ross
1030	Presentation of Working Paper	Michael Ikonomou
1110	***Break***	
1130	Reviewer Presentation & Author Response	Les Burridge
1215	***Lunch Break***	
1330	Reviewer Presentation & Author Response	Andrew Ross (for Kenny Black)
1415	Group Discussion to identify issues and topics needing further discussion	RAP Participants
1445	***Break***	
1500	Discussion and resolution - Issues & Topics	RAP Participants
1630	***Adjournment***	

Day 2 - Wednesday, October 19, 2011

Time	Subject	Presenter
0930	Introductions & Housekeeping	Andrew Ross
0940	Day 1 Review & Confirm Agenda for the day	Andrew Ross
0950	Discussion and resolution - Issues & Topics	RAP Participants
1030	***Break***	
1100	Discussion and resolution - Issues & Topics	RAP Participants
1200	***Lunch Break***	
1315	Science Advisory Report: Develop Consensus on: • Key Findings & Conclusions • Uncertainties • Ecosystem Considerations • Advice for Management / application of protocols • Recommendations for Future Work • Other	RAP Participants
1630	***Adjournment***	

APPENDIX B: PARTICIPANTS

Last Name	First Name	Affiliation
DFO		
Burgetz	Ingrid	Aquaculture Science
Burridge	Les	Science (Reviewer)
Chamberlain	Jon	Aquaculture Management
Hoyseth	Kerra	Aquaculture Management
Ikonomou	Michael	Science OSD (Author)
Joyce	Marilyn	Science CSAP
Lange	Krista	Aquaculture Management
McPhee	Dan	Ecosystem Science
Parsons	Jay	Aquaculture Science
Ross	Andrew	Science OSD (Chair)
Rutherford	Dennis	Science MEAD
Sheppard	Mark	Aquaculture Management
House	Nancy	Aquaculture Science
Taccogna	Gary	Aquaculture Management
Taekema	Bernie	Aquaculture Management
EXTERNAL		
Dedominicis	Sharon	Marine Harvest
Endris	Dick	Merck Animal Health
Johnston	Guy	Pacific Prawn Fishermans Association
Park	Ashley	University of Victoria
Pearsall	Isobel	Pacific Salmon Foundation
Roth	Myron	BC Ministry of Agriculture
Werring	John	David Suzuki
Veldhoen	Nik	University of Victoria
Abbott	Matt	Atlantic Coalition for Aquaculture Reform
Blain	Francoise	Merck
Enwright	William	Merck
House	Betty	Atlantic Coalition for Aquaculture Reform
McLaughlin	Alison	Health Canada
Ratzlaff	Deborah	Health Canada
Haddon	Paul	Scottish Government (Marine Scottland)
McKenzie	Peter	Mainstream Canada

APPENDIX C: TERMS OF REFERENCE

Assessment of the environmental impact of the treatment of sea lice with the pesticide SLICE® at aquaculture facilities in British Columbia

Pacific Regional Science Advisory Process

October 18 – 19, 2011
Sidney, B.C.

Chairperson: Andrew Ross

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 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 very young salmon. Consequently sea lice levels are carefully controlled on British Columbia's salmon farms, thereby reducing the risk of infecting fish that live outside the farm. Current sea lice management strategies include fallowing (modifying production cycles to minimize farmed fish presence during key periods), harvest and preventative treatment of farms with anti-parasitic chemotherapeutants. The main chemical used to treat sea lice at farms sites in British Columbia is SLICE®, in which the active ingredient is Emamectin Benzoate (EB). The application of such treatments to manage sea lice levels at marine cage finfish sites can lead to the release of these chemicals into the broader environment through a variety of pathways (e.g. dissolution, particle transport and sedimentation). This presents the potential for chemical residence in the water column, accumulation in benthic ecosystems and exposure of non-target organisms. In recent years, DFO Science Branch has been engaged in research to investigate the fate and environmental concentrations of EB following the use of SLICE® at a number of finfish aquaculture sites in B.C. This research has been supported in part by the Program for Aquaculture Regulatory Research (PARR), an internal DFO program that funds research aimed at increasing the science knowledge base used to inform ecosystem-based environmental regulation and decision-making with regard to the aquaculture sector. DFO Fisheries and Aquaculture Management (FAM) Division has requested that a Regional Advisory Process be undertaken to report on the results of current DFO research on the fate of EB following SLICE® applications at marine cage finfish sites. A Science review is sought to corroborate the above-mentioned pathways, and Science Advice requested on the potential distribution and environmental interactions of EB in the vicinity of B.C. finfish farms.

Objectives

The main objective of this Regional Advisory Meeting (RAP) will be to answer the general Question:

Does the current use of SLICE® in B.C. finfish farms result in the exposure of non-target organisms to levels of emamectin benzoate (EB) that could result in biological effects? The following working paper will be reviewed and provide the basis for discussion and advice on the specific questions outlined below.

Ikonomou, M.G. Environmental Fate and Potential Biological Effects of the Anti-Parasitic Chemo-therapeutant Emamectin Benzoate (active ingredient of SLICE®). CSAP Working Paper 2011/P14.

Specific questions to be addressed:

1. Is emamectin benzoate (EB) detectable in water and sediment surrounding Finfish Farms and at distant Reference Sites in B.C. coastal waters?
2. If so, what is the spatial and temporal distribution of EB in the vicinity of Finfish Farms following the application of SLICE®, and how does this compare with the Reference Sites?
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?
4. How do the concentrations of EB measured in water and sediment at Reference Sites and within 300 metres of Finfish Farms compare with those measured in non-target organisms (e.g. shrimp) and with those found, through toxicological studies, to result in biological effects?

The goal of the first day will be to review the findings of a recent study carried out by DFO Science to measure EB concentrations in water and sediment from Reference Sites and Finfish Farms before, during and after the application of SLICE®. Day 1 will also include a brief review of the analytical methodology developed for EB analysis, and of the available information concerning the toxicity of EB to benthic marine organisms. The goal of Day 2 will be to address as many of the aforementioned questions as possible, based on the available information, and to provide advice and recommendations regarding the use of SLICE® to control sea lice at aquaculture facilities in B.C.

Outputs

Expected outputs include a CSAS Research Document, Science Advisory Report, and Proceedings.

Participation

Participants will be invited from DFO (Science, Aquaculture Management, Ecosystem Management), Environment Canada, Health Canada, First Nations, academic and non-government organizations, Aquaculture Pharmaceutical producers, and the Aquaculture Industry, based on the knowledge and expertise to be contributed through this advisory process.

APPENDIX D: SUMMARY OF THE WORKING PAPER

SLICE® is one of the chemotherapeutants used to control sea lice infestations at marine finfish aquaculture facilities in Canada. Concerns regarding the uptake and potential effects of the active ingredient of SLICE®, Emamectin Benzoate (EB), in non-target organisms have been raised by several stakeholder groups. EB was measured at parts per trillion (ppt) levels in water samples collected in the immediate vicinity of two salmon aquaculture sites in British Columbia following SLICE® treatment. EB was also detected at parts per billion (ppb) levels in sediment samples and in the muscle tissue of spot prawns collected within a 150 m radius of the net-pen. Sediment concentrations were related to the specific oceanographic conditions at each site. EB was found to be present in sediments up to 1.5 years following SLICE® treatment. Potential sub-lethal effects of EB on spot prawn were examined in a series of laboratory exposure experiments. Results suggest that short-term exposure to EB can impact this non-target crustacean.

APPENDIX E: WRITTEN REVIEWS

REVIEWER: DR. LES BURRIDGE

Fisheries and Oceans Canada

Centre for Science Advice Pacific

Regional Advisory Process

Working Paper: Ikonomou, M.G. "Environmental Fate and Potential Biological Effects of the Anti-Parasitic Chemo-therapeutant Emamectin Benzoate (active ingredient of SLICE®)". CSAP Working Paper 2011/P55

General comments

There are a few typos and comments on sentence structure but very minor and I've identified them on the accompanying .pdf.

Similarly there are a couple of inconsistencies between text and figures but also minor.

The paper addresses the science questions identified in the terms of reference although the question of modelling in question #3 does not seem to be answered directly.

The paper is well written and easily understood which is essential since the target audience covers a wide range of expertise.

I would prefer that some references be included in the CSAS paper. I agree that the supporting documents have all the references but I think that the inclusion of some key references would greatly strengthen the paper, provide a smaller package and make it a stand-alone document, eliminating the necessity of working through 2 other papers to find the references of interest. For example: while the author states that up to 5 EB treatments can occur during a grow-out cycle Veldhoen et al. state that, in BC, a cage site treats an average of 1.2 times in a grow-out cycle. This type of information needs to be in the summary document as it has significant implications for discussion of exposure and risk.

Specific comments:

1. The author shows the presence of EB in samples from reference sites. Is this a function of the analytical sensitivity or is EB becoming ubiquitous in some areas of BC? This has consequences for exposure, effects and risk.
2. The author states that the sediment contamination is limited to the near field (150 m) around the farm site. Data presented show EB at all sites where measurements took place. EB was measured in water samples as far away as 200 m and the proposed route of uptake for EB in prawns is from water. It doesn't seem that a clear limit has been identified.
3. I think there should be a discussion of feed pellets. Are there pellets in the sediment samples? This will have an effect on exposure as the concentration of EB in pellets is high. While it is stated that prawns prefer other food, it seems from Veldhoen et al. they preferred squid over pellets. Is squid a normal part of the prawn's diet? Following up on this could be an additional research objective.
4. I'd like to see more discussion about availability of EB to prawns. The paper suggests that one of the reasons EB appears in samples taken over a year after the last treatment is the depth of the subsample collected. Is the suggestion then that once the EB is "buried"

under a subsequent organic load, it is no longer bioavailable? Is there natural re-suspension of sediments under cage sites? Does the industry provide data regarding historical use of EB?

5. In a low flow regime do the authors expect a significant build up of EB with time?
6. Are there data that indicate where the organic waste from site B may go? Is it likely to be widely dispersed or is there a possibility of it sinking out in one area and affecting far field non targets? Again, as the author suggests that a short-term exposure may have consequences for prawn, where the product goes may have implications for far field risk assessment.
7. The author states that anecdotal evidence suggests there are fewer prawns near cage sites, yet sampling provided prawns that have spent enough time in the area to accumulate higher levels of EB that at other sites. This could suggest that prawns are not avoiding cage sites. If there are indeed fewer prawns near cage sites, the inference is that there is some toxicity. I'd be interested in the author's comments.
8. The paper could be strengthened with a discussion of expected zones of impact therefore expected consequences. For example, in a low flow situation EB may be present at concentrations expected to result in biological effects but the area where those effects could occur may be quite small. There is no mention of a medium flow situation and that could be included in the research objectives.

REVIEWER: DR. KENNY BLACK

Scottish Association for Marine Science

Centre for Science Advice Pacific

Regional Advisory Process

Working Paper: Ikonomou, M.G. "Environmental Fate and Potential Biological Effects of the Anti-Parasitic Chemo-therapeutant Emamectin Benzoate (active ingredient of SLICE®)". CSAP Working Paper 2011/P55

I have read the three documents provided and offer a number of observations here.

1. A method is presented with unparalleled sensitivity for the detection of emamectin benzoate (EB) and its metabolite (AB). This is a significant step forwards as present methods operate too near their limits of detection when it comes to EB and AB concentrations typical of fish farm sediments.
2. In several respects the results presented differ from previous work and change our understanding of the processes affecting EB around fish farms. Thus these results should have international significance. Unfortunately the authors have not adequately contrasted their findings with those of previous studies.

   For example, a few years ago a review was conducted of Scottish data collected by SEPA during audit surveys (Wells, 2007). I quote below some paragraphs from the executive summary:

   "Residue data from individual farms collected over several years showed that there was no evidence of accumulation of emamectin benzoate in sediments over time.

   No relationship was established between farm site current speeds and emamectin benzoate concentration in sediments."

   As these are different results to those in the present report some discussion is required.

Also, quoting from Black et al (2005):

"Excretion of emamectin continues for a considerable time post-treatment, with the depuration half-life in salmon estimated as 36 days at 10 ˚C. Metabolites of emamectin benzoate, which may display similar or reduced toxicity, will also be excreted for a considerable time post-treatment (SEPA, 1999a)."

This is not quite how it is described in the report under review and this is important as the pathway into non-target species is discussed in explanation of the tissue concentrations of EB and AB. I would strongly recommend the use of sediment traps under cages to consider the temporal loss of particulate EB.

3. The discussion of the temporal change in EB concentration in surface sediments is interesting but handicapped by the method chosen to sample the sediments. I do not think that scraping sediment from the top of a van Veen grab is an appropriate technique for sampling sediments. It seems to me that the excellent work on producing a highly sensitive analytical method is rather spoiled by the fact that sampling with a van Veen is likely to remove the surface layer where much of the EB is likely to reside. Thus the results from Site A, which presumably has an easily disturbed sediment surface, will likely be underestimated by this technique. It may be good enough for monitoring but I don't think it is good enough for research.

   Also, it would have been easy to have collected redox, sulphide and TVS data from the same samples. This would have allowed a more penetrating analysis of the environmental factors that affect the distribution of EB around fish farms. It might have been useful to have run DEPOMOD (first used by SEPA to predict the concentration of EB in sediments) as that would have allowed the differing physics and bathymetry of the 2 sites to have been normalised.

4. The aquarium based research has provided interesting results and the molecular work is promising – although I am no expert on this. However, I think that this would have been better if the concentration range had been started somewhat lower. The remarkable result that toxicity decreases with increased concentration requires verification. If there are parallels in the literature these should be given together with more information on statistical significance. Given the reported persistence of EB in sediments, it would make sense to conduct future experiments over longer periods to consider chronic effects. Also, if the anecdotal evidence that prawns avoid fish farms after SLICE® treatment can be verified, it would crucial to find out by how much this offers them protection. Given the present results, they may be swimming from the frying pan into the fire!

5. Toxicity tests should be carried out to establish LC-50 etc values for this species – if possible taking into account the different potential pathways of exposure: ingestion and absorption through the gills. This will be very important in establishing the real level of risk posed to prawn fisheries.

6. The point is made that humans may eat the tail muscle tissue and thus be exposed to contaminant EB. Is there a threshold for EB in human food? How many prawns would I need to eat to cross the level?

7. At site A, a calculation showed that only 1% of the added EB was expected in the sediment around the farm. My understanding is that most of the added EB is eventually lost from the fish and enters the environment. The authors have data on water column concentrations. It would be very interesting to try to produce a mass balance, even with big uncertainties. However, I would urge the authors to measure EB in cores taken from farms where the depth distribution of EB can be considered.

8. Finally, I commend the authors on an excellent programme of work that moves us considerably forwards. I understand that this area of work is fraught with difficulty and there are always many more things to do than there is time or money

I now move on briefly to the points raised in the TOR.

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

Yes, but surely this was already known. I suppose the purpose of this question is to do with issues of Canadian legislation but it does not seem a very sensible question for an expert panel!

If so, what is the spatial and temporal distribution of EB in the vicinity of Finfish Farms following the application of SLICE®, and how does this compare with the Reference Sites?

The present research goes some way to answer this although sites with a wide range of environmental setting need to be monitored. However, the fact that we can only account for a small percentage of added EB is of concern.

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?

The review cited above (Wells, 2007) found that Depomod predicted results within the same order of magnitude as measurements but tended to significantly over predict sediment concentrations. Future work is required to consider the relationships between sediment conditions pH, Eh [S], TVS etc. and EB.

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

We need further toxicological studies to establish safe sediment and water column EQS for this species.

I hope these (mercifully for you) brief comments have proved useful and I look forward to hearing something of your deliberations.

References

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