USE OF EMAMECTIN BENZOATE IN THE CANADIAN FINFISH AQUACULTURE INDUSTRY:

A REVIEW OF ENVIRONMENTAL FATE AND EFFECTS

Prepared For:

Environment Canada
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*Photo Courtesy of the BC Salmon Farmers Association

EXECUTIVE SUMMARY

UMA Engineering Ltd. was requested by Environment Canada (**EC**) to prepare a comprehensive literature review pertaining to the use of emamectin benzoate (**EB**) for sea lice control in coastal finfish aquaculture in Canada. EB is used under the trade name Slice®, developed by Schering Plough Animal Health. The review will consider:

- EB's use patterns and characteristics of application;
- analytical methods and detection limits for EB and its desmethyl metabolite;
- physicochemical properties, environmental fate and transport, aquatic toxicity and effects of EB and its desmethyl metabolite; and
- the current relevant Canadian and international standards and regulations.

The review identifies specific knowledge gaps and provides recommendations on future research requirements including pre-requisites for any field studies.

The preferred chemotherapeutant for sea lice in Canada, at the present time, is "Slice®", which is a trade name for a product developed by Schering-Plough Animal Health (SPAH) that has EB (CAS No. 155569-91-8, formerly 137512-74-4) as its active ingredient. Internationally, Slice® has been developed as an alternative to the use of other sea lice control products, including ivermectin, dichlorvos, azamethiphos, hydrogen peroxide, cypermethrin, teflubenzuron and diflubenzuron.

Emamectin belongs to the avermectin group, a family of closely related compounds produced by the fungus *Streptomyces avermitilis,* which share broad spectrum toxicity against nematodes, arthropods, and several other pest taxa. Slice® is currently being used in British Columbia and Atlantic Canada under an "Emergency Drug Use" basis, for controlling sea lice at coastal finfish aquaculture operations. The recommended dosage of EB, administered as Slice® is 50 $\mu g\ kg^{-1}\ day^{-1}$ for a duration of 7 consecutive days.

In New Brunswick, treatment for sea lice is often initiated when infection rates reach > 5 pre-adult sealice per fish, or > 1 overigerous female per fish, depending on the water temperature and the season. Federally, the *Feeds Act and Regulations* require Canadian feed mills to maintain copies of records for prescriptions administered through feed at their manufacturing sites. In 1998 in Atlantic Canada, 4% of all manufactured fish feed was medicated, representing about 3,600 metric tonnes of feed. EB accounted for 38.1% of the prescriptions, while tetracyclines accounted for 52.4% and sulfonamides accounted for 9.5%. EB usage records were difficult to obtain for both the Pacific and Atlantic coasts of Canada. In British Columbia, it is estimated that use of EB as Slice® nearly quadrupled from the year 2000 (2.4 kg total quantity used) to 2002 (8.9 kg total quantity used), followed by a drop in 2003 to about 5 kg used. The significance of this amount on the marine environment is unknown at this time.

Overall, there appears to be a strong dependence on the use of Slice® for sea lice control in finfish aquaculture in Canada and in Europe, and the available accounts suggest that multiple applications within grow out cycles may be the norm rather than the exception. This is important, since previously completed environmental risk assessments for Slice® use in the marine environment have focused on predicted environmental concentrations base on a one-time rather than repeated applications at a site. In addition, some jurisdictions have recommended moving to a coordinated application of sea louse therapeutants across all farm sites in a single region, for a more integrated pest management approach. This practice, if implemented, might have negative consequences for non-target organisms in light of short-term EB concentrations associated with releases from multiple sites.

The strong lipophilicity of EB (log K_{OW} = 5) suggests that the major portion of environmental releases will partition to, or remain in, suspended and settled particles. The potential for dissociation of some functional groups on the EB molecule, however, at a pH typical of seawater may result in greater tendency to partition into water than would be expected based on examination of the octanol- water partition co-efficient in isolation. The water solubility is expected to be in the range of 5 to 24 mg/L depending on salinity, and solubility limits are not expected to impose restrictions on leaching of EB or its metabolites from medicated feed or faecal pellets into the water column or sediment interstitial water.

Scientific data on concentrations of EB in the Canadian aquatic/marine environment are extremely sparse. Limited data may become available shortly based on studies in progress. There are significant knowledge gaps about expected or documented concentrations of EB and its metabolites in the environment on a global basis, and this imposes perhaps the greatest limitation on the ability of scientists and managers to accurately assess environmental risks from the use of Slice® at this time.

There is a reasonable amount of data on the short-term toxicity of EB to crustaceans and other aquatic organisms; however, substantial knowledge gaps were noted for: (i) data on chronic (as opposed to acute) toxicity, ii) ecologically relevant effects other than mortality, (iii) endocrine disruption effects (e.g., altered moulting and reproduction in lobsters exposed to EB); and (iv) toxicity data for benthic meiofauna such as nematodes which are potentially sensitive and ecologically important indicator species.

Recommendations for follow-up studies include:

- Determining representative chemical concentrations in the Canadian coastal environment (i.e. water, sediment and biota) for both EB and related compounds such as the desmethyl metabolite, and

- Conducting ecotoxicity studies on sensitive Canadian indigenous species under ‘real world’ conditions for a range of toxic effects including chronic and sub-lethal end-points.

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1. INTRODUCTION

Modern commercial finfish aquaculture in Canada began in the 1970s, although some have traced its Canadian origins back to the aboriginal peoples, who used to transfer fish between rivers and streams. The earliest written records of fish farming are actually from China, where the practice has been known for at least 3,500 years. Today, Canadian aquaculture has evolved into a multi-million dollar industry, with revenues for the year 2000 of approximately $675 million Canadian. Of this, production in New Brunswick and British Columbia accounted for 83.2% of all Canadian aquaculture revenues (CAIA, 2004).

The main commercial finfish aquaculture species in Canada include Atlantic salmon (*Salmo salar*), Pacific salmon (*Oncorhynchus* spp.), Rainbow trout (*Oncorhynchus mykiss*), tilapia (*Oreochromis* spp.) and Arctic charr (*Salvelinus alpinus*) (CAIA, 2004). Atlantic salmon comprises 60% of world salmonid production, of which 91% (54.6% of world production) is produced in Canada (Hargrave, 2004).

In British Columbia, the industry produced over 73 million metric tonnes of salmon in 2002, of which Atlantic salmon accounted for 82%, followed by Chinook (15%) and Coho (3%). The BC Salmon Farmers Association (**BCSFA**) estimates that salmon farming creates 1,800 direct, full-time jobs and over 2,000 indirect jobs. According to Land and Water BC, which is responsible along with the British Columbia Ministry of Agriculture Food and Fisheries (**BC MAFF**) for leasing fish farm tenure sites in British Columbia, aquaculture is now the fourth largest agribusiness industry in BC, based on farm-gate value. Only dairy, floriculture/nursery, and poultry produce more income province-wide.

Often-stated concerns about the finfish aquaculture industry revolve around possible consequences for biota within the receiving environment in which open net pens are operated. Cultured salmon are maintained at much higher densities than non-domesticated fish populations (except perhaps during rare periods when salmon congregate near river mouths during return migrations but are temporarily prevented from moving up river as a result of low flow conditions), and become susceptible to epidemics of infectious bacterial, viral and parasitic diseases (Hargrave, 2004). Parasitic copepods (sea lice) are common on wild marine finfish, and although many parasitic species have long been recognized to have the potential to affect the growth, fecundity, and survival of their hosts, it has only been since recent developments in intensive aquaculture that their importance as disease-causing agents has come to the fore (Johnson et al., 2004). There is a growing but still limited understanding of conditions that can result in higher density epizootic rather than lower density endemic populations of sea lice in nearshore marine ecosystems and on host fish.

Parasitic sea lice infestations frequently occur at aquaculture operations. Sea lice not only threaten the health of the farmed salmon, but also have the potential to endanger wild salmon stocks. While low numbers of sea lice cause only minimal damage to the host

fish, high numbers can result in severe effects and even death of the host fish (SPAH, 2004).

In February 2003, the British Columbia Minister of Agriculture, Food and Fisheries, Stan Hagen, announced that all BC coastal fish farms must begin monitoring and treating sea lice, after drastic declines were noted in the number of native pink salmon returning to spawn in watersheds that enter the Broughton Archipelago area of coastal BC. Regional declines in the native pink salmon stocks have been hypothesized to result from abnormally high rates of sea lice infection of out-migrating smolts. It has been suggested that salmon aquaculture operations serve as reservoir areas for sea lice, and that the proximity of operations to estuarine and nearshore areas that are important foraging grounds of post-smolt native salmon prior to out-migration to offshore areas may result in unnaturally high rates of sea lice infection. Research is currently underway by Fisheries and Oceans Canada (DFO) scientists and others to test this hypothesis.

The preferred chemotherapeutant for sea lice control in Canada, at the present time, is "Slice®". Slice® is a trade name for a product developed by Schering-Plough Animal Health (SPAH) that has EB (CAS No. 155569-91-8, formerly 137512-74-4) as its active ingredient. It is currently recommended by BC MAFF fish health managers for use in the control of sea lice on farmed salmon in fish farms located within the Broughton Archipelago, because of its effectiveness against both adult and immature stages of sea lice parasites. Health Canada has authorized the use of EB, administered as Slice®, as an Emergency Drug Release (**EDR**), and the Veterinary Drug Directorate (VDD) of Health Canada is currently reviewing an application from Schering-Plough for the formal registration of Slice®, under the Pest Control Products Act of 2002.

A few of the questions that underlie risk management decisions about the use of Slice® in British Columbia or other Canadian coastal waters are:

- whether there exists the possibility that repeated applications could be made within a locale or larger ecosystem;
- whether risks to non-target marine life are adequately characterized based on a one-time application; and
- whether the evaluation of risks to non-target biota based on published acute or sub-chronic toxicity data adequately address important impact hypotheses.

Slice® has been developed as an alternative to the use of other sea lice control products, several of which have now been phased out for use in finfish aquaculture. These include (Rae, 2000) dichlorvos (Aquaguard®), Azamethiphos (Salmosan®), hydrogen peroxide, cypermethrin (Excis®), Teflubenzuron (Calcicide®), and Diflubenzuron (Lepsidon®). Several of these are applied to the water in and immediately around the net pen, after installing a water tight curtain around the facility, while EB, Teflubenzuron, and Diflubenzuron are used as systemic therapueutants, delivered in the feed. Of these, several (e.g. Excis®) have not been approved for use in Canada.

Members of the family Caligidae are the most commonly reported sea lice species on fish reared in brackish and marine waters. The species that are primarily responsible for infestations on farmed salmon in Canada include *Lepeophtheirus salmonis* (circumpolar distribution), C*aligus elongatus* (Atlantic Ocean) and *C. clemensi* (Pacific Ocean). *L. salmonis* is by far the more important of the two parasites for domesticated and wild salmonid stock from a perspective of disease transmission, not just in British Columbia but also in Atlantic Canada, United Kingdom countries and elsewhere in Europe.

Damage to the fish is caused by the feeding activity of the sea lice. The most damaging stage of *L. salmonis* tends to be the pre-adults, particularly as these concentrate on the head region, which has no protective scales and is therefore more susceptible to damage (SPAH, 2004). Sea lice typically eat the epidermis (skin) along with mucus, blood and cells. The subsequent exposure of delicate underlying tissues can cause death due to bacterial infections, stress, and osmotic regulation problems (UPEI, 2004). Coho salmon are known to be far less susceptible to sea lice infestation than Atlantic salmon (Johnson et al., 2004).

EC initiated this comprehensive literature review of EB use for sea lice control in finfish aquaculture in Canada for several reasons: First, an Advisory Group for Aquaculture, composed of members representing EC, DFO, BC MAFF, BC MWLAP, B.C. Salmon Farmers Association, (**BCSFA**), the salmon aquaculture industry, and veterinarians noted to EC that there is incomplete understanding of research to date and the regulatory framework relating to EB as well as its desmethyl metabolite. A detailed review would ensure that future studies build on rather than duplicate previous research. Second, a detailed ecotoxicity review is merited for EB inputs to Canadian coastal waters, since much of the previous information was developed in consideration of the use of Slice® in other areas of the world, such as Scottish fjords, where the physical oceanographic conditions and ecosystems might not be adequately representative of the Canadian situation. As discussed below, registration and use of a new therapeutant in Canada should satisfy similar review requirements to those mandated under the *Canadian Environmental Protection Act,* 1999 (**CEPA**).

The overall objective of this report is to provide a summary of the available information on the following:

- Patterns and trends for the use of EB for sea lice control in Canadian coastal waters, with a special focus on British Columbia,
- Chemical properties of EB and its associated degradation or metabolic byproducts once released to the environment,
- Analytical methods for EB and major byproducts, along with detection limits,
- Documented concentrations of EB and major byproducts in various marine environmental media,

- Environmental persistence and multi-media partitioning behaviour,
- Toxicity to non-target biota (including taxa of concern given the settings, mode of toxicological action, and toxicity thresholds),
- Current management regime for EB used in aquacultural operations in Canada, and
- Important knowledge gaps for introductions to the marine environment in Canada, and recommendations on research priorities.

2. BACKGROUND INFORMATION

2.1 Physical and Chemical Properties

Slice®, developed by Schering-Plough Animal Health (**SPAH**), contains 0.2% EB by weight, which is the active ingredient against both adult and immature forms of sea lice. Some other major constituents of Slice® are butylated hydroxyanisole (0.01%), propylene glycol (2.5%), maltodextrin (47.4%) and corn starch. Ingredients other than EB have not been evaluated as part of this review. A "semi-synthetic" process is used to manufacture EB from abamectin (SEPA, 1999).

Emamectin belongs to the avermectin group, a family of closely related 16-membered macrocyclic lactones produced by the fungus *Streptomyces avermitilis*. Nearly all the avermectins exhibit a broad spectrum of activity against nematodes and arthropods, with the B1a compound being the most efficacious for control of a variety of terrestrial and aquatic pest species (Korystov et al., 1999). Up to the late 1980s, there were basically two types of such avermectin-based active ingredients, i.e. ivermectin (consisting mainly of avermectin H2B1a) and abamectin (predominantly containing avermectin B1a).

The benzoate salt of emamectin, EB, is a white to cream coloured powder and is a mixture of two avermectin homologues:

- = 90% of 4'-epimethyamino-4'-deoxyavermectin B_{1a} benzoate (MAB_{1a})
- = 10% of 4'-epimethyamino-4'-deoxyavermectin B_{1b} benzoate (MAB_{1b})

The benzoate salt confers stability on the molecule (SPAH, 2004). The molecular formula of MAB_{1a} is $C_{49}H_{75}NO_{13}$, with a corresponding molecular weight of 1008.26 g/mol. Similarly, the MAB_{1b} homologue can be written as $C_{48}H_{73}NO_{13}$, with a molecular weight of 994.24 g/mol. The components differ only in having a methylene group on the isobutyl side chain of the B_{1a} component, as illustrated in Figure 2-1. MAB_{1a} has an ethyl group on the C26 position of the molecule, while MAB_{1b} has a methyl group in the same position.

Table 2-1 outlines some of the key chemical and physical properties of EB.

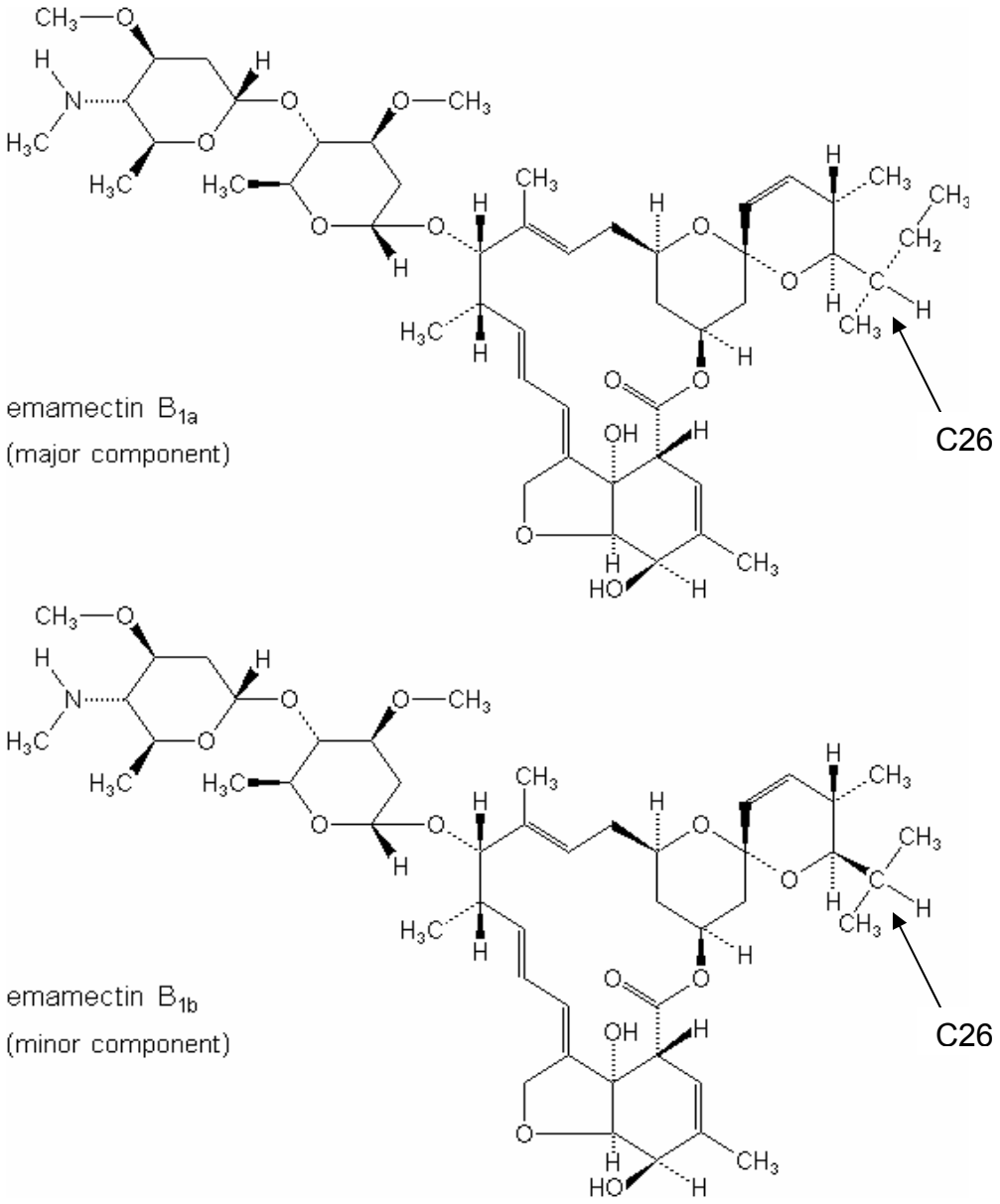

Figure 2-1: Emamectin homologues (Wood, 2004)

Table 2-1: Properties of Emamectin Benzoate

Scientific Name	(4"R)-5-O-demethyl-4"deoxy-4"(methylamino)avermectin A1a and (4"R)-5-O-demethyl-25-de (1-methylpropyl)-4"-deoxy-4"-(methylamino)-25-(1-methylethyl) avermectin A1a (9:1)
Molecular Formula	B_{1a} component $C_{49}H_{75}NO_{13}C_7H_6O_2$ B_{1b} component $C_{48}H_{73}NO_{13}C_7H_6O_2$
Molecular Weight	B_{1a} component: 1008.26 g/mol B_{1b} component: 994.24 g/mol
Vapour Pressure	3×10^{-8} mm Hg (torr)
Water Solubility	Fresh: 24 mg/L (pH 7.04) to 320 mg/L (pH 5.03) Salt: maximum 5.5 mg/L
Log K_{ow}	5.0
Stability (half-life)[1]	*Hydrolysis* – 19.5 weeks at pH 9, 25˚C (stable at pH 5.2 to pH 8.0) *Photolysis* – 1.4 to 22.4 days for EB in solution. 5 days when EB was bound to microbially active soil *Soil* - 193.4 days (aerobic), - 427 days (anaerobic), - 174 days (aerobic for 30 days then anaerobic) *Marine Sediment* – 164 to 175 days

[1]Excerpted from McHenery and Mackie, 1999. A more detailed review of environmental persistence, including critical review of the available studies is provided in Section 6.

At the neutral pH values typical of estuarine and marine areas, the dissociation constants for the benzoic acid and methylamino moieties (4.2 and 7.6, respectively) suggest that EB will occur in a dissociated form. EB, therefore, may interact with other molecules/receptors by ionic interactions (SEPA, 1999), in spite of the high K_{OW}. No information was found on the specifics of the dissociated forms of EB, although it is assumed that there are a diverse range of possible dissociations, given the presence of multiple methylamino moieties.

Based on the information provided in Table 2-1, the following inferences can be made with respect to the environmental fate and transport of EB:

- EB is unlikely to volatilize, be transferred to, and persist in the atmosphere as its vapour pressure is less than 1 mm Hg;

- Although the Log K_{OW} does indicate a potential for bioaccumulation, the very high molecular weight suggests that bioavailability of EB may be inhibited relative to its

strong lipophilicity owing to steric hindrance from entry across lipid bilayer membranes. On the other hand, EB can be taken up into biota and be circulated systemically, which accounts for its efficacy after oral administration. The lack of evidence for biomagnification of EB may be more related to the ability of various biota to metabolize it and excrete relatively more polar metabolites, than the lipophilicity of the parent compounds.

- The K_{OW} value indicates the potential for EB to become tightly bound to soil/sediment organic matter in the receiving environment.

2.2 History of Use and Registration

Major regions of marine salmonid aquaculture activity worldwide include Japan, the east and west coasts of Canada, the northeastern coast of the United States, Ireland, Scotland, Norway, Chile, New Zealand and Tasmania (Johnson et al., 2004). Sea lice have not been reported as aquacultural pests in New Zealand and Tasmania. In areas where sea lice infections are common, secondary infections [e.g., with other diseases such as infectious pancreatic necrosis, bacterial kidney disease, and salmonid rickettsial septicemia (Thompson et al, 2004)] and reduced growth are issues of concern. Secondary infections associated with sea lice infestations has been identified as a serious issue on the east coast of Canada, but not yet on the west coast (Johnson et al, 2004).

EB received its first global registration in Japan in 1998, under the trade name Affirm®. Its use was for the control of lepidopteran pests on leafy vegetables, brassicas and as a trunk injection in pine trees to control the pine sawfly (PMAC). EB is not widely used, however, for sea lice control in Japan. Instead, problems associated with sea lice are avoided through rearing of coho salmon, which are less vulnerable to sea lice infestations than Atlantic salmon, and the restriction of grow-out periods to about one year.

The EB-based insecticide Proclaim® was granted emergency exemption in Hawaii and used in 1996 and 1997. Full registration for use was approved in 1999 (Syngenta). In the United States, EB is used in terrestrial agriculture to control pests on head lettuce, celery, cauliflower, broccoli, cabbage, and other crops. For example, about 260 kg of EB was applied to edible crops in California in 2002 (http://www.pesticideinfo.org; accessed October 2004). EB has also come into widespread use in some countries as an anti-fungal agent, sold under the trade name Proclaim®. Overall, EB first came into use in the United States and several other countries as a pesticide against terrestrial pests, and its use was shortly thereafter extended to use in finfish aquaculture.

EB, formulated as Slice®, was approved for use in the United Kingdom in 2000. EB, as Slice®, was provided an "Animal Test Exemption" in 1999 in the UK by the Veterinary Medicine Directorate (VMD) in order to allow the conductance of field trials (Rae, 2000). The European Medicines Evaluation Committee prior to this developed maximum residue levels (MRLs) for EB in foods intended for human consumption.

In Canada, Slice® is currently being used in British Columbia and Atlantic Canada, under an "Emergency Drug Use" basis (see Chapter 3). In addition SPAH has applied for full registration through the Veterinary Drug Directorate (**VDD**) of Health Canada.

Roth (2000) provides a summary of the history of registration and use of various chemotherapeutants for sea lice control on a world wide basis. Four chemicals (EB, azamethiphos, teflubenzuron, hydrogen peroxide) have been registered or provisionally registered for use in Canada for sea lice control. In addition, ivermectin was available as an "off-label" veterinary prescription (i.e., for use in pesticidal applications other than the control of sea-lice), but apparently has not been used in Canada since the late 1990s. Ivermectin, although structurally very similar to EB, has an effective dose based on oral administration which is very close to its lethal dose for Atlantic salmon (Table 2-2) while the margin of application error is greater for EB.

Since its introduction for use in Canada, Slice® has become the major component of sea lice control strategies at marine finfish aquaculture operations in Canada. According to Johnson et al. (2004):

> "At present, outbreaks of disease caused by sea lice are rarely reported, although rates of sea lice infection remain high as evidenced by the frequent requirement for treatments. The lack of disease is due to the use of management strategies that rely on medicines and husbandry practices to maintain sea lice at low levels of abundance."

Westcott et al. (2004) express concern about the heavy reliance of farms in the Bay of Fundy on Slice® for sea lice control, given the potential for sea lice to develop resistance to the drug.

2.3 Efficacy and Resistance in Sea Lice

The recommended dosage of EB, administered as Slice®, is 50 µg/kg/day for a duration of 7 consecutive days. Since the late 1990s, considerable effort has been directed toward the evaluation of the efficacy of EB, based on route of administration, tissue residue concentrations in salmon and post-dosing efficacy following oral administration, and potential for the development of resistance to EB by target organisms.

Table 2-2: Comparison of the Effective Versus Lethal Dose and Other Properties of Sea Louse Pesticides Used in Finfish Aquaculture (from Roth, 2000, unless indicated otherwise).

Pesticide	Therapeutic Dose	Toxic Dose to Atlantic Salmon (*Salmo salar*)	Therapeutic Margin of Safety	Prescribed Withdrawal Days, by Country	Maximum Residue Levels (MRLs) for Fish Tissue[2]	Sea Lice Life Stage Affected
Topical (Bath Applications)						
Dichlorvos	1.0 mg/L	> 4 mg/L	> 4 X	4 (UK), 14 (Norway)		Adult + Pre-adult
Azamethiphos	0.1 mg/L	>0.5 mg/L	> 5 X	2 (Canada) 7 (Norway)	0.1 mg/kg (EEC)[1]	Adult + Pre-adult
Hydrogen Peroxide	1,500 mg/L	1,500 to 4,000 mg/L	0 to 3 X	1 (Canada, UK) 0 (Norway)	No MRL recommended (EEC)[1]	Adult + Pre-adult??
Pyrethrum	0.01 to 10,000 mg/L	??	??	30 (Canada) 7 (Norway)		Adult + Pre-adult
Cypermethrin	0.005 mg/L	> 0.5 mg/L	> 100 X	3 (Norway, US)	0.02 mg/kg, 0.2 mg/kg in fat (EEC)[1]	Adult + Pre-adult
Deltamethrin	0.003 mg/L	0.003 mg/L, > 0.01 mg/L	0 to 3.5 X	3 (Norway)	0.01 mg/kg 0.05 mg/kg in fat (EEC)[1]	Adult + Pre-adult
Oral (With Feed)						
Emamectin	0.05 mg/kg for 7 d	0.36 mg/kg for 7 d	7 X	25 (Canada)	0.1 mg/kg (EEC)[1]	Adult, pre-adult, larvae

Table 2-2 (continued)

Ivermectin	0.2 mg kg^{-1} one time 0.02 to 0.2 mg/kg 1-2 X/wk; 9-40 wk	0.4 mg/kg one time 0.05 mg/kg for 2 d, 2 wk	2 X ??	180 (Canada) 1,000 degree days (Canada, UK)	0.1 mg/kg (bovine liver) 0.015 mg/kg (liver of other livestock) 0.04 mg/kg (bovine fat) 0.02 mg/kg (fat of other livestock)(EEC)[1]	Adult, pre-adult, larvae
Diflubenzuron	3 mg/kg over 14 d	??	??	60 (Norway)	1 mg/kg (EEC)[1]	Adult, pre-adult, larvae
Teflubenzuron	10 mg/kg over 7 d	??	??	21 to 42 (Canada) 60 (Norway	0.5 mg/kg (EEC)[1] 3.2 mg/kg (Canada)	Adult, pre-adult, larvae

[1] European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit. EMEA/MRLs (http://www.emea.eu.int/pdfs/vet/mrls); [2] In Canada, a generic MRL of 0.1 mg kg^{-1} for all pesticide residues was withdrawn by PMRA in 2003, with interim replacement by United States MRLs.

In 1999, Stone *et al.* conducted laboratory studies to determine the efficacy of Slice® administered to Atlantic salmon (*Salmo salar*) at three different concentrations (25, 50 and 100 µg/kg/day), and compared to a control group fed un-medicated pellets. Sea lice (*Lepeophtheirus salmonis*) were counted at 7, 14 and 21 days following treatment. In comparison to the control group, total numbers of sea lice were significantly reduced at all concentrations of EB, although the 25 µg/kg/day concentration was significantly less effective than the 50 and 100 µg/kg/day doses. As there was no significant reduction in sea lice between the two latter doses, 50 µg/kg/day was determined to be the optimum therapeutic dose.

Laboratory studies by Stone *et al.* (2000), have shown that the administration of Slice® as directed, prevented the development of sea lice (*Lepeophtheirus salmonis*) copepodites for up to 62 days from start of treatment, while chalimus numbers remained low for 69 days. The study involved Atlantic salmon (*S. salar*) divided into two groups: one group administered the EB as medicated food pellets; and one group that was fed un-medicated pellets (control group). Sea lice were introduced into both tanks on eight separate occasions, and fish were observed for lice infestation. Efficacy of Slice® was determined to range between 97.3% on day 43 of the study, to 35.4% on day 98.

Rainbow trout (*Oncorhynchus mykiss*) were exposed to *L. salmonis* in a laboratory study in Scotland, following treatment with Slice®. Both the treatment and control group were challenged with sea lice copepodids on four different occasions (days 35, 49, 65 and 77 from start of treatment). Treatment of rainbow trout with Slice® prevented the development of settled copepodids to chalimus and treated fish had significantly fewer lice than control fish when challenged with copepodids between days 35 and 49 from the start of treatment. Following challenge at Day 35, many of the lice found on Slice® treated fish were still copepodids whereas most of the lice found on control fish had developed to adults. Efficacy ranged from a high of 83% on day 63 to 40% on day 76 (SPAH, 2001).

Duston and Cusack (2002) administered EB as Slice® to brook trout (*Salvelinus fontinalus*) at the recommended dosage in order to determine the reduction in the numbers of the ectoparasite *Salmincola edwardsii*. The fish were purchased from a fish hatchery previously infested with the lice. Results from two studies indicated that EB significantly reduced the number of *S. edwardsii* on the brook trout. In the first experiment, fish were euthanized seven days following treatment and the mean number of lice per fish had decreased from 118 to 49, compared with an increase in the control fish from 109 to 125. Likewise, the second study indicated that between 17 and 31 days post-treatment, the mean number of lice decreased from 56 to 35, while the control group numbers increased from 67 to 82. Both reductions were determined to be statistically significant.

Stone et al. (2000) also conducted field studies on the northwest coast of Scotland to determine the efficacy of Slice® administered to Atlantic salmon. Field trials were carried out in experimental pens on a commercial fish farm, observing salmon that were naturally infested with both *L. salmonis* and *Caligus elongatus*. Each study included a

treatment group and a control group, in which observations were made on days 7, 14 and 21 following the administration of Slice® to the treatment group. In three separate trials, treatment with EB was effective against both chalimus and motile stages of sea lice, even though the treatment group were surrounded by pens containing salmon heavily-infested with sea lice. In all three trials, *L. salmonis* numbers increased over time on control fish by 87–284%, whereas over the same period, *L. salmonis* were reduced on treated fish by 68–98%. In the two summer trials, large numbers of *C. elongatus* were rapidly reduced by treatment with 82–84% efficacy by day 21. The study concluded that despite the potential for continuous re-infestation, oral treatment with EB presented an effective means of controlling all parasitic stages of *L. salmonis* and *C. elongatus* on farmed salmon, and in one trial, numbers remained lower on treated fish for at least 55 days.

Similar field trials conducted by Stone *et al.* (2000) on the west coast of Scotland determined that the efficacy of EB (administered as Slice® as per directions) was 89% at 35 days following treatment. Numbers of sea lice (*L. Lepeophtheirus* and *C. elongatus*) were also lower for the treatment group than the control group 64 days following treatment.

Seawater temperature in both field studies varied between trials, with a range from 5.5˚C to 15.5˚C. Reductions in sea lice numbers were slower during the colder temperature trials, but good efficacy (90% and 89%) was observed by days 21 and 35, respectively.

Ramstad *et al.* (2002) conducted four field studies on the west coast of Norway to determine the efficacy of EB (Slice®), and compared it with another commercially available product (teflubenzuron 2 g/kg, administered as Ektoban®). The fish species was *S. salar*, while the sea lice was *L. lepeophtheirus*. Sea lice numbers were counted two days prior to, and 1, 7, 14 and 21 days post treatment. Pens treated with EB were found to harbour significantly fewer lice 14 and 21 days post-treatment. Twenty-one days following treatment with EB the lice abundance was reduced on average by 94%, when compared to the control group.

Schering-Plough Animal Health (2001) reports efficacy numbers for field trials in Canada and Chile - 91% at ten weeks post-treatment and 93% at six weeks post-treatment, respectively. Numbers of *C. elongatus* were still 48% lower in the latter study, 14 weeks following treatment with Slice®.

SPAH concluded that while it appears that temperatures affect the rate of drug clearance from the skin and muscle of fish, the duration of efficacy cannot be predicted at different temperatures as other factors such as fish size, maturity, health and condition may also have an influence. While differences in the duration of efficacy between individual fish may be partly related to drug uptake, different rates of metabolism may also play a role. Trials confirm that the protective benefits of treatment with Slice® extend far beyond the seven day medication period in Atlantic salmon and rainbow trout, reducing the need for frequent repeat treatments, thereby reducing concerns regarding costs and environmental impact of repeat applications (SPAH, 2001).

It has been noted in some cases that *Lepeophtheirus salmonis* has developed resistance to other sea lice compounds, such as azamethiphos, deltamethrin, cypermethrin and hydrogen peroxide. For example, treatment failures were documented in the early 1990s for the organophosphate dichlorvos and azamethiphos in Norway and Scotland (Devine et al., 2000), attributed in part to pest resistance.

Schering-Plough defines resistance as "an increase in the quantity or dose rate of a chemotherapeutant required to elicit a given response due to a change in gene frequency in a population of the gene(s) that control susceptibility" (SPAH, 2000). Resistance to ivermectin, used to control helminthes in sheep, was first documented 33 months following its introduction into one location. Likewise, resistance to abamectin by Colorado potato beetles and several species of mites were noted within five years of the first commercial use of this pesticide.

Resistance mechanisms employed by arthropods against avermectins include penetration, excretion, oxidative metabolism, esteratic metabolism/sequestration, altered target site, and glutathione S-transferase-dependent conjugation (SPAH, 2000). There also exists a risk of cross-resistance, whereby a pest demonstrates a resistance to compounds of the same chemical class or that utilize the same modes of action. Since ivermectin has been used for the control of sea lice for the past 10 years, there is the possibility that a resistance to avermectins, including EB, may develop. Schering-Plough Animal Health (2000) recommends the following measures to maintain the susceptibility of sea lice to chemotherapeutants:

1. Administration of the correct dosage rate over the full treatment period;
2. Medication of an appropriate amount of feed to ensure complete and homogeneous consumption;
3. Careful feeding practices to monitor feed consumption;
4. Use of the product in the absence of any inter-current disease affecting appetite;
5. Simultaneous treatment of all fish on a site;
6. Coordination of treatments of all farms in a bay system or coherent hydrographic entity to reduce cross infestation; and,
7. Strategic rotation of chemotherapeutants with different modes of action.

Anderson and Kvenseth (1999) recommend that de-lousing should not be conducted based on an over-reliance on any one de-lousing compound, and two or more pesticides should be routinely employed to minimize potential for the development of pesticide resistance.

2.4 Sources to the Environment

2.4.1 Application Regimes

EB is administered in Canada as the active ingredient in Slice®, manufactured by the Schering-Plough Animal Health Corporation. The product is supplied as a pre-mix containing 0.2% EB in a 99.8% inert[1] carrier, which is comprised of 0.01% butylated hydroxyanisole, 2.5% propylene glycol, 47.40% maltodextrin and corn starch (to 100%) (SEPA, 1999). The premix is coated onto non-medicated fish feed pellets to achieve an intended dose of 50 µg EB/kg of fish biomass per day for seven days. The suggested feeding rate is 0.5% of fish biomass per day. It can be used up to 3 times/year (maximum 5 treatments in any 2 year growth cycle). A withdrawal period of 25 days is required in Canada for EB, under its current emergency registration.

EB may enter the environment through two main routes:

- Deposition of uneaten food pellets to the sea floor below the salmon pens
- Deposition of fecal matter containing both EB and its metabolites

The degree of environmental risks associated with EB deposition will depend on factors such as (i) the quantity of active ingredient, (ii) the frequency of administration, (iii) the biological activity of the active ingredient, (iv) the biological activity of any metabolites, (v) the degree of deposition, and (vi) the sensitivity of the receiving environment (McHenry and Mackie, 1999).

2.4.2 Canadian Usage Patterns

In New Brunswick, treatment for sea lice is often initiated when infection rates reach > 5 pre-adult sealice per fish, or > 1 ovigerous female per fish, depending on the water temperature and the season (Johnson et al., 2004). Costello and Chang (2003) provide an overview of the sea lice situation in New Brunswick: Sea lice infestations in Bay of Fundy operations are ranked by operators as one of the major three issues facing the industry, along with ISA and "fish performance" issues. In 2002, it was estimated that there were one to three sea lice treatments required per cage per grow out cycle, and this was less than in previous years. The report also indicates that similar issues were identified in Maine. Fallowing of sites has not generally proven to be an effective sea lice control technology owing to the close proximity of adjacent operations.

Health Canada (2001) conducted a review of the testing of chemotherapeutants in fish tissue by the Canadian Food Inspection Agency (**CFIA)**, and provide estimates of chemotherapeutant use in Atlantic Canada. Federally, the *Feeds Act and Regulations* require Canadian feed mills to maintain copies of records for prescriptions administered through feed at their manufacturing sites. In 1998 in Atlantic Canada, 4% of all

[1] Text from Schering-Plough Animal Health documentation

manufactured fish feed was medicated, representing about 3,600 metric tonnes of feed. EB accounted for 38.1% of the prescriptions, while tetracyclines accounted for 52.4% and sulfonamides accounted for 9.5%. In 1998, the CFIA tested for ivermectin in farmed salmon tissue, but not EB.

Virtually all marine finfish sites in British Columbia are located on tenured Crown foreshore (http://www.agf.gov.bc.ca/fisheries/Finfish_main.htm: Accessed Oct. 2, 2004). There are currently 129 registered farms (Appendix A). Their general location within British Columbia coastal waters is as follows:

(http://www.agf.gov.bc.ca/fisheries/images/marine_fishfarms.jpg MAFF, MWLAP, 2004):

- Southern Georgia Basin (N. of Puget Sound)
  - Sechelt coastal waters: 10 sites
- Central Georgia Basin
  - East Coast of Vancouver Island: 31 sites
- Northern Georgia Basin
  - Northern Vancouver Island: 41 sites
- West Coast of Vancouver Island
  - Clayoquot Sound: 26 sites
- Mainland North of Cape Caution: 6 sites
- Plus 15 sites scattered in various locations throughout British Columbia coastal waters

Table 2-3 provides an estimate of quantities of EB used in British Columbia in recent years, based on personal communication with representatives from EC and the BC MWLAP.

Table 2-3: Emamectin Benzoate Use as Slice ® in British Columbia, 2000 to 2003.

Year	Total Quantities Used in BC (grams)
2000	2,440
2001	4,190
2002	8,890
2003*	4,950

* Data provided by BC MWLAP

Based on Best Management Practice Plan reporting requirements, BC MWLAP received information submitted by the industry for EB uses in 2003, as summarized in Figure 2-2. Based on reports received by BC MWLAP, 30 sites received EB therapeutant use in 2003 of the approximately 80 that were in operation at the time on a coast-wide basis. On a site-by-site basis, the minimum reported mass of EB used was 8 g, while the maximum

reported use at a site was 460 g. For all 30 sites, the arithmetic mean of the amount applied was 165 g, while the median was 139 g. The total reported use in 2003 for BC was 4.95 kg.

Site use patterns were bimodal, with one major group of farm sites utilizing from about 8 g. to 110 g. of EB in fish feed and another group utilizing from 167g. to 350 g. of EB (Figure 2-2).

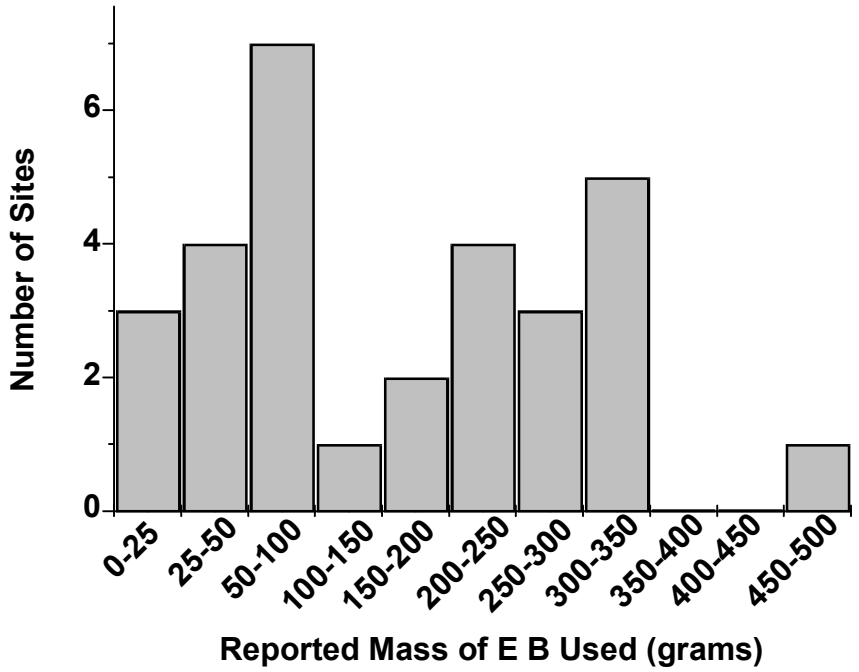

Figure 2-2: Emamectin benzoate reported used in British Columbia, 2003, based on industry reporting to BC MWLAP

No data were available for 2004 to-date.

2.4.3 EB Use in Other Maritime Countries

Some northern hemisphere countries have developed voluntary or mandatory guidance for the monitoring and/or treatment of sea lice in farmed salmon. A review is provided by Johnson et al. (2004). Industry treatment thresholds for sea lice in Ireland are set at 0.3 to 0.5 egg-bearing females per fish on average during the spring, and 2 egg-bearing females per fish during other seasons. The Norwegian treatment threshold is an average of 1 to 5 adult females per fish, depending on season, site location and water temperature. In Scotland, voluntary treatment is recommended when sea lice densities in farmed salmon approach one ovigerous female per ten fish on average during the spring. In Chile, parasiticide treatment is initiated after infection rates reach 10 sea lice per fish.

Thompson et al. (2004) provide cost estimates (US $0.22/kg fish) for parasiticidal treatment of sea lice on Atlantic salmon in a Chilean case study, where treatment with Slice® occurred three times per year.

2.4.4 Overarching Issues for EB Use and Release

Independent of regulatory requirements to limit sea lice infections of farmed salmon to limit transmission to wild salmonid stocks, there is a strong financial incentive for the salmon aquaculture industry to apply sea lice parasiticides and control strategies. Johnson et al. (2004), citing conclusions from Sinnott (1999), Mustafa (2001) and Rae (2002), provide a review of estimated economic losses to the industry from sea lice infestations on farmed salmon, which is summarized in Table 2-4.

Table 2-4: Estimated Economic Losses to the Finfish Aquaculture Industry from Sea Lice.

Region of Operation	Financial Loss Estimates	Basis	Source
Scotland	US $31-45 M/yr	Based on harvest of 130,000 t. Stress and growth reduction (US $20 M). Cost of therapeutants (US $6.8-7.2M)	Rae (2002)
Scotland	US $0.18-0.45 per harvested kg of salmon		Sinnott (1999)
Norway	US $67 M/yr		
New Brunswick, Canada	US $0.08-0.11 per harvested kg of salmon	With treatment	Mustafa et al. (2001)
New Brunswick, Canada	US $0.35 per harvested kg of salmon	Without treatment	Mustafa et al. (2001)
Chile	US $0.30 per kg fish	Treatment during grow-out, growth reductions, and de-lousing of carcasses prior to market.	Carvajal et al. (1998)

Regulatory guidance in both Norway and Ireland, and voluntary guidance elsewhere (e.g. see Rae, 2000) encourages an integrated pest management (IPM) approach[2] to the treatment of sea lice. In particular, recognizing the potential for sea lice transmission between different operations within a larger discrete ecosystem, it has been suggested by some jurisdictions that –

1. treatment of sea lice with Slice® or other parasiticides should be tied to routine monitoring observations of sea lice prevalence at aquaculture operations (discussed in Section 2.4.3);

2. fallowing of farm sites be considered as a management tool against sea lice infections;

3. individual year classes should be separated. Grow-out areas for juveniles and adults be segregated to limit disease and sea lice transmission between the different life stages;

4. individual grow-out areas should be separated by a minimum distance to limit transmission of sea lice between host populations; and

5. the treatment of all farm operations for sea lice within a larger geographic region be coordinated, so that control measures at one location are not undermined by transmission of the pest from adjacent reservoir areas.

Formal veterinary and regulatory guidance on such issues is currently lacking in Canada; however, items 1 and 5 in particular are very important in the context of assessing the risks of use of Slice® to non-target organisms in the adjacent marine environment. Synchronized application of Slice® across different net-pens in a contiguous area might serve to decrease the absolute mass of parasiticide required (and by extension releases to the environment); however, the instantaneous concentration resulting from such cumulative inputs might conceivably result in peak concentrations in the surface microlayer, water column or sediment that would exceed expectations based on single 7-day applications at a single operation.

The determination of application timing for Slice® or other pesticides based on sea lice build-up on farmed stocks, in order to limit the pool of ovigerous female sea lice, makes good sense from a pest control perspective. A likely consequence of such practice, however, would be repeated application of sea lice pesticides at an individual site across multiple grow-out cycles, or even within a single grow-out cycles. The potential for cumulative environmental loading and effects based on repeated applications at any given aquaculture site has not been formally assessed in Canada. No other discussion of the issue of multiple applications for Slice® were found for other jurisdictions, either.

[2] See, however, Thompson et al., 2004, for a detailed review of environmental/siting and other factors beyond the use of pesticides that influence see lice infestations on domesticated fish stocks.

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3. CURRENT REGULATORY REGIME

3.1 Canada

While not fully approved for use in Canada, Schering-Plough has applied for approval of the use of Slice® through the VDD of Health Canada. The VDD is part of the Health Products and Food Branch of Health Canada. The VDD is responsible for ensuring the safety of foods such as milk, meat, eggs, fish, and honey from animals treated with veterinary drugs. The VDD has authorized the use of Slice® on an Emergency Drug Release (**EDR**) basis, in which a licensed veterinarian can apply for and oversee the administration of the drug. After completion of the treatment, the veterinarian must provide a report to the VDD documenting when the treatment was administered, clinical observations, and whether any adverse reactions were noted. Apparently, there is no requirement for public or standardized reporting of therapeutant uses in Canada authorized through EDR authorizations. The VDD has set a withdrawal period of 25 days when using Slice® under the EDR process. In other words, farmed finfish cannot be sacrificed for market prior to 25 days from the last application of EB (Burridge, 2003).

A registration is normally granted for a term of five years, subject to renewal. Once a chemical therapeutant is formally registered for use, it is regulated under the *Canadian Food and Drugs Act*, which provides standards for veterinary drug use and fish destined for market. In addition, the Canadian Food Inspection Agency (**CFIA**) maintains responsibility for the testing of pesticide residues in domestic and imported livestock, fish and shellfish.

Health Canada was not able to provide comment on EB to the authors of this report, given that the registration application for Slice® is pending.

The issue of EB use for sea lice control in Canada is limited mostly to New Brunswick and British Columbia. Finfish aquaculture also occurs in other Atlantic provinces and eastern provinces (Burridge, 2003); however, the Labrador and Prince Edward Island industry produces mainly Arctic charr (*Salvelinus alpinus*) in freshwater operations, as well as rainbow trout. The Nova Scotia industry produces rainbow/steelhead trout in land-based facilities and marine cage sites. The Quebec industry is primarily focused on producing rainbow trout for the food market and speckled trout (*Salvelinus fontinalis*) for enhancement of the sport-fish trade. Regulatory regimes for EB use in aquaculture, therefore, have not been developed in these jurisdictions.

Within British Columbia, the provincial *Fisheries Act* provides the authority for the Ministry of Agriculture, Food and Fisheries (BC MAFF) to regulate on-site farming activities. The *Aquaculture Regulation*, last modified in April 2002, establishes regulatory requirements for finfish aquaculture operations, including minimum acceptable standards of operation. BC MWLAP staff are involved in reviewing and auditing environmental monitoring data submitted by fish farms to verify compliance with the environmental standards established in the *Finfish Aquaculture Waste Control*

Regulation (**FAWCR**), which was adopted under the *Waste Management Act* (superceded in 2004 by the *Environmental Management Act*). Under the FAWCR, fish farm operators have been required since March of 2003 to implement a Best Management Practices Plan (**BMPP**) to address the management of potentially harmful materials, promote the reduction of the discharge of wastes and pollutants, prevent the attraction of wildlife to feed, foodstuffs and mortalities, collect and dispose of mortalities in a timely fashion and in a manner to prevent spillage to the environment, and minimize odours during storage and transportation (MAFF and MWLAP, 2004).

Under a service agreement between BC MAFF and BC MWLAP, each operating finfish aquaculture site (those that are not being fallowed) must be visited by BC MAFF inspectors at least once per year to assess compliance with the Management Plan, which includes maintaining the appropriate on-site records of escapes, adequacy of escape contingency plans, stock inventory records, routine inspection records, compliance with Best Management Plans, net cage configuration, *et cetera*. On-site inspections provide an opportunity to verify that therapeutant use (Slice®, for example) on the farm site is properly documented and these records are properly maintained. The BC MAFF inspections are also used to assess compliance with the FAWCR.

For examination of chemotherapeutant use, BC MAFF inspections evaluate whether the appropriate paper work has been completed to document and track the administration of any therapeutics. This includes records of the following:

- Aquaculture license number, name of holder and location of the operation;
- Species being cultivated;
- Name of veterinarian as well as person responsible for administering the therapeutant(s);
- Name of administered drug(s);
- Particulars of administration (date, treatment schedule, delivery method, date of last treatment.

If the treated fish have been harvested, the aquaculture licence holder must be able to produce a statement with specific information regarding the treatment history of harvested fish, which must then accompany the fish to the processing plant.

According to MAFF and MWLAP (2004), provincial government inspectors conducted reviews in 2003 of drug record keeping requirements only at the 74 sites (of 77 operational sites total; the remainder were in fallow in 2003) where fish had been medicated and where these records were available on-site for inspection. The inspections revealed that 73 sites were in compliance with all drug reporting requirements under the *Aquaculture Regulation*. Sixteen sites were inspected where therapeutants were in use.

One requirement of BMPPs at finfish operations, enabled under the FAWCR, is the reporting of chemical therapeutant use to BC MWLAP (B. Takaema, BC MWLAP, *pers. com.).* Some of the information captured in on-site records must be reported to BC MAFF and BC MWLAP; however, the major portion of the records are not publicly accessible,

and some of the information may be proprietary in light of business competition considerations.

BC MAFF recently released guidelines for a sealice monitoring program at coastal finfish aquaculture sites (http://www.agf.gov.bc.ca/fisheries/health/Sealice/Sealice_Monitoring_Program.pdf: accessed December 2nd, 2004). For Atlantic Salmon, the program specifies sampling once per month of 20 fish in at least three pens. Anaesthetized fish are then analyzed for *Lepeophtheirus* spp. and counts are made of adult females (with and without egg strings), mobile lice (adult female/male and pre-adult male and female), *Chalimus* (total), and *Caligus* (total).

Salmon farms first appeared in New Brunswick in the late 1970s, and are currently regulated under the *Aquaculture Act* of 1988. The Aquaculture Registrar of the Department of Agriculture, Fisheries and Aquaculture is responsible for the licensing and leasing of all aquaculture in the province, and administers commercial, private and institutional licences, as well as occupation permits and leases for aquaculture sites situated on Crown Land. Under Section 11(1) of the act:

> **"11**(1) Upon issuing, renewing or amending an aquaculture licence, the Registrar may, in addition to any terms and conditions established by or in accordance with the regulations, make the licence subject to terms and conditions in relation to
>
> > *(a)* adherence to an aquaculture site development plan approved by the Registrar,
> >
> > *(b)* standards relating to site utilization, stocking densities and production at aquaculture sites,
> >
> > *(c)* measures to be taken to minimize the risk of environmental degradation,
> >
> > *(d)* measures to be taken to prevent the escape of aquacultural produce,
> >
> > *(e)* measures to be taken to minimize the risk of disease, parasites, toxins or contaminants spreading to other aquaculture sites,
> >
> > *(f)* measures to be taken to ensure the maintenance of applicable health, grade and genetic standards, and
> >
> > *(g)* any other matter the Registrar considers necessary for the purposes of this Act and the regulations."

The province of New Brunswick and DFO signed a memorandum of understanding (**MOU**) in 1989 intended to facilitate the orderly development of aquaculture and the establishment of a coordinated system of licensing and leasing of commercial aquaculture ventures (Salmon Aquaculture Review, 1997, Vol. 4, accessed at http://www.intrafish.com/laws-and-regulations/report_bc/v4c_iv.htm). The province is

responsible for the promotion, training and development of aquaculture and the management and issuance of leases and operating licences for aquaculture facilities. The MOU provides for the establishment of coordinating committees to ensure interagency cooperation regarding the management, promotion and development of aquaculture.

For chemotherapeutant application, an aquaculture licence holder in New Brunswick must submit a written report to the Minister within seven days after receiving written or verbal information about any diagnostic work or treatment. The report must contain the name, dosage and total amount of any drug or chemical agent administered, the time period in which the drug or chemical agent was administered, the temperature of water at the time, and the number of fish treated.

According to Westcott (2004), there are no regulations for the reporting of lice burdens on salmon farms in Atlantic Canada, nor are there officially standardized protocols for conducting sea lice counts in the field.

3.2 Other Maritime Countries

Slice® premix is fully approved in the UK, Chile, Ireland, Iceland and Norway, Finland, Spain, Portugal, and the Faroe Islands. The following summarizes information that was readily accessible. While similar information may exist for France, Chile, Iceland and Norway, the level of effort involved in retrieving the information was beyond the scope of this review.

The Scottish Environment Protection Agency (**SEPA**) has developed sediment and water Maximum Allowable Concentration (**MAC**) standards for EB. The sediment standards are further divided into a *far-field* standard, and a *near-field* standard. Far-field includes the area beyond 100 m from the fish pen edges, and down to a 5 cm depth into the sediment. Near-field is defined as the immediate area under and surrounding the fish pens, up to 25 m from the cage edge. Standards were based on previous toxicological studies, and were developed by using the geometric mean of the Lowest Observed Effect Concentration (LOEC) and the highest No Observed Effect Concentration (NOEC) of the most sensitive species tested for a given media (sediment, water column, etc.). The most sensitive species tested for exposure to EB in the sediment was the polychaete worm (*Arenicola marina*), while the crustacean *Mysidopsis bahia* was used for developing the water standard.

A safety factor of 100 was applied to the far-field sediment standard and the water standard, while a factor of 10 was used in the development of the near-field standard. The far-, near-field, and water standards for EB adopted by SEPA are 0.763 µg/kg (w/w), 7.63 µg/kg (w/w) and 2.2 x 10^{-4} µg/L, respectively. Application to administer Slice® must be accompanied by data from running the model DEPOMOD to determine the estimated deposition rates of EB to the surrounding environment (SEPA, 2004a).

The maximum number of treatments that SEPA will allow are:

- Three treatments in any 12 calendar months, and

- Five treatments in any two year growth cycle.

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4. ANALYTICAL METHODS

The principle analytical method for determining EB concentrations in sediment and water media is by high pressure liquid chromatography (HPLC) with fluorescence detection. Detection limits for trace amounts of EB in water are in the order of 10 ng/L, while limits of quantification of 20 and 24 ng/L for fresh and seawater, respectively, have been developed (Hicks *et al.*, 1997).

HPLC/fluorescence detection has also been adapted for the analysis of EB in Atlantic salmon tissue (Kim-Kang et al., 2002), in medicated fish feed (Farer et al., 1999), for the simultaneous determination of EB and ivermectin residues in Atlantic Salmon (van de Riet et al., 2001), for the simultaneous determination of residues of emamectin and its metabolites, as well as milbemectin, ivermectin, and abamectin in crops (Yoshi et al, 2001), quantification of abamectin and doramectin in sheep feces (Kolar et al., 2004) and for other media.

In 2004, Pereira and Chang reported on a method for analysis of ivermectin in rat or human plasma using a protein precipitation method followed by LC-tandem mass spectrometry.

Several studies on the environmental fate or metabolism of EB have been based on the use of radiolabeled compounds followed by quantification in a scintillation counter. For example, Mushtaq et al. (1996) examined soil sorption affinity of EB using [5-^{3}H]-labeled and [3, 7, 11, 13 or 23-^{14}C]-labeled EB. Eluants were extracted from spiked soils (six different types) with 0.01 M calcium chloride.

Chukwudebe et al. (1997) similarly used [3, 7, 11, 13 or 23-14C]-labeled EB in a study of fate in spiked soils, but evaluated degradation products by analyzing extractable radioactivity of the parent compound as well as metabolites by a combination of reverse phase-HPLC (high pressure liquid chromatography), NP-HPLC, and reverse-phase-HPLC/MS/MS (mass spectrometry). [^{14}C]-MAB1a, 8aOH-MAB1a, 8aoxo-MAB1a, and an early-eluting polar fraction were initially identified by RP-HPLC and then re-analyzed by NP-HPLC. Fractions collected from RP-HPLC were also analyzed by RP/HPLC/MS/MS on a Zorbrax ODS HPLC column and important metabolites identified based on ion spray MS in the positive ion mode.

Other studies based on radioactively labeled abamectins and metabolites have been included that by Kim-Kang et al (2004) examining EB pharmacokinetics and tissue residues in Atlantic salmon.

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5. LEVELS IN THE CANADIAN ENVIRONMENT AND OTHER MARITIME COUNTRIES

In order to assess risks of EB use in Canadian coastal environments, it is necessary to have accurate estimates of possible exposure concentrations in seawater, sediment, marine biota tissues and other exposure media. Burridge (2003) provides a comprehensive review of chemical use in marine finfish aquaculture in Canada. There is limited existing information on EB levels in Canadian marine environments. Unfortunately, published data are very sparse not just for Canada, but for all regions where EB has been used to control sea lice. Summaries provide in Sections 5.1 through 5.4 pertain to all areas of the globe, and the lack of data relevant to Canadian waters is identified as a major knowledge gap in the collective ability to evaluate environmental risks associated with the use of Slice®.

5.1 Seawater

Ernst et al. (2001) simulated the release of sea lice treatments from salmon aquaculture operations in the lower Bay of Fundy, New Brunswick. The study focused on chemotherapeutants applied to the water column (azamethiphos, cypermethrin) EB, which is administered in the feed, was not included in the study. Dilution of a conservative dye tracer (Rhodamine) or cypermethrin suggested that pesticide concentrations were generally between 1/200 and 1/2000 of the pre-release concentration (i.e. within the water curtain, prior to its removal), over distances of 900 to 3,000 m from the point of release.

There are no data on EB or its metabolites in waters surrounding Canadian marine finfish aquaculture operations. According to SPAH (2002), predicted environmental concentrations (**PECs**) of EB in waters downstream of salmon farms can be derived from model predictions. Using a residual flow of 0.018 m/s and "a 7-day feeding period plus 1 day for the ingested medication which is not absorbed to be excreted, then the concentration in the water passing the farm would have been 4.16×10^{-6} µg/L."

Such modeled predictions may not be applicable to some Canadian sites with dissimilar net pen configurations, physical oceanographic regimes, or area-wide stocking densities. Using PECs from modeled and very limited measured estimates of waterborne EB concentrations, SPAH (2002) estimated risk quotients from 0.01 to 215 (the latter being based on interstitial water concentrations). Note that such estimates do not account for synchronized use of EB-medicated pellets over a larger area, as recommended by some management agencies (Chapter 2). Nor do they account for possible benthic-pelagic flux from sediments under net pens where Slice® has been used repeatedly. The risk estimates are also not applicable to possible risks to larval crustaceans, fish, and other taxa that might be exposed to the lipid-rich surface microlayer.

5.2 Sediments

Parker and Mallory (2003) oversaw pre- and post-treatment sediment sampling in the vicinity of a salmon farm in the Bay of Fundy, New Brunswick in 2002. Prior to the study, the last sea lice treatment took place in August 2001, and consisted of a treatment of 0.2% EB added at a ratio of 1.67 kg per tonne of feed over a 7 day period. A total of 4,250 kg of medicated feed containing 14 g of EB was used during the 2001 treatment. A 'potential zone of impact' area, along with three control areas, was selected for the 2002 sampling program. Transects were established, and six pre-treatment composite sediment samples were taken from within the designated areas (three within the impact zone and one from each of the three controls). The samples were diver-collected cores from fine grained areas of the seabed. Concentrations of emamectin were lower than the analytical detection limit of 0.4 µg/g in all of the "pre-treatment samples" from the potential zone of impact or the control areas.

The 2002 treatment consisted of 0.2% EB administered over a 7 day period (September 25 to October 3, 2002). The chemical was added at a ratio of 2.5 kg per tonne of feed. Due to the larger size of the fish in the cages, approximately 50,000 kg of medicated feed was used over the 7 day period. The feed contained a total of 250 g of EB, which was approximately 18 times more than the previous treatment. At 10 weeks post-treatment, six composite sediment samples (3 cores each) were obtained again along the same transects established within the potential zone of impact and the control areas. The post-samples also did not contain detectable concentrations of emamectin; however, the analytical method employed had a detection limit of 0.4 µg/g, or 400 µg/kg. This value is approximately 5-times greater than the Maximum Acceptable Toxicant Concentration (MATC) for EB 76.3 µg/kg for the marine polychaete, *Arenicola marina* (see Section 7.10). Note also that the Scottish Environmental Protection Agency (SEPA, 1999) developed a "far-field predicted no effects concentration" (PNEC) for sediments based on this MATC of 0.76 µg/kg, since there is a limited availability of emamectin toxicity data for sediment dwelling organisms. The PNEC is based on applying a 100-fold uncertainty factor to the *A. marina* MATC in order to account for variability in the sensitivity of different species. It will be necessary to achieve an analytical detection limit for EB in sediments of ≤ 0.5 µg/kg in order ascertain associated levels of ecological risk.

SPAH (2002) provided estimates of EB concentrations in the marine environment as part of an environmental risk assessment submitted in Scotland in support of the registration of Slice®. The predictions were developed using the model DEPOMOD (J. Chamberlain, *pers.com*) with the assistance of the Scottish Environmental Protection Agency. Predicted environmental concentrations (PECs) in sediment were derived considering the case of 37 g of EB administered over 7 d under a series of 12 cages, each 15 m x 15 m in dimension. This assumed release can be compared with 2003 usage data for 30 British Columbia sites (Section 2.4.2), for which the median EB use was 139 g and the maximum documented use at a site was 460 g.

For the SEPA risk assessment, assumed PEC concentrations in sediment were further derived by assuming 10% of pellets were not consumed and ended up in bottom

sediments, wherein the EB would be incorporated uniformly in the top 1.5 cm of sediment (sediment density assumed to be 1.5 g/cm^3). Post-release breakdown of EB was assumed to be negligible. The predicted sediment PEC, therefore, was 3 μg kg^{-1} following the initial feeding losses to the seabed, followed by an increase to about 14-37 μg kg^{-1} after further additions of EB from faecal waste. Averaging inputs from the multiple cages, a maximum PEC of 76 μg kg^{-1} was predicted.

Using predictions from the DEPOMOD fate model, McHenry and Mackie (1999) predicted surface sediment concentrations of about 14-17 μg kg^{-1} EB beneath the net pens and 1.7-2.6 μg kg^{-1} at a distance of 50 m away. These predictions were validated against measurements in field-collected sediments. One week after treatment ended, only one sample, collected at a distance of 10 m from the net pen, exhibited a quantifiable EB concentration of 2.2 μg kg^{-1}, with the desmethyl metabolite quantified at 0.6 μg kg^{-1}. Four months post-treatment, however, EB was detected at 2.73 and 0.62 μg kg^{-1} at upstream stations at 10 and 100 metres, respectively. The desmethyl metabolite was detected at one sample point within 10 metres of the cages, at a concentration of 0.71 μg kg^{-1}, where the highest level of the parent compound was also detected. Twelve months after treatment, 1.8 μg kg^{-1} EB was detected at the same site.

Risk quotients derived from the resulting PECs (SPAH, 2002) were in the range of <0.23 to 67. It should be noted, however, that such risk estimates do not account for multiple applications of Slice® at a site, and possible cumulative loading in sediments. Note also that the SEPA DEPOMOD predictions were based on a total mass release during and following EB application of 37 g EB, while the study by Parker and Mallory (2003) was at an Atlantic Canada site where the estimated total input was 250 g EB.

SEPA (2004a) provides guidance on the use of EB at marine sites in Scotland. SEPA has an audit function for the use of EB, but no particulars are provided about whether this involves routine assessments of EB concentrations in the environment around sites where EB has been used.

In 2001 and 2002, SEPA conducted monitoring surveys of the occurrence in sediment of active ingredients of sea lice treatments near Scottish marine aquaculture sites (SEPA, 2004b). In 2001, a total of 76 sediment samples were collected in the vicinity of 44 fish farms. In 2002, a total of 66 samples were collected at 30 sites. EB and ivermectin were Soxhlet extracted from sediments, derivatized using trifluoroacetic anhydride and analyzed by HPLC-fluorometric detection. EB was not detected in any of the sediment samples collected in 2001. In 2002, EB was detected beneath the south-east corner of one fish farm at a concentration of 21.3 μg/kg, in excess of the previously established 7.63 μg/kg monitoring trigger value within 25 m of the cage edges (see Section 7.10). EB was also detected in two samples, with concentrations of 6.12 μg/kg in Loch Seaforth and 6.40 μg/kg at Scotasay in Loch Tarbet.

5.3 Marine Organisms other than Salmon

Studies involving bivalve molluscs positioned adjacent to and downstream of a net cage system receiving treatment for sea lice revealed that no Slice® residue was detected during a complete fish production cycle (Cross, 2004; *pers. com.*); however, the analytical detection limits achieved by the commercial analytical laboratory were too high to preclude bioaccumulation. The data from this research is not yet publicly available.

Some anecdotal information was received on an in progress study of EB concentrations in the Scottish environment, the results from which may be available in 2005. Similarly, DFO and other researchers are involved in a limited study of EB and metabolite concentrations in media near Canadian aquaculture operations; however, the data are not yet available.

6. ENVIRONMENTAL FATE

6.1 Multi-media Partitioning

The K_{OW} value for EB indicates that upon entering the soil or sediment environment, it is likely to be tightly bound. Adsorption data indicate that EB-derived materials in feed and feces will be bound to particulates (SPAH, 2002).

In laboratory studies with marine sediments and seawater, only two to three percent of the EB was recovered from the seawater, with a similar proportion being recovered in the water following the desorption phase from sediments. It has been determined that up to 5% of EB can leach off medicated feed over a six-hour period, and approximately 25% after 7 days, following shaking in seawater for 5 minutes (SPAH, 2002). Davies *et al.* (1997) determined that less than 5% of ivermectin leached off medicated feed over a 48-hour period, and that its physicochemical properties suggest that leached ivermectin would adsorb onto surrounding sediments. Field studies involving silt traps adjacent to fish cages showed that about 1% of the total EB in the traps was in the water phase. This material may consist of both soluble and fine particle-associated material (SPAH, 2002).

EB in a soluble form in water may arise by equilibration from the sediment-bound material into interstitial water, and then potentially into overlying waters. This action, assuming that input mechanisms are no longer active, has the potential to dilute the sediment concentrations over time. This is supported by adsorption/desorption and marine degradation studies whereby residual levels of EB were found in the seawater phase throughout the study. This was further supported by reports that EB did not significantly accumulate in the sediments, despite being detected in settling material (SPAH, 2002).

6.2 Transformations and Byproducts

Emamectin has various metabolites such as the 8,9-Z isomer, *N*-demethylated, *N*-formylated and *N*-methylformylated emamectins (Yoshii, 2002). Gavage feeding of radio-labeled EB to salmon, and subsequent analysis, revealed that higher proportions of metabolites were found in gut contents at all time points than were found in tissue samples. This may indicate that the metabolites are excreted more rapidly than the parent compound, or that the parent compound is subject to more enterohepatic circulation than the metabolites (SPAH, 2002).

Similar studies involving variations in water temperature revealed that when temperatures were 10˚C, almost all of the excreted material was metabolites, whereas only 30% of excreted material (from treatment to 90 days post-treatment) was metabolites at 5˚C (SPAH, 2002).

Kim-Kang *et al.* (2004) administered radio-labeled emamectin benzoate EB to *S. salar*, maintained at 5˚C (+/- 1˚C), and collected tissue, blood and bile from fish at 3 and 12 hours, and 1, 3, 7, 15, 30, 45, 60 and 90 days post treatment (final dose). Feces were also collected daily from the tank and monitored for total radioactive residues (TRR). The residue components of liver, kidney, muscle, and skin samples pooled by post dose interval were emamectin B1a (81-100% TRR) and desmethylemamectin B1a (0-17% TRR) with N-formylemamectin B1a seen in trace amounts (<2%) in some muscle samples.

In rats, approximately 80 percent of the radio-labeled material in feces and tissue was un-metabolized emamectin B_{1a}. An N-demethylated product of emamectin B_{1a} was the only metabolite found in feces, liver, kidneys, muscle and fat. The amount of this metabolite represented about one to two percent of the radioactivity one day post-treatment, but increased to 18 to 19 percent of radioactivity on day seven post-treatment. The percentage of this metabolite was found to be independent of the dose level administered, the route of administration, or the sex of the animal (EMEA, 1999).

6.3 Environmental Persistence

6.3.1 Tissue

In a study where Atlantic salmon (*Salmo salar*) were administered labeled EB at a dose of 50 µg/kg BW/day for 7 days, mean total radioactive residues (TRR) during 90 days post-treatment ranged from 1.4 to 3.0 mg/kg (kidney), 1.0 to 2.3 mg/kg (liver), 0.04 to 0.09 mg/kg (skin), 0.02 to 0.06 mg/kg (muscle) and <0.01 mg/kg in bone (Kim-Kang, 2004). Acceptable maximum residue levels (MRLs) established within the European Economic Community are 0.1 mg/kg (Table 2-2).

6.3.2 Water

SEPA (1999) reports that EB is stable to hydrolysis at a pH range from 5.2 to 8.0 (six week test at 25˚C), but breaks down at pH 9.0 with a half-life of 19.5 weeks. When EB in solution was exposed to natural autumn illumination, photolysis half-lives ranging from 1.4 to 22.4 days have been determined. The rate of photolysis is dependant on the aqueous media, with a half-life of 6.9 days determined for natural water, although there was no reference to the water source. Calculated half-lives for EB in water during summer and winter were 0.7 to 35.4 days, respectively (SPAH, 2002).

6.3.3 Sediment

EB's low marine water solubility (maximum 5.5 mg/L) and relatively high K_{OW} (5000) indicate that it will have a tendency to absorb to particulate material and surfaces, and that it will be tightly bound to sediments with little or no mobility (Hargrave, 2004). Adsorption data indicate that EB-derived materials in feed and feces will be bound to particulates, which might be expected to become incorporated in the sediments, unless they are re-suspended (SPAH, 2002).

Field trials conducted adjacent to an EB-treated cage indicated that only 4 of 59 collected sediment samples had detectable levels of EB. It was reported that the EB persisted in the sediment, and the highest concentration was measured at 10 m from the cage four months post-treatment (Hargrave, 2004).

In aerobic soils, EB has been shown to initially degrade at a half-life of 79 days, followed by a slower anaerobic phase whereby its half-life degradation is reduced to 349 days. The cumulative aerobic/anaerobic half-life degradation is 174 days (SPAH, 2002). SEPA (1999) reports the same cumulative degradation (aerobic for 30 days then anaerobic) of 174 days, but reports an aerobic soil half-life of 193.4 days, and an anaerobic half-life of 427 days. Investigations of the fate of radio-labeled EB in two types of marine sediments were conducted, and results indicate that the proportion of the applied radioactivity recovered as parent compound after 100 days was 66-68%. From this, the half-life of EB in marine sediments was calculated to be 164-175 days. Recently, SEPA (2004a) ruled that an assumed EB degradation half-life of 175 days for modeling purposes was not sufficiently conservative, given the supporting studies, and have opted instead to use an assumed degradation half-life of 225 days.

6.4 Bioavailability and Bioaccumulation

Bioaccumulation is the term describing a process whereby a substance is accumulated by organisms directly from the surrounding media and through consumption of food containing the substances. Bioconcentration is a process whereby there is a net accumulation of a substance directly from water into aquatic organisms resulting from simultaneous uptake (e.g., gills or epithelial tissue) and elimination. In the categorization process, bioaccumulation factors (BAF) are preferred over bioconcentration factors (BCF), however, in the absence of BAF or BCF data, the octanol–water partition coefficient (log K_{OW}) may be used. The octanol-water partition coefficient (log K_{OW}) is the ratio of the concentration of a material in the octanol phase to the concentration in the aqueous phase of a two-phase octanol/water system (Environment Canada, 2004).

A bioaccumulation factor (BAF) of 5000 is typically used as the threshold value, whereby a chemical is or is not likely to accumulate in an organism during an exposure period. Values greater than or equal to 5000 are likely to accumulate within body tissue,

whereas values less than 5000 are more likely not to. Likewise, BCF values of 5000 are used as a threshold, as is a log K_{OW} value of 5. The reported log K_{OW} value for EB is 5.

SEPA (1999) reported data for EB bioconcentration and depuration laboratory studies using the bluegill sunfish (*Lepomis macrochirus*). Study results are shown in Table 6-1.

Table 6-1: Tissue distribution of emamectin benzoate in bluegill sunfish.

Component	BCF	Study Length	50% Depuration Time	Study Length
Whole Body	80	28 days	3.9 days	14 days
Edible Tissue	30	28 days	3.8 days	14 days
Non-edible Tissue	116	28 days	4.0 days	14 days

The above study was conducted by Chukwudebe *et al* (1996). In a second trial, the study also recorded BCFs of 69, 31 and 98 for whole body, flesh and viscera, respectively. Both trials involved exposure to EB at 1.1 to 1.4 µg/L in a flow-through system.

Similarly, a study using avermectin B_{1a} (abamectin) and *L. macrochirus* found BCF values of 56 for the whole fish, 84 for viscera (non-edible tissue) and 28 for fillet (edible tissue). The study concluded that abamectin "does not strongly bioconcentrate in aquatic organisms and would not be expected to biomagnify".

Davies *et al.* (1997) studied BCF and depuration potential of ivermectin (also an avermectin), whereby mussels (*Mytilus edulis*) were exposed to 6.9 µg/L of ivermectin over 6 days, resulted in the calculation of a BCF of 752. The peak tissue concentration of 5.2 mg/kg (w/w) was reduced to half following 22 days of immersion in clean seawater, and a concentration of 0.06 mg/kg was detected after 150 days.

It has been speculated that the reason abamectin exhibits limited tendency to bioconcentrate is due to its large molecular size. EB has a large molecular weight (1008.26 for MAB_{1a} and 994.23 for MAB_{1b}), similar to abamectin (873), and thus EB's molecular size would also inhibit bioconcentration (SEPA, 1999). However, the limited tendency for avermectins to undergo food-web mediated transfer may be due more to the ability of many biota to metabolize them.

6.5 Pharmacokinetics in Marine Biota

Limited information is provided in Sections 6.2 and 6.4 on the kinetics and outcome of uptake, depuration, and tissue distribution of EB in various species. Few studies have examined in any detail the pharmacokinetics of EB and metabolites in aquatic species.

7. TOXICITY TO NON-TARGET ORGANISMS

Much of the literature on toxicity of EB to various non-target biota is difficult to critically evaluate (e.g. in terms of methodology), since it is contained in restricted circulation reports and papers produced by or on behalf of Sherring-Plough Animal Health (SPAH). The following review, therefore, relies in part on limited technical summaries of the original work produced by SPAH, or the Scottish Environmental Protection Agency (SEPA). The major portion of independent work on the toxicology of EB has been conducted by Fisheries and Oceans research scientists.

7.1 Mode of Toxicological Action in Sea Lice and Insect Pests

Avermectins act by binding to specific high-affinity binding sites, and at least part of their toxicological action is attributed to their tendency to open glutamate-gated chloride channels resulting in increased membrane permeability to Cl^-, hyperpolarization of muscle and nerve tissue, and inhibition of nerve transmission (Roy et al. 2000; SEPA, 1999). EB is absorbed from the gut and distributed to the tissues of the fish to which it is administered. According to SPAH (2004), when sea lice feed on tissues of treated fish, emamectin is taken up into the tissues of the louse. Emamectin then binds to ion channels of nerve cells and disrupts transmission of nerve impulses, which results in paralysis and death of the parasite. Studies have shown that EB is effective at killing all parasitic life-stages of sea lice, including both motile and non-motile.

Avermectins tend to be broad-spectrum toxicants for nematodes and arthropods, and modes of toxic action other than through disruption of chloride ion channels are poorly understood. The observations by Waddy et al. (2000) that EB induces molting in lobsters suggests the possibility of other modes of toxic action, including some that can be categorized as endocrine disruption effects. The relevance of such modes of toxicological action remain unclear, however, since altered molting in lobsters occurred when EB was administered by gavage at doses that likely exceed possible field exposures.

EB has been found to be less toxic than ivermectin where comparable data are available for a species (Burridge, 2003). The recent availability of EB in Canada has resulted in little if any interest in use of ivermectin for sea-lice control, an “off-label” application.

7.2 Non-target Species and Communities of Concern

Evaluation of the risks of EB release to the Canadian environment requires an appreciation of the non-target organisms and biotic consortia that are important from an ecological and/or economic perspective. There are at least four environmental compartments that represent important exposure pathways/points of exposure in the receiving environmental around an operation that may administer EB in feed pellets:

- The water column: EB can be released through limited dissolution from medicated feed pellets, as well as from fish excretion, especially where fecal elimination is not accompanied by rapid settling to the seabed;

- The seabed: EB can be introduced to benthic environments especially in unconsumed medicated feed, and in fish fecal pellets. The seabed can be soft-substrate, hard-substrate, or transitional between the two (e.g. shell gravel and shell hash environments). While most of the attention on soft-bottom environments has been on macrofauna and megafauna, an ecologically important component of this compartment are smaller meiofaunal biota such as nematodes and harpacticoid copepods < 0.5 mm in length.

- The surface microlayer: The top few μm to mm of the sea surface are recognized to be unique habitat. This is an area where biogenic and other lipids rise to the surface, and may contribute to the capture of other lipophilic substances. It is also an area of nearshore coastal environments that is very important from the perspective of larval fish and invertebrate transport and feeding, and – by extension – for larval recruitment in the coastal zone. The surface microlayer is at the top of the photic zone, and tends to be an area of intense primary and secondary productivity.

- Macro and megafauna or macrophytes: Biota that capture substances from the water column are possible secondary sources of exposure to their consumers. The potential for exposures via this route for EB are probably worst-case for bivalve mollusks, which tend to bioaccumulate environmental chemicals to a high degree based on the volume of water that they filter, and which have very limited MFO-like activity resulting in a more limited ability to metabolize heterocyclic macromolecules.

Decapod crustacean such as crabs, lobsters, and pandalid shrimp are important scavengers that tend to be drawn to eutrophic seabeds under finfish aquaculture operations, and are also of economic interest. In Atlantic Canada, therefore, it was important to evaluate effects of EB on lobsters (Burridge et al, 2000., 2004; Waddy et al., 2002). On the Pacific coast, Dungeness crabs, red rock crabs, and prawns are obvious non-target biota of particular concern.

Research on EB uptake into Dungeness crabs and prawns from feeding on medicated feed pellets has been conducted (Linssen et al., 2002). Contrary to the understanding of some veterinarians involved in the administration of EB in BC, toxicity thresholds for these species have not been experimentally derived. More details on this situation is discussed in the next section.

Some of the infaunal macrofauna (e.g. worms, clams and crustaceans living in the sediments) that are dominant in the vicinity of five aquaculture operations, but outside of the immediate area of influence of organic waste deposition, are listed in Appendix B.

7.3 Toxicity to Non-target Crustaceans

Table 7-1 summarizes the available emamectin benzoate toxicity data for crustaceans and other aquatic organisms.

The data are limited to that derived primarily from studies on acute toxicity, rather than chronic or sub-chronic effects on mortality, fecundity, reproductive success, growth, or other sub-lethal responses. Figure 7-1 shows the distribution of ecotoxicity data for EB exposures in water (including both freshwater and marine species).

Table 7-1: Ecotoxicity data for emamectin benzoate.

Test Location	Scientific name	Common name	Endpoint	Effect Measurement	Media Type	Duration	Exposure Type	Concentration	Reference
Crustacea									
Lab	*Americamysis bahia*	Opossum shrimp	EC50	Immobilization	seawater	96 h	Flow-through	0.00004 mg/L	OPP, 2000
Lab	*Corophium volutator*	Sand flea	LC50	Mortality	seawater	10 d		0.0063 mg/L	SEPA, 1999
			NOEC	Mortality	seawater	10 d		0.0032 mg/L	SEPA, 1999
Lab	*Mysidopsis bahia*	mysid	LC50	Mortality	seawater	96 h		0.000043 mg/L	SEPA, 1999
			NOEC	Mortality	seawater	96 h		0.000018 mg/L	SEPA, 1999
Lab	*Nephrops norvegicus*	lobster	LC50	Mortality	seawater	192 h		0.572 mg/L	SEPA, 1999
			NOEC	Mortality	seawater	192 h		0.440 mg/L	SEPA, 1999
Lab	*Crangon crangon*	shrimp	LC50	Mortality	seawater	192 h		0.161 mg/L	SEPA, 1999
			NOEC	Mortality	seawater	192 h		<0.161 mg/L	SEPA, 1999
Lab	*Daphnia magna*	Water flea	EC50	Immobilization	freshwater	48 h	Static	>0.728 mg/L	OPP, 2000
			EC50	Immobilization	freshwater	48 h	Flow-through	0.001 mg/L (0.00084 - 0.0012 mg/L)	OPP, 2000
			LOEC	Reproduction	freshwater	21 d	Static	0.00016 mg/L	OPP, 2000
			NOEC	Reproduction	freshwater	21 d	Static	0.000088 mg/L	OPP, 2000
Lab	*Corophium volutator*	Sand flea	LC50	Mortality	sediment	10 d		0.19 mg/kg sed. (ww)	SEPA, 1999
			NOEC	Mortality	sediment	10 d		0.11 mg/kg sed. (ww)	SEPA, 1999
Lab	*Homarus americanus*	Lobsters -adults	Lethality	Mortality	food	7 d	feeding	644 (CI: 428-1275) mg/kg food	Waddy, 2000; Burridge et al., 2004
		Lobsters –Stage V, VI juveniles	Lethality	Mortality	food	7 d	feeding	598) mg/kg food	Burridge, 2000
Lab	*Nephrops norvegicus*	Lobsters	LC50	Mortality	food	192 h	feeding	>68.2 mg/kg food	SEPA, 1999

Table 7-1 (Continued)

Test Location	Scientific name	Common name	Endpoint	Effect Measurement	Media Type	Duration	Exposure Type	Concentration	Reference
Lab	*Nephrops norvegicus*	Lobsters	NOEC	Mortality	food	192 h	feeding	68.2 mg/kg food	SEPA, 1999
Lab	*Crangon crangon*	shrimp	LC50	Mortality	food	192 h		>69.3 mg/kg food	SEPA, 1999
			NOEC	Mortality	food	192 h		69.3 mg/kg food	SEPA, 1999
Other Invertebrates									
Lab	*Crassostrea virginica*	American or virginia oyster	EC50	Immobilization	seawater	96 h	Flow-through	0.49 mg/L (0.41 - 0.59 mg/L)	OPP, 2000
			LC50	Mortality	seawater	96 h		0.67 mg/L	OPP, 2000
			NOEC	Mortality	seawater	96 h		0.26 mg/L	OPP, 2000
Lab	*Arenicola marina*	Lug worm (polychaete)	LC50	Mortality	sediment	10 d		0.11 mg/kg sed. (ww)	SEPA, 1999
			NOEC	Mortality	sediment	10 d		0.056 mg/kg sed. (ww)	SEPA, 1999
Fish									
Lab	*Cyprinodon variegatus*	Sheepshead minnow	LC50	Mortality	seawater	96 h	Flow-through	1.43 mg/L (1.25 - 1.67 mg/L)	OPP, 2000
			NOEC	Mortality	seawater	96 h	Flow-through	0.86 mg/L	OPP, 2000
Lab	*Lepomis macrochirus*	Bluegill	LC50	Mortality	freshwater	96 h	Flow-through	0.180 mg/L (0.04 - 0.24 mg/L)	OPP, 2000
			NOEC	Mortality	freshwater	96 h	Flow-through	0.087 mg/L	OPP, 2000

Table 7-1 (Continued)

Test Location	Scientific name	Common name	Endpoint	Effect Measurement	Media Type	Duration	Exposure Type	Concentration	Reference
Lab	*Oncorhynchus mykiss*	Rainbow trout, Donaldson trout	LC50	Mortality	freshwater	96 h	Flow-through	0.17 mg/L (0.15 - 0.21 mg/L)	OPP, 2000
			NOEC	Mortality	freshwater	96 h	Flow-through	0. 049 mg/L	OPP, 2000
Lab	*Pimephales promelas*	Fathead minnow	LC50	Mortality	freshwater	96 h	Flow-through	0.19 mg/L (0.16-0.26 mg/L)	OPP, 2000
			NOEC	Mortality	freshwater	96 h	Flow-through	0.16 mg/L	OPP, 2000

Figure 7-1: Toxicity of emamectin benzoate to marine and freshwater crustaceans.

The threshold of effects for acute mortality for a range of species is likely to be in the range of 10^{-5} to 10^{-4} mg/L EB. The lowest NOEC, from Table 7-1, is for the mysid *Mysidopsis bahia*, exposed for 96 h and observed for signs of mortality (1.8×10^{-5} mg/L). Thresholds of effects for effects mediated through disruption of ecdysis, other endocrine type effects, and/or other reproductive effects cannot be confidently ascertained at the present time. It can reasonably be assumed, however, that such effects might occur at EB concentrations in the range of 10^{-6} mg/L, or in the low ng/L range. Since the achievable detection limits for EB and its metabolites is likely to be $\geq$ 10 ng/L, there remains a possibility that subtle non-target effects could occur at or below the detectable environmental concentrations.

EB was administered by oral gavage to female American lobsters (*Homarus americanus*) at a nominal dose 1 µg/g bodyweight in a slurry containing salmon pellets, seawater and propylene glycol. The lobsters were exposed on three different occasions: once each in June (pre-ovigerous), July (ovigerous) and October (ovigerous and resorbing). Forty-four percent (44%) of the treated lobster molted prematurely, compared with 0% of the control

group. These results were the first example of a crustacean molting in response to chemical exposure (Waddy *et al.*, 2002).

A follow-up study (Burridge et al., 2004) better establishes levels of EB in feed that are acutely toxic to juvenile and adult lobsters. Commercial salmon feed was coated with SLICE® at a range of concentrations and provided to the animals for 7 d in the laboratory. The LC_{50} was estimated to be 644 µg/g in food for adult lobsters and 589 µg/g food for stage V and VI juvenile lobsters. Consumption of medicated pellets by adult lobsters decreased significantly with increasing concentration of EB. Adult lobsters that died during the study had a significantly greater concentration of emamectin B1a in their muscle tissue than those that survived. The authors concluded that salmon feed medicated with EB at the concentrations used by the aquaculture industry is unlikely to pose an acute lethal threat to adult and small juvenile American lobsters.

Results from studies of avermectin effects on non-target organisms, based on delivery in food, have been equivocal owing to the internalized dose achieved in key toxicological studies. Burridge and Haya (1993) found that sand shrimp (*Crangon septemspinosa*) exposed to ivermectin in salmon food pellets for 96 h in running water died when the food was available to and consumed by the shrimp. The resulting LC_{50} was estimated to be 8.5 mg kg^{-1} food. Haya et al. (2001) note that ivermectin is likely lethal to sand shrimp at concentrations below the recommended dosage for systemic control of sea lice in Atlantic salmon. In particular, when the researchers limited the exposure by sand shrimp to only 2 h, then monitored the shrimp for 94 h, the resulting 96 h LC_{50} was equivalent to a dosage of 190 g ivermectin kg^{-1} fish d-1, which is very close to the maximum recommended effective dosage. Toxic responses were not observed in shrimp exposed to ivermectin in the water column.

Linssen et al. (2002) attempted to evaluate the toxicity of EB in medicated pellets to two common Pacific coast decapods: the Dungeness crab (*Cancer magister*) and the spot prawn (*Pandulus platyceros*). In these laboratory studies, prawns or crabs were offered feed medicated with EB at concentrations of 0, 1, 10, 100 and 500 mg kg^{-1} food (6 h per day x 7 days), and behaviour and food consumption were observed. There was no acute mortality in any of the tests conducted; however, the realized dose for these trials was very low. Medicated food pellets were provided to either of the two test organisms for up to four hours, after which uneaten pellets were recovered to calculate ingestion rates. Medicated pellets did not break down prior to 4 h in the feeding exposure trials (G. van Aggelen, *pers. com.*). It was noticed that both crabs and prawns tended to hoard, but not ingest, food pellets. Estimated ingestion rates of the pellets, therefore, were very low (for example, from 0.001 to 0.05 g/prawn), which was one to two orders of magnitude lower than consumption rates for a 'preferred' diet comprised of filets of sub-year rainbow trout (about 0.5 g/prawn). In addition, prawns and crabs tended to ingest less of the higher dose medicated pellets relative to control or lower dose pellets. Owing to the dislike of prawns and crabs for fish pellets, especially those medicated with EB, a toxicity benchmark could not be established.

The toxicity of EB to larval crustaceans has not been examined in detail. Effects on the reproduction of the freshwater *Daphnia magna* have been examined (Table 7-1). In addition, laboratory studies were conducted whereby Slice® was administered to three

life stages (nauplii, copepodites, adult) of four marine copepods – *Acartia clausie, Pseudocalanus elongatus, Temora longicornis, Oithona similes*. Exposures occurred for 48-hours. Nauplii and copepodite EC_{50} values were observed to be lower than adult stages. Observed EC_{50} values ranged from 0.00012 mg/L (*P. elongatus* nauplii) to 0.232 mg/L (*O. similes* adults), and the primary toxic effect was immobilization. In addition, a seven-day sub-lethal test was conducted with adult *A. clause*, which resulted in a NOEC of 0.00005 mg/l, and LOEC of 0.00016 mg/L (Willis and Ling, 2003).

7.4 Toxicity to Other Marine Invertebrates

The Office of Pesticide Programs (2000) recorded EC_{50} endpoints in a 96-hour flow-through study involving the American oyster (*Crassostrea virginica*) (Table 7-1). The observed effect was immobilization, and EC_{50} values of 0.04 ppb and 0.49 ppm, respectively, were obtained.

7.5 Toxicity to Fish

Toxicity data for fish species are summarized in Table 7-1. Roy *et al*. (2000) conducted laboratory tank studies with both *S. salar* and *O. mykiss* in 1994 and 1997, respectively. In both studies the treatment fish were administered Slice® medicated food pellets at 0.7% BW/day. Corrected minimum dose rates of 0, 70, 173 and 356 µg/kg BW/day were administered to *S. salar* for a duration of seven days. The study concluded that Atlantic salmon could tolerate EB at doses up to 173 µg/kg BW/day (3.4x recommended Slice® dosage), and that toxicity effects were observed only at doses of 356 µg/kg BW/day (7.1x recommended dose). Rainbow trout were administered corrected minimum dose rates of 0, 88, 218 and 413 µg/kg BW/day for seven days. This study concluded that *O. mykiss* could tolerate EB at doses up to 218 µg/kg BW/day (4.3x recommended Slice® dosage), and that toxicity effects were observed at doses of 413 µg/kg BW/day (8.3x recommended dose).

The Office of Pesticide Programs summarized a study from 2000, in which the researchers conducted a 96-hour flow-through study involving the exposure of Sheepshead minnow (*Cyprinodon variegates*) to EB in salt water. The average lethal concentration to 50 percent of the population (LC_{50}) was 1.43 ppm, with a range of 1.25 ppm to 1.67 ppm. The same studies were also conducted in freshwater involving Bluegill sunfish (*Lepomis macrochirus*), Rainbow trout (*Oncorhynchus mykiss*) and Fathead minnow (*Pimephales promelas*). Average LC_{50} values of 180, 174 and 194 ppb, respectively, were observed.

7.6 Toxicity to Birds

Chukwudebe *et al.* (1998) conducted laboratory tests on Mallard Duck (*Anas Platyrhyncos*) and Bobwhite Quail (*Colinis virginianus*) whereby the birds were administered EB by gavage (with a corn oil carrier) and through dietary intake of six

different concentrations, plus a control. Results indicated an LD_{50} of 76 and 254 mg/kg, respectively, with NOEC of <25 and 25 mg/kg for oral dosing. For dietary intake studies, results indicated a 5-day LC_{50} of 570 and 1,318 mg/kg, respectively, with NOEC of 20 and <125 mg/kg for dietary intake.

Similarly, O'Grodnick *et al.* (1998) also tested varying dietary concentrations of EB on both *A. platyrhyncos* and *C. virginianus*. The study tested maximum EB dietary concentrations 40 ppm and 125 ppm, respectively, and monitored for feed consumption, weight, general health and reproductive parameters. The study was conducted over a 20-week period for *A. platyrhyncos* and 22 weeks for *C. virginianus*. The authors concluded that the NOECs for mallards and bobwhites was 40 mg/kg and 125 mg/kg, respectively.

The US EPA Office of Pesticide Programs (2000) also conducted LD_{50} and LC_{50} studies on Bobwhites and Mallards. In the dose studies, the birds were administered EB through orally administered capsules and observed for 21 and 14 days, respectively. Average LD_{50} values of 264 and 46 mg/kg, respectively were recorded. Average lethal concentration (LC_{50}) for Bobwhite and Mallards was determined to be 1318 and 570 mg/kg, respectively, in a 21-day study where the birds were administered EB through dietary intake.

7.7 Toxicity to Mammals

In 1997, Wise *et al.* conducted laboratory tests on four groups of 25 pregnant female Sprague-Dawley rats were orally gavaged EB once daily at rates of 0, 0.1, 0.6 or 3.5 mg/kg/day, from gestation day 6 through lactation day 20. From gestation day 17 to 20, the high dose was reduced from 3.5 to 2.5 mg/kg/day due to pup tremors. Both maternal females and pups were observed during the study. Significant maternal weight gains were observed in the higher dose females (0.6 and 3.5/2.5 mg/kg/day), but no other effects were observed. Tremors were observed in high-dose pups, beginning on postnatal day six, and hind-limb splay was observed for all high-dose pups for post-natal days 15 through 26. However, these sign disappeared by observation day 34. The calculated NOAEC for developmental neurotoxicity of EB was determined to be 0.6 mg/kg/day.

Roy *et al.* (2000) reports that toxicity data generated in support of terrestrial plant applications have shown that rodent LD_{50} values have ranged from 22 to 120 mg/kg.

7.8 Toxicity to Algae

Freshwater EC_{50} studies involving Green algae (*Selenastrum capricornutum*) revealed that 50 percent of the algae were affected (observed as abundance) at a mean EB concentration of 0.0039 mg/L. The study was conducted over five days under static exposure conditions (Office of Pesticide Programs, 2000).

7.9 Toxicity to Microbes

No information was found on toxicity of SLICE® to aquatic microbes. In general, though, bacteria and fungi have been found to be insensitive to avermectins, where the targeted use has been in terrestrial/soil settings. (Campbell, 1989).

7.10 Predicted No-effects Concentrations

SEPA (1999) derived a "Predicted No Effect Concentration" (PNEC) for both water and sediment, based on the available toxicity data for EB. For water, the maximum acceptable toxicant concentration (**MATC**), which is the geometric mean of the LOEC and NOEC concentration, for *Mysidopsis bahia*, was used to derive a water-based PNEC by applying an uncertainty factor of 100, to achieve a PNEC of 2.2×10^{-4} μg/L. The PNEC is derived from the MATC for the most sensitive species for which data are available. Toxicity data are summarized in Table 7.1. The NOEC for *M. bahia* was determined to be 1.8×10^{-5} mg/L.

For sediment, SEPA based a PNEC on the toxicity results for the polychaete *Arenicola marina*, also with a 100-fold uncertainty factor, to derive a PNEC of 0.76 μg/kg.

Although SEPA did not derive a PNEC based on dietary intake, studies by Burridge et al. (2004) on acute mortality in American lobsters, *Homarus americanus*, are instructive. Estimated LC_{50} values for adults or stage V and VI juveniles were 644 mg/kg food and 598 mg/kg food respectively. These feeding doses cannot easily be converted to doses on a body weight basis, since the EB was administered in food at a standardized series of concentrations, and the test animals exhibited minor variations in body weights. No other toxicity data is available with which to establish an ECx, LCx or LOEC concentration based on dietary intake. For the lobster *Nephrops norvegicus* and the shrimp *Crangon crangon*, mortality was not observed at the maximum dose administered (68.2 mg/kg food and 69.3 mg/kg food, respectively). Toxicity thresholds for smaller scavenging marine species based on dietary intakes are needed to establish PNECs for EB based on the oral uptake route.

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8. KNOWLEDGE GAPS

Adequate evaluation of the potential environmental risks from EB use on sensitive marine foodchain and ecosystem species at finfish aquaculture operations requires an understanding that sensitive non-target organisms merit protection, the predicted extent of their exposure, as well as accurate estimates of the levels of EB which can cause toxic effects.

Slice® has become an important tool for sea lice control in both Atlantic Canada and British Columbia since 1999. Very little information is available, however, on the concentrations of EB in the sediment, water, surface microlayer, or biota resulting from current usage patterns, for Canada or other regions of the world where Slice® is used. This is of concern in light of the fact that feed pellets treated with EB may be fed to fish several times per year, and repeatedly on those occasions across different grow-out cycles, particularly in operations located in the Bay of Fundy. In addition, no information is available on environmental concentrations of EB metabolites, including the desmethyl metabolite.

The current state of knowledge results in considerable uncertainty regarding the degree of exposure and chemical concentrations to which non-target marine organisms are subjected. SEPA (1999) and others have predicted maximum expected sediment and water column concentrations, based on limited data. As a minimum, such predictions should be re-evaluated to assess predicted versus actual values in light of current EB usage patterns. More information on EB usage patterns, in terms of temporal and spatial trends, as well as magnitude and frequency of use is needed for both the Atlantic and Pacific coasts of Canada.

Knowledge about the effects of EB and its metabolites is also very limited. According to Burridge (2003):

> "Most work on pesticides to date has been conducted in the laboratory and has focused on determining the acute responses of aquatic organisms (non-target species) to exposure(s) to anti-sea lice chemicals. Limited field trials have focused on lethality of single treatments. Short-term responses to pesticide applications and long-term studies to establish the natural variability in local populations and measures of change in biodiversity need evaluation. Currently, commercially important non-target species have attracted much of the attention regarding effects of chemicals. There are apparently no data regarding the effects of these chemicals on microorganisms and planktonic species that form the foundation of the marine food chain in the near-shore environment."

Ecotoxicity data are mostly limited to lethality tests conducted over short time frames (96 h or less). More research is needed to determine thresholds of effects based on chronic lethal and sublethal endpoints for indigenous species. Of particular interest is effects on ecdysis and moulting in decapod and other crustaceans, which in turn might affect

growth, fecundity, and sub-population fitness. To the present time, the work of Waddy et al (2002) of American lobsters remains the only detailed research of this type.

The major portion of toxicity data has been developed for Atlantic marine species. While there is no *a priori* reason to expect major differences in species sensitivities between Atlantic and Pacific species, data of immediate relevance to British Columbia coastal ecosystems would be very useful.

Field studies of EB effects would be very useful as well; however, such studies are likely to be confounded by the influence of organic waste discharge effects from aquacultural operations. On the other hand, there remains a possibility that sub-lethal effects on ecdysis, histology and biochemistry of hepatopancreatic cell populations in crustaceans, reproductive output or other indicators might occur near operations using EB for sea lice control.

Prioritized data needs for improving confidence in assertions about environmental risks from EB introductions to the Canadian coastal environment include –

- Improved public and researcher access to usage patterns, facilitated by access to reported applications;

- Additional measurements of EB and its metabolites in sediments, water, the surface microlayer, and in key marine ecosystem indicator species such as filter-feeding bivalves and crustaceans in the vicinity of finfish aquaculture operations;

- Chronic toxicity data for ecologically important and sensitive indigenous species. This is especially important for species on the BC coast which serve as food items for local First Nations peoples;

- Scientifically conducted surveys to test for possible endocrine disruption effects of EB in field populations of crustaceans;

- Additional toxicity data for sensitive juvenile life stages of larval invertebrates and fish and other sub-adult forms;

- Additional toxicity data for other key ecologically important, and potentially sensitive species such as marine nematodes, harpacticoid copepods, mollusks, and marine algae;

- Field studies of persistence, environmental compartmentalization, and cumulative loading across multiple operations and application cycles.

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Appendix A: List of Currently Registered Coastal Salmon Farms in British Columbia

Licencee	MAFF#	Landfile#	Location
622335 British Columbia Ltd.	456	193432	West Redonda Island, Doctor Bay
Connors Bros. Limited	306	1403267	Venture Point, Sonora Island
Connors Bros. Limited	1401	1403267	Okisollo Channel, N of Quadra Island
Creative Salmon Company Ltd.	233	1401621	Indian Bay, Tofino Inlet
Creative Salmon Company Ltd.	244	1401643	Tofino Inlet (Eagle site)
Creative Salmon Company Ltd.	1048	1406335	McCaw Peninsula, Tranquil Inlet
Creative Salmon Company Ltd.	1419	1408125	Ridout Islets & McCall Island
Creative Salmon Company Ltd.	1596	1409666	Dawley Passage, Fortune Ch., Dark Isl.
Ewos Aquaculture Ltd.	314	1405933	Northeast McKay Island, Ross Passage
Ewos Aquaculture Ltd.	520	1403980	East Shore of Bedwell Sound
Ewos Aquaculture Ltd.	526	1403262	Rant Point, Clayoquot Sound
Ewos Aquaculture Ltd.	527	1401590	Saranac Island
Ewos Aquaculture Ltd.	540	1403914	East side Warn Bay, Fortune Channel
Ewos Aquaculture Ltd.	543	1401589	Mussel Rock, Clayoquot Sound
Ewos Aquaculture Ltd.	753	1401974	Hecate Bay, Cypress Bay, S-5 (Cormorant)
Ewos Aquaculture Ltd.	1148	1406648	Herbert Inlet, NE of Binns Island
Ewos Aquaculture Ltd.	1291	1407342	McIntyre Lake, Bare Bluff, Bedwell Sound
Ewos Aquaculture Ltd.	1472	1408492	West Side, Bedwell Sound
Ewos Aquaculture Ltd.	1507	1408719	Millar Channel, 2km S Hayden Passage
Ewos Aquaculture Ltd.	1537	1403979	Clayoquot Snd, Bedwell Snd, Bare Bluff
Ewos Site Co. Ltd.	227	1403647	Bawden Point, Herbert Inlet
Ewos Site Co. Ltd.	234	1403293	Dixon Point, Shelter Inlet
Ewos Site Co. Ltd.	507	210067	Obstruction Island, Shelter Inlet

Appendix A (Continued)

Licencee	MAFF#	Landfile#	Location
Grieg Seafood BC Ltd.	404	1405007	Across from Steamers Pt. (Cliff Cove)
Grieg Seafood BC Ltd.	1078	1404968	Hecate Channel (Lutes Creek & Hecate)
Grieg Seafood BC Ltd.	1079	1404969	Hecate Channel (Steamer Pt & Esperanza)
Grieg Seafood BC Ltd.	1700	1411064	Muchalat Inlet, Nootka District
Grieg Seafood BC Ltd.	1705	1411068	Williamson Passage, Nootka Sound
Grieg Seafood BC Ltd.	1738	1411084	Atrevida Point, Hanna Channel
Hardy Sea Farms Inc.	219	2402613	Hardy Isl, Jervis Inl (Power Bay-Site B)
Hardy Sea Farms Inc.	408	2402490	North Salmon Inlet (Kunechin-Site 5)
Hardy Sea Farms Inc.	412	2402492	North Salmon Inlet, Site 9
Hardy Sea Farms Inc.	746	2402591	Sechelt Inlet (Site 13)
Hatfield Biotechnology Ltd.	56	1401514	Cusheon Cove, Captain Passage
Heritage Salmon Limited	106	1403895	Simoom Sound, N. Wishart Peninsula
Heritage Salmon Limited	136	1403929	Cliff Bay Simoom Sound Wishart Peninsula
Heritage Salmon Limited	169	1401284	San Mateo Bay, Barkley Dist.
Heritage Salmon Limited	224	1404438	South Side San Mateo Bay, Alberni Inlet
Heritage Salmon Limited	304	2403035	Raza Island, Raza Passage
Heritage Salmon Limited	458	1405381	Cypress Hrbr, Harbour Pt, Sutlej Channel
Heritage Salmon Limited	728	1404179	Sir Edmond Bay, NE Shore Broughton Inlet
Heritage Salmon Limited	819	1405181	Cecil Island, Greenway Sound
Heritage Salmon Limited	869	1405739	SE Broughton Is., Greenway Snd, Maude Is
Heritage Salmon Limited	1070	1406618	Macktush Bay, Alberni Inlet
Heritage Salmon Limited	1144	1406650	Raleigh Passage, Burdwood Group
Heritage Salmon Limited	1335	1407731	Wehlis Bay, Wells Passage

Appendix A (Continued):

Licencee	MAFF#	Landfile#	Location
Heritage Salmon Limited	1336	1407730	Well Passage, Mount Simmonds Bay
Middle Bay Partnership	1770	1409460	Middle Point Bay, N. of Duncan Bay
Nutreco Canada Inc.	108	1405412	Orchard Bay, Kanish Bay, Quadra Island
Nutreco Canada Inc.	112	1404284	Whiteley Island, Kyuquot Sound
Nutreco Canada Inc.	137	1401597	Conville Bay, Hoskyn Channel
Nutreco Canada Inc.	138	1401659	Dunsterville Bay, Hoskyn Channel
Nutreco Canada Inc.	248	1403859	Conville Point, Hoskyn Channel
Nutreco Canada Inc.	380	1403144	Sonora Pt., Nodales Channel
Nutreco Canada Inc.	547	1401611	Bear Bay, Read Island
Nutreco Canada Inc.	733	1406292	Cyrus Rocks, Okisollo Channel
Nutreco Canada Inc.	769	1405768	Young Passage, Sonora Island
Nutreco Canada Inc.	884	6403484	Lockalsh Bay, Jackson Passage
Nutreco Canada Inc.	1158	1405003	South Point of Hohoae, Pinnace Channel
Nutreco Canada Inc.	1159	1405005	Amai Inlet, Amai Pt.
Nutreco Canada Inc.	1191	1406837	Shelter Inlet, E of Steamer Cove
Nutreco Canada Inc.	1554	1409081	E. Pinnace Ch, Kyuquot Sound (Charlie's)
Nutreco Canada Inc.	1580	6406814	Jackson Passage S.of Finlayson Channel
Nutreco Canada Inc.	1598	6406836	Arthur Island, Mathieson Channel
Nutreco Canada Inc.	1626	2407932	Church House, Calm Channel
Nutreco Canada Inc.	1691	6406984	Kid Bay, Roderick Island
Nutreco Canada Inc.	1702	6407324	Goat Cove, Roderick Island
Nutreco Canada Inc.	1724	1411078	Hohoae Island, Markale Passage
Omega Pacific Seafarms Inc.	270	1403261	Jane Bay, Barkley Sound

Appendix A (Continued):

Licencee	MAFF#	Landfile#	Location
Pan Fish Canada Ltd.	78	2403170	Phillips Arm, Cardero Channel
Pan Fish Canada Ltd.	100	1401949	Lees Bay, N. Shore, West Thurlow Is.
Pan Fish Canada Ltd.	303	2402751	Jervis Inlet near Glacial Creek
Pan Fish Canada Ltd.	402	2403015	Mouth of Homfray Ck., Homfray Channel
Pan Fish Canada Ltd.	553	2402966	SE Frederick Arm
Pan Fish Canada Ltd.	790	1405245	West Thurlow Island, Chancellor Channel
Pan Fish Canada Ltd.	831	1404091	Shelter Passage, Wishart Island
Pan Fish Canada Ltd.	892	1404918	Goletas Channel, S.E. Bell Island
Pan Fish Canada Ltd.	1110	1406566	Loughborough Inlet, Poison Creek
Pan Fish Canada Ltd.	1117	1406566	Griffin Cove, Loughborough Inlet
Pan Fish Canada Ltd.	1136	1406628	Shaw Point, Sunderland Channel
Pan Fish Canada Ltd.	1288	1407325	Doyle Island, Gordon Group
Pan Fish Canada Ltd.	1293	1407326	Duncan Island, Goletas Channel
Pan Fish Canada Ltd.	1300	1407426	Althorpe, Sunderland Channel
Pan Fish Canada Ltd.	1308	1403715	Mayne Passage, East Thurlow Island
Pan Fish Canada Ltd.	1350	1407748	Shelter Bay, Richards Channel
Pan Fish Canada Ltd.	1351	1407749	Marsh Bay (Stuart Rock) N. of P. Hardy
Pan Fish Canada Ltd.	1376	1407743	Cleagh Creek, Quatsino Sound
Pan Fish Canada Ltd.	1382	1407822	Robertson Island, Richards Channel
Pan Fish Canada Ltd.	1581	1409321	Hardwicke Is. Site B, Chancellor Channel
Pan Fish Canada Ltd.	1755	6407365	Anger Anchorage, S.of Petrel Channel
Pan Fish Canada Ltd.	1757	6407366	Petrel Point, Petrel Channel
S.K.M. Enterprises Ltd.	871	1405542	Barnes Bay, Sonora Island

Appendix A (Continued):

Licencee	MAFF#	Landfile#	Location
Seven Hills Aquafarm Ltd.	706	1401561	Hardy Bay, Port Hardy
Seven Hills Aquafarm Ltd.	1198	1404089	Varg Island, Raynor Group
Sonora Sea Farm Ltd.	211	1403325	Sonora Island, Okisollo Channel
Stolt Sea Farm Inc.	95	1404264	Mound Island, Indian Channel
Stolt Sea Farm Inc.	140	1403326	Deep Harbour, Broughton Island
Stolt Sea Farm Inc.	141	1403104	Port Elizabeth, Gilford Island
Stolt Sea Farm Inc.	142	1403313	Blunden Passage, Baker Island
Stolt Sea Farm Inc.	143	1408560	Larsen Island, Indian Channel
Stolt Sea Farm Inc.	144	1401722	Koskimo Bay, Quatsino Sound
Stolt Sea Farm Inc.	377	1404309	Bickley Bay, East Thurlow Island
Stolt Sea Farm Inc.	378	1403300	Thurlow Point South, Nodales Channel
Stolt Sea Farm Inc.	388	1403301	Brougham Point, East Thurlow Island
Stolt Sea Farm Inc.	465	1404381	North side Swanson Island
Stolt Sea Farm Inc.	466	1404681	Arrow Passage, Bonwick Island
Stolt Sea Farm Inc.	467	1404380	Spring Passage, Midsummer Island
Stolt Sea Farm Inc.	468	1404780	Mistake Island, Havannah Channel
Stolt Sea Farm Inc.	469	1405897	Havannah Channel, Bockett Pnt/Lily Islet
Stolt Sea Farm Inc.	739	1404379	Upper Retreat Passage
Stolt Sea Farm Inc.	817	1405184	Smith Rock, Tribune Channel
Stolt Sea Farm Inc.	820	1405183	Wicklow Point, Broughton Island
Stolt Sea Farm Inc.	821	1405180	Watson Cove, Tribune Channel (Glacial F)
Stolt Sea Farm Inc.	1031	2402924	Frederick Arm
Stolt Sea Farm Inc.	1059	1403328	Tribune Channel, Sargeaunt Passage

Appendix A (Continued):

Licencee	MAFF#	Landfile#	Location
Stolt Sea Farm Inc.	1145	1406655	Potts Bay, Midsummer Island
Stolt Sea Farm Inc.	1237	1406960	Quatsino Sound near Monday Rocks
Stolt Sea Farm Inc.	1238	1406961	Koskimo Islands, Quatsino Sound
Stolt Sea Farm Inc.	1299	1407385	Thorpe Point, Holberg Inlet
Stolt Sea Farm Inc.	1338	1403748	2km NE of Mahatta River, Quatsino Sound
Stolt Sea Farm Inc.	1586	1408758	Doctor Islets, Knight Inlet
Stolt Sea Farm Inc.	1618	1409707	Humphrey Rock, Tribune Channel
Target Marine Products Ltd.	221	2402095	Sechelt Inlet (Vantage Point)
Target Marine Products Ltd.	332	2402424	Northwest Sechelt Inlet (Salten)
Target Marine Products Ltd.	572	2402738	East Newcomb Point, Salmon Inlet
Target Marine Products Ltd.	1697	2408043	Culloden Point, Jervis Inlet
Tofino Aquafarms Ltd.	776	1405980	Baxter Islet, Dawley Passage
Totem Oysters Ltd.	247	298167	St. Vincent Bay, Jervis Inlet
Yellow Island Aquaculture (1994) Ltd	216	1401748	East of Maud Island, Discovery Passage

Appendix B: List of dominant infaunal macroinvertebrate taxa under (0-22.5 m), near (30-60 m), farther away (7-225 m) from aquaculture sites, or from local reference sites (>300 m away)

Stations 0-22.5 m from operation (n=36 stns)				**Stations 30-60 m from operation (n=46 stns)**			
Taxon	Abund.	% of total abund.	Cum. %		Abund.	% of total abund.	Cum.%
Capitella capitata (1)	59230	48.9%	48.9%	*Capitella capitata* (1)	19519	33.9%	33.9%
Nebalia pugettensis (1)	41311	34.1%	83.0%	*Nebalia pugettensis* (1)	13987	24.3%	58.1%
Schistomeringos annulata or *pseudorubrovittata* (1)	6644	5.5%	88.5%	*Schistomeringos annulata* or *pseudorubrovittata* (1)	7203	12.5%	70.6%
Diopatra ornata (1)	4070	3.4%	91.9%	*Eusirus* sp.(1)	5243	9.1%	79.7%
Eusirus sp.(1)	2579	2.1%	94.0%	*Pseudotanais oculatus* (2)	4388	7.6%	87.3%
Sigambra tentaculata (1)	2140	1.8%	95.8%	*Sigambra tentaculata* (1)	3769	6.5%	93.8%
Brada villosa (1)	2138	1.8%	97.6%	*Platynereis bicanaliculata* (2)	258	0.4%	94.3%
Axinopsida serricata (1)	268	0.2%	97.8%	*Lucina tenuisculpta* (2)	249	0.4%	94.7%
Alvania sp. (1)	172	0.1%	97.9%	*Glycymeris subobsoleta* (2)	208	0.4%	95.1%
Scalibregma inflatum (1)	109	0.1%	98.0%	*Nephtys cornuta* (2)	166	0.3%	95.4%
				Axinopsida serricata (1)	154	0.3%	95.6%
				Alvania sp. (1)	151	0.3%	95.9%
				Jassa falcata (2)	143	0.2%	96.1%
				Metacaprella kennerli (2)	112	0.2%	96.3%
				Pinnixa occidentalis, eburna or *schmittii* (2)	96	0.2%	96.5%
				Acila castrensis (2)	95	0.2%	96.7%
				Lumbrineris luti or *lagunae* (2)	91	0.2%	96.8%
				Cancer magister or gracilis (2)	84	0.1%	97.0%
				Ophiodromus pugetensis (2)	82	0.1%	97.1%
				Alia gaussipauta (2)	80	0.1%	97.3%
				Leitoscoloplos pugettensis or Orbinidae (2)	71	0.1%	97.4%
				larval shrimp (2)	61	0.1%	97.5%
				Lucinoma annulata (2)	60	0.1%	97.6%
				Unidentified bivalves and juveniles (2)	55	0.1%	97.7%
				Lepida longicorrata (2)	47	0.1%	97.8%
				Orchomene obtusa or cf. *pinguis* or *dicipiens* (2)	43	0.1%	97.8%
				Chaetozone setosa (2)	42	0.1%	97.9%
				Armandia brevis (2)	39	0.1%	98.0%

Appendix B (Continued):

Stations 75-225 m from operation (n= 129 stns)	Abund.	% of total abund.	Cum.%	**Stations > 300 m from operation (n= 44 stns)**	Abund.	% of total abund.	Cum.%
Schistomeringos annulata or *pseudorubrovittata* (1)	18988	24.5%	24.5%	*Axinopsida serricata* (1)	2153	14.6%	14.6%
Pseudotanais oculatus (2)	10989	14.2%	38.7%	*Glycymeris subobsoleta* (2)	1019	6.9%	21.5%
Capitella capitata (1)	10255	13.2%	51.9%	*Lumbrineris luti* or *lagunae* (3)	807	5.5%	26.9%
Nebalia pugettensis (1)	4886	6.3%	58.2%	*Cooperilla subdiaphana* (3)	607	4.1%	31.0%
Eusirus sp.(1)	4145	5.3%	63.6%	*Leitoscoloplos pugettensis* or Orbinidae (2)	605	4.1%	35.1%
Sigambra tentaculata (1)	3741	4.8%	68.4%	*Chaetozone spinosa* (3)	568	3.8%	38.9%
Axinopsida serricata (1)	2729	3.5%	71.9%	*Chaetozone setosa* (2)	452	3.1%	42.0%
Glycymeris subobsoleta (2)	2298	3.0%	74.9%	*Acila castrensis* (2)	387	2.6%	44.6%
Lumbrineris luti or *lagunae* (3)	1461	1.9%	76.8%	*Sigambra tentaculata* (1)	366	2.5%	47.1%
Leitoscoloplos pugettensis or Orbinidae (2)	1114	1.4%	78.2%	*Nuculana minuta* or *cellulitaa* (3)	282	1.9%	49.0%
Acila castrensis (2)	1014	1.3%	79.5%	*Euclemene zonalis* (3)	259	1.8%	50.8%
Rhepoxynius cf. variatus (3)	741	1.0%	80.5%	*Schistomeringos annulata* or *pseudorubrovittata* (1)	254	1.7%	52.5%
Pinnixa occidentalis, eburna or *schmittii* (2)	673	0.9%	81.3%	*Alia gaussipauta* (3)	248	1.7%	54.2%
Chaetozone setosa (2)	670	0.9%	82.2%	*Pseudotanais oculatus* (2)	239	1.6%	55.8%
Ophiodromus pugetensis (2)	456	0.6%	82.8%	*Pinnixa occidentalis, eburna* or *schmittii* (2)	209	1.4%	57.2%
Euclemene zonalis (3)	450	0.6%	83.4%	*Cumella vulgaris* or *Lucon* sp. (4)	209	1.4%	58.6%
Peisidice aspera or similar (3)	439	0.6%	83.9%	*Exogone molesta* (3)	170	1.1%	59.7%
Unidentified bivalves and juveniles (2)	424	0.5%	84.5%	*Tachyrhynchus lacteolus* (3)	170	1.1%	60.9%
Alvania sp. (1)	404	0.5%	85.0%	*Unidentified bivalves and juveniles (2)*	168	1.1%	62.0%
Scalibregma inflatum (1)	359	0.5%	85.5%	*Spio cirrifera* (3)	161	1.1%	63.1%
Prionospio cirrifera or *multibranchiata* (3)	348	0.4%	85.9%	*Rhepoxynius cf. variatus* (3)	153	1.0%	64.2%
Chaetozone spinosa (3)	339	0.4%	86.3%	*Prionospio steenstrupi* (3)	152	1.0%	65.2%
Exogone molesta (3)	311	0.4%	86.7%	*Prionospio cirrifera* or *multibranchiata* (3)	146	1.0%	66.2%
Lepida longicorrata (2)	303	0.4%	87.1%	*Peisidice aspera* or similar (3)	143	1.0%	67.1%
Lucinoma annulata (2)	293	0.4%	87.5%	*Heterophoxus oculatus* (3)	142	1.0%	68.1%
Pandora filosa or *bilirata* (3)	290	0.4%	87.9%	*Lucina tenuisculpta* (2)	137	0.9%	69.0%
Lucina tenuisculpta (2)	278	0.4%	88.2%	*Lucinoma annulata* (2)	126	0.9%	69.9%
Terebellides sp. or *Lanassa venusta* (3)	277	0.4%	88.6%	*Dentalium* sp. (3)	126	0.9%	70.7%
Heterophoxus oculatus (3)	269	0.3%	89.0%	*Pandora filosa* or *bilirata* (3)	122	0.8%	71.6%
Platynereis bicanaliculata (2)	263	0.3%	89.3%	*Ischyrocerus* sp. (3)	120	0.8%	72.4%
Orchomene obtusa or cf. *pinguis* or *dicipiens* (2)	263	0.3%	89.6%	*Syllis elongata* (3)	114	0.8%	73.1%
larval shrimp (2)	243	0.3%	89.9%	*Ophiodromus pugetensis* (2)	106	0.7%	73.9%
Harmothoe sp.(3)	242	0.3%	90.3%	*Nephtys ferruginea* (3)	106	0.7%	74.6%

Appendix B (Continued):

Stations 75-225 m from operation (n= 129 stns)				**Stations > 300 m from operation (n= 44 stns)**			
	Abund.	% of total abund.	Cum.%		Abund.	% of total abund.	Cum.%
Mysella tumida (3)	236	0.3%	90.6%	*Cirratulidae* (3)	105	0.7%	75.3%
Tachyrhynchus lacteolus (3)	224	0.3%	90.9%	*Scalibregma inflatum* (1)	103	0.7%	76.0%
Spio cirrifera (3)	224	0.3%	91.1%	*Harmothoe* sp.(3)	95	0.6%	76.6%
Goniada brunnea or *maculata* or *annulata* (3)	222	0.3%	91.4%	*larval shrimp (2)*	92	0.6%	77.2%
Dentalium sp. (3)	218	0.3%	91.7%	*Lepida longicorrata* (2)	82	0.6%	77.8%
Syllis elongata (3)	209	0.3%	92.0%	*Macoma secta* (3)	79	0.5%	78.3%
Ischyrocerus sp. (3)	198	0.3%	92.2%	*Onuphis iridescens* or *elegans* (3)	79	0.5%	78.9%
Alia gaussipauta (3)	193	0.2%	92.5%	*Solariella vancouverensis* (4)	77	0.5%	79.4%
Nephtys cornuta (2)	186	0.2%	92.7%	*Cossura* sp.(4)	76	0.5%	79.9%
Armandia brevis (2)	184	0.2%	93.0%	*Terebellides* sp. or *Lanassa venusta* (4)	75	0.5%	80.4%
Macoma nasuta (3)	182	0.2%	93.2%	*Ampharete* sp. (3)	75	0.5%	80.9%
Ophelina breviata (3)	169	0.2%	93.4%	*Amage anops* (4)	72	0.5%	81.4%
Cooperilla subdiaphana (3)	167	0.2%	93.6%	*Eteone tuberculata* (4)	68	0.5%	81.9%
Cirratulidae (3)	158	0.2%	93.8%	*Eusyllis* sp.(4)	67	0.5%	82.3%
Westwoodilla caecula (3)	155	0.2%	94.0%	*Euclemene reticulata* (3)	66	0.4%	82.8%
Macoma secta (3)	152	0.2%	94.2%	*Mysella tumida* (3)	65	0.4%	83.2%
Nuculana minuta or *cellulitaa* (3)	149	0.2%	94.4%	*Yoldia scissurata* (3)	64	0.4%	83.6%
Prionospio steenstrupi (3)	143	0.2%	94.6%	*Platynereis bicanaliculata* (2)	63	0.4%	84.1%
Nereis procera (3)	141	0.2%	94.8%	*Sternaspis scutata* (4)	62	0.4%	84.5%
Ampharete sp. (3)	138	0.2%	95.0%	*Pectinaria granulata* (3)	61	0.4%	84.9%
Glycera capitata,robusta or *convoluta* (3)	132	0.2%	95.1%	*Laonice cirrata* or *pugettensis* (3)	61	0.4%	85.3%
Polydora (3)	127	0.2%	95.3%	*Tiron biocellata* (4)	59	0.4%	85.7%
Monoculoides sp. (3)	122	0.2%	95.5%	*Terebellides stroemi* (3)	53	0.4%	86.1%
Pectinaria granulata (3)	121	0.2%	95.6%	*Nicomache lumbricalis* (3)	53	0.4%	86.4%
Diplodonta impolita or *orbella* (3)	108	0.1%	95.8%	*Eteone longa* (4)	52	0.4%	86.8%
Yoldia scissurata	104	0.1%	95.9%	*Alvania sp.* (1)	51	0.3%	87.1%
Crab zoea or megalopae (3)	102	0.1%	96.0%	*Macoma inquinata* (4)	50	0.3%	87.5%
Nereis juveniles or *Nereis brandti* (3)	102	0.1%	96.1%	*Glycera capitata,robusta* or *convoluta* (3)	49	0.3%	87.8%
Glycinde picta (3)	101	0.1%	96.3%	*Lumbrineris bicirrata* or *similibris* (3)	48	0.3%	88.1%
Onuphis iridescens or *elegans* (3)	100	0.1%	96.4%	*Sphaerodoropsis biserialis* (4)	48	0.3%	88.4%
Euclemene reticulata (3)	99	0.1%	96.5%	*Monoculoides* sp. (3)	46	0.3%	88.7%
Nephtys ferruginea (3)	95	0.1%	96.7%	*Orchomene obtusa* or cf. *pinguis* or *dicipiens* (2)	44	0.3%	89.0%
Kefersteinia cirrata (3)	93	0.1%	96.8%	*Crab zoea or megalopae (3)*	44	0.3%	89.3%

Appendix B (Continued):

Stations 75-225 m from operation (n= 129 stns)				**Stations > 300 m from operation (n= 44 stns)**			
	Abund.	% of total abund.	Cum.%		Abund.	% of total abund.	Cum.%
Lumbrineris bicirrata or *similibris* (3)	91	0.1%	96.9%	*Eusirus* sp.(1)	43	0.3%	89.6%
Phyllodoce sp.(3)	87	0.1%	97.0%	*Westwoodilla caecula* (3)	42	0.3%	89.9%
Megaluropsus sp.(3)	85	0.1%	97.1%	*Goniada brunnea* or *maculata* or *annulata* (3)	41	0.3%	90.2%
Axiothella rubrinocincta (3)	83	0.1%	97.2%	*Macoma nasuta* (3)	40	0.3%	90.5%
Maldanidae or Notoproctus pacificus (3)	81	0.1%	97.3%	*Glycera* sp. or *Glycera americana* (4)	40	0.3%	90.7%
Eunoe depressa (3)	80	0.1%	97.4%	*Diplodonta impolita* or *orbella* (3)	39	0.3%	91.0%
Praxillella affinis or *P.* (3)	79	0.1%	97.5%	*Nitidiscala cf. tincta* (4)	39	0.3%	91.3%
Nephtys longosetosa or *punctata* (3)	78	0.1%	97.6%	*Maldanidae or Notoproctus pacificus* (3)	38	0.3%	91.5%
Pinnixa tubicola (3)	76	0.1%	97.7%	*Ophelina breviata* (3)	36	0.2%	91.8%
Laonice cirrata or *pugettensis* (3)	74	0.1%	97.8%	*Unidentified amphipods*	36	0.2%	92.0%
Terebellides stroemi (3)	73	0.1%	97.9%	*Nephtys cornuta* (4)	35	0.2%	92.2%
Nicomache lumbricalis (3)	71	0.1%	98.0%	*Polydora* sp.(4)	35	0.2%	92.5%
				Syllis juveniles (*3)*	35	0.2%	92.7%
				Eunoe depressa (3)	34	0.2%	92.9%
				Nebalia pugettensis (1)	33	0.2%	93.2%
				Nereis juveniles or *Nereis brandti* (3)	32	0.2%	93.4%
				Lumbrineris sp. (3)	31	0.2%	93.6%
				Decamastus gracilis or *Heteromastus fillobranchus* (4)	29	0.2%	93.8%
				Diopatra ornata (4)	29	0.2%	94.0%
				Byblis millsi (4)	29	0.2%	94.2%
				Parandalia fauveli (4)	28	0.2%	94.4%
				Nephtys longosetosa or *punctata* (3)	27	0.2%	94.6%
				Cylichna sp. or *Crepidula* sp.(4)	27	0.2%	94.7%
				Thyasira gouldi or *Thracia trapezoides* (4)	27	0.2%	94.9%
				Capitella capitata (1)	26	0.2%	95.1%
				Cirratulus cirratulus (4)	26	0.2%	95.3%
				Axiothella rubrinocincta (4)	25	0.2%	95.4%
				Spionidae (4)	25	0.2%	95.6%
				Maera simile (4)	24	0.2%	95.8%
				Crenella decussata (4)	24	0.2%	95.9%
				Lumbrineris zonata	23	0.2%	96.1%
				Ostracoda (4)	23	0.2%	96.2%
				Nereis procera(3)	22	0.1%	96.4%

Appendix B (Continued):

Stations 75-225 m from operation (n= 129 stns)	Abund.	% of total abund.	Cum.%	Stations > 300 m from operation (n= 44 stns)	Abund.	% of total abund.	Cum.%
				Odostomia tennuisculpta (4)	22	0.1%	96.5%
				Syllis spongiphila (4)	22	0.1%	96.7%
				Oregonia gracilis (4)	21	0.1%	96.8%
				Kefersteinia cirrata (3)	20	0.1%	97.0%
				Megaluropsus sp.(3)	20	0.1%	97.1%
				Glycinde picta (3)	19	0.1%	97.2%
				Phyllodoce sp. (3)	18	0.1%	97.4%
				Ampelisca sp. (4)	18	0.1%	97.5%
				Phaline bakeri or *Cephalaspidea* (4)	17	0.1%	97.6%
				Lyonsia californica or *pugettensis* (4)	17	0.1%	97.7%
				Armandia brevis (2)	16	0.1%	97.8%