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An updated review of hazards associated with the use of pesticides and drugs used in the marine environment by the finfish aquaculture industry in Canada

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

The purpose of this CSAS document is to update the current knowledge relating to the exposure and potential biological effects (hazards) of pesticide and drugs used in the marine environment on non-target organisms during finfish aquaculture activities. This document will provide peer-reviewed science advice to DFO's Aquaculture Management Directorate. In this paper we reviewed the available literature on biological effects of two pesticide formulations currently in use in Canada: Salmosan® (active ingredient: azamethiphos) and Paramove 50® (active ingredient: hydrogen peroxide) In general, new peer-reviewed published data (since 2013) are relatively rare although there are a number of relevant documents in the grey literature. In 2013 we concluded that the degree of toxicity was pesticide specific with Paramove 50® being the least toxic of these formulations. Recent publications show, however, that sublethal responses to Paramove 50® may occur in shrimp and mysids at low concentrations. Anti-parasitic drugs were not part of the 2013 CSAS review process. Available data are reviewed dealing with lethal and sublethal responses (moulting, growth and behaviour) of non-targets to emamectin benzoate (EB) and ivermectin. Two new compounds, selamectin and lufenuron are mentioned but next to no data are published regarding these compounds and aquaculture use. Finally, a section on antibiotics is presented focusing on biological effects. The concentration of these compounds required to affect non-targets is often greater than the prescribed treatment levels. The key concern with antibiotic use in aquaculture is the potential for antibiotic resistance to develop both in the target species, fish and in the microbial populations in the marine environment. This topic will be covered in a separate CSAS research document. There remain inconsistencies with respect to biological effects research and anti-parasitic compounds. Some authors use technical grade chemicals, others use anti-sea louse formulations. Authors, surprisingly, still are able to publish effects data using nominal concentrations with no analytical chemistry conformation of concentrations. While there are recommended treatment doses of therapeutic drugs (anti-parasitic and antibiotic), there are no labels prescribing use as there are for pesticides. The experience and expertise of prescribing veterinarians plays a major role in how fish are treated, and courses of treatment can vary considerably.

INTRODUCTION

As is the case in of most forms of farming, fish are affected by diseases and infestations of parasites which must be treated. Drugs are used to treat bacterial infections as well as infestations of parasites on farmed fish. Pesticides are applied as anti-parasitics. The consequences of untreated disease and parasite infestations relate not only to the loss of product, but serious fish welfare issues.

A number of reviews discussing the use of these compounds and the potential for them to have negative impacts in the environment are large. The work by Burridge et al. (2010) covered all types of chemical inputs from aquaculture activities. Burridge and Van Geest (2014) reviewed the use of anti-sea louse pesticides in the Canadian context. These documents and others describe sea lice biology and the types of bacterial infections that are common in salmon aquaculture. Readers are encouraged to see these papers for details.

Recently, Bentley et al. (2019a, 2019b, in press) prepared a thorough review of biological effects of pesticides and drugs used to treat salmon against sea lice infestations. Their paper covers several of the topics covered herein.

Our purpose in preparing this paper was to review the literature focusing on biological effects research conducted since Burridge and Van Geest published their CSAS document in 2014. In addition to updating the state of knowledge on aquaculture pesticides, this paper includes a review of the in-feed aquaculture drugs and some information on in-feed aquaculture antibiotics used for cultured fish in the marine environment. This paper does not cover hazards associated with chemicals used as disinfectants, antifoulants or anaesthetics.

Tables 1 and 2 show the products applied to marine finfish aquaculture sites in 2018 to treat bacterial diseases and infestations of parasites (Department of Fisheries and Oceans 2019).

Table 1. Antiparasitic Drugs and Pesticides registered for use or commonly used in marine finfish aquaculture in Canada and the frequency of use and quantity of product used in 2018. From Department of Fisheries and Oceans 2019.

Active Ingredient	Purpose	Mode of action	Dose/Concentration*	Frequency of use (Canada 2018)	Quantity (Kg of active)
Ivermectin	Anti-parasitic Drug	Affects chloride channels in nerves	In-feed treatment 50 µg·Kg^{-1} BW/day x 2 weekly	41 (NB, NL)	3.35
Emamectin Benzoate	Anti-parasitic Drug	Affects chloride channels in nerves	In-feed treatment 50 µg·Kg^{-1} BW/day x 7 days	120 (BC, NB, NL)	73.98
Lufenuron	Anti-parasitic Drug	Chitin synthesis inhibitor	In-feed treatment 5 mg·Kg^{-1} BW/day until 35 mg·Kg^{-1} BW is achieved. Minimum 7days	NA	NA

Active Ingredient	Purpose	Mode of action	Dose/Concentration*	Frequency of use (Canada 2018)	Quantity (Kg of active)
Praziquantel**	Anti-parasitic Drug	Affects calcium metabolism	In-feed treatment 75 mg·Kg^{-1} BW X 6 days (in freshwater)	0	0
Azamethiphos	Anti-parasitic Pesticide	Acetylcholinesterase inhibitor	Bath treatment 100 µg·L^{-1}	69 (NB, NL)	502.13
Hydrogen Peroxide	Anti-parasitic Pesticide	Mechanical paralysis, peroxidation of lipid membranes, inactivation of enzymes	Bath treatment 1200-1800 mg·L^{-1}	65 (BC, NB, NL)	418747.08

*Application of drugs and pesticides is under control of veterinarians who prescribe doses according to local conditions and their expertise. As such the doses listed may not be reflective of those used in the field.

**This product was prescribed in NL in 2017 and used twice during that year. We were unable to determine the dose prescribed for these seawater treatments

BW = body weight; NB = New Brunswick; NS = Nova Scotia); NL = Newfoundland and Labrador; BC = British Columbia; NA = data not available

Table 2. Antibiotic Drugs registered for use or commonly used in marine finfish aquaculture in Canada and the frequency of use and quantity of product used in 2018. From Department of Fisheries and Oceans 2019.

Active Ingredient	Purpose	Mode of action	Dose/Concentration*	Frequency of use (Canada 2018)	Quantity (Kg of active)
Oxytetracycline	Antibiotic	Inhibits protein synthesis	In-feed treatment 75 mg·Kg^{-1} BW/day x 10 days	25 (BC, NB, NS)	11097.09
Florfenicol	Antibiotic	Inhibits protein synthesis	In-feed treatment 10 mg·Kg^{-1} BW/day x 10 days	118 (BC, NB, NS, NL)	4120.58
Erythromycin	Antibiotic	Inhibits genetic translation	In-feed treatment 50 – 100 mg·Kg$^{-1\ -1}$ BW/day x 21 days	1 (BC)	0.81

Active Ingredient	Purpose	Mode of action	Dose/Concentration*	Frequency of use (Canada 2018)	Quantity (Kg of active)
Ormetoprim	Antibiotic	Inhibits folic acid metabolism	In-feed treatment 50 mg·Kg^{-1} BW/day x 7-10 days	2 (BC)	0.13
Trimethoprim	Antibiotic	Inhibits folic acid metabolism	In-feed treatment 30 mg·Kg^{-1} BW/day x 7-10 days	1 (NB)	28.29

*Application of drugs and pesticides is under control of veterinarians who prescribe doses according to local conditions and their expertise. As such the doses listed may not be reflective of those used in the field.

BW = body weight; NB = New Brunswick; NS = Nova Scotia; NL = Newfoundland and Labrador; BC = British Columbia

ANTIPARASITIC DRUGS

Tables 3 and 4 shows threshold values for antiparasitics and aquatic species. Table 3 has data for toxicity following exposure in water and Table 4 has toxicity data following exposure in food or in sediment. While the focus of this paper is presenting data published, or available since 2013 older data are presented in these tables as well.

PRAZIQUANTEL

Praziquantel (2-cyclohexylcarbonyl-1,2,3,6,7, 11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one) is a synthetic heterocyclic broad-spectrum anthelminthic agent effective against parasitic schistosome species as well as most other trematodes and adult cestodes (Alsaqabi and Lofty 2014). In fish it is usually used to treat against infestations of cestodes (Iles et al. 2012). Forwood et al. (2013) report a recommended dosage for freshwater fish of 75 mg·Kg^{-1} BW for six days but also mention that palatability problems reduced efficacy.

Praziquantel is soluble in water (400 mg·L^{-1}) and has a Log K_{ow} of 2.5 (DrugBank 2021). It is internationally accepted that log $K_{ow} \geq 3$ indicates a potential to bioaccumulate and the Canadian Environmental Protection Act (CEPA) recognizes log $K_{ow} \geq 5$ as indicative of potential to persist in the environment (Beek 2000). An *ad hoc* committee of the Canadian Government has recommended that a product be deemed persistent if its half-life in air is equal to or greater than 2 days, its half-life in water is equal to or greater than 6 months or its half-life in sediment is equal to or greater than 1 year. The exact mode of action of praziquantel is unknown but it is thought to affect calcium metabolism. Tapeworms appear to lose their ability to resist digestion by the host and consequently are destroyed. The drug is ineffective against the parasite's eggs (Iles et al. 2012). It is rapidly absorbed and metabolized in mammals and fish (70-80%) and quickly excreted (Tubbs et al. 2008, Alsaqabi and Lofty 2014). Thomas et al. (2016) added praziquantel to seawater under various conditions and showed a rapid degradation of the compound, except under sterile conditions.

Frohberg (1984) reported on mammalian toxicity of praziquantel and concluded it had low acute and chronic toxicity and is a safe compound for use. The author was unable to find any

published data on non-mammalian toxicity. The rapid metabolism of the product within the vertebrate system, its apparent lack of persistence and the fact that the parent compound is rapidly degraded in seawater suggest that the product is not hazardous. However, there are limited data available regarding biological effects on non-target organisms and therefore it cannot be concluded that use of this drug is risk free. No product was prescribed in 2018, however, several treatments were conducted in Newfoundland and Labrador in 2017 (DFO, 2019). This author has been unable to determine the dosage used in these applications. However, application of drugs and pesticides is under control of veterinarians who prescribe doses according to local conditions and their expertise.

AVERMECTINS

The avermectins are effective in the control of internal and external parasites in a wide range of host species, particularly mammals (Campbell 1989). In invertebrates, they generally open glutamate-gated chloride channels at inhibitory synapses resulting in an increase in chloride concentrations, hyperpolarization of muscle and nerve tissue, and inhibition of neural transmission (Roy et al. 2000, Grant 2002). Avermectins can also increase the release of the inhibitory neurotransmitter γ-amino-butyric acid (GABA) in mammals.

Two avermectins are used in Canada in marine finfish aquaculture. Ivermectin is used in an "extra-label" manner as an anti-parasitic under a veterinary prescription. Extra-label drug use, also referred to as "off-label use", refers to the use of a drug approved by Health Canada in a manner that is not in accordance with the label or package insert. Emamectin Benzoate (Slice®) is fully registered for use in Canada.

Two other avermectins, selamectin and abamectin have been mentioned as active ingredients in drugs that are, or may be, used as anti-sea louse compounds.

The antiparasitic drugs are mixed with feed and delivered to affected fish via the medicated feed. Therefore, the drug may reach the aquatic environment either through uneaten feed pellets or in excreta from treated fish (*c.f.* Samuelson et al. 1992, Kim-Kang et al. 2004).

IVERMECTIN

A typical treatment with ivermectin ranges from 50 $\mu g \cdot Kg^{-1}$ of fish biomass twice weekly within a one-week interval to 200 $\mu g \cdot Kg^{-1}$ of fish biomass every two weeks from June to November (Davies and Rodger 2000). DFO's latest use statistics show that ivermectin was applied 41 times in 2018 with a total of 3.35 Kg of active ingredient used (Department of Fisheries and Oceans 2019).

Ivermectin has a low solubility in water (4 $mg \cdot L^{-1}$) and a strong affinity to lipid (log K_{ow} = 3.2-4.1), soil, and organic matter (Tomlin 1997, Pub Chem 2018). It is readily photo-degraded, but the half-life for hydrolysis in the dark is quite long (Hoy et al. 1992). Within the marine environment, ivermectin is expected to be associated with sediments and particles and to show low mobility. The half-life of ivermectin in sediment is at least three months (Davies et al. 1998). The calculated bioconcentration factor of ivermectin is 74 for fish and 750 for mussels (Carvajal et al. 2000). A "withdrawal period" is recommended prior to harvesting salmon for the elimination of ivermectin from edible tissue. Consequently, ivermectin is routinely used only to treat fish during their first year in sea pens (Whyte et al. 2019).

Biological Effects of Ivermectin

As ivermectin has been used to treat infestations of sea lice in Canada for over 20 years a body of literature exists for LC_{50}s and LD_{50}s for ivermectin to fish and marine invertebrates.

Over a 27-day period, there was a cumulative mortality of 10% and 80% of the Atlantic salmon (wt = 800 g) exposed to 0.05 and 0.2 $mg \cdot Kg^{-1}$ ivermectin in food, respectively (Johnson et al. 1993). Atlantic salmon was the most sensitive of several salmonid species tested and behavioural changes, such as cessation of feeding and lethargy, were observed in fish exposed to lower concentrations. The 96-h LD_{50} was 0.5 $mg \cdot Kg^{-1}$ for Atlantic salmon administered ivermectin by intubation and the 96-h LC_{50} was 17 $\mu g \cdot L^{-1}$ when the salmon were immersed in a sea water solution of ivermectin (SEPA 1999).

Sand shrimp (*Crangon septemspinosa*) were exposed to fish feed treated with various concentrations of ivermectin for 96 h in running seawater (Burridge and Haya 1993). When the food was accessible to the shrimp, mortality occurred. When the feed was present in the water but not accessible by the shrimp, no mortality occurred, suggesting that the feed must be ingested by the shrimp before lethality occurs. The nominal 96-h LC_{50} was 8.5 $mg \cdot Kg^{-1}$ (CI = 6.2-10.8) food and the No Observed Effect Concentration (NOEC) was 2.6 $mg \cdot Kg^{-1}$ food.

The 10-day LC_{50} for ivermectin in sediment to the marine amphipod, *Corophium volutator* was estimated to be 180 (95% CI = 130-240) $\mu g \cdot Kg^{-1}$ dry weight (Davies et al. 1998). The 10-day LC_{50} to the lugworm, *Arenicola marina* was 23 (95% CI = 18-27) $\mu g \cdot Kg^{-1}$ (Thain et al. 1997). The 10-day LC_{50} for the starfish, *Asterias rubens* was 23600 (95% CI = 20300-27300) $\mu g \cdot Kg^{-1}$ (Davies et al. 1998).

Black et al. (1997) spiked sediment cores with ivermectin and recorded the effects on the annelid worm, capitella which are commonly found under active cage sites. The cores were incubated for three weeks and ivermectin was only toxic at concentrations between 8.1 and 81 $\mu g \cdot m^{-2}$. The authors suggest that, while these concentrations are unlikely to be found in undercage environments after a single treatment, as ivermectin may remain in sediments for long periods, there may be a risk to annelids over time (Black et al. 1997).

Daoud et al. (2018) exposed stage IV American lobster (*Homarus americanus*) to ivermectin in sediment and estimated a 10-day LC_{50} of 212.4 (±202.6) $\mu g \cdot Kg^{-1}$ wet weight.

Two NOECs are available for use in predictive models, one for concentration on the food and one for a concentration in sediment. If we assume a pre-market fish (2 Kg) is fed at a rate of 1.5% BW per day the concentration of ivermectin on food should be 6 $mg \cdot Kg^{-1}$ or slightly over two times the NOEC for sand shrimp (2.6 $mg \cdot Kg^{-1}$). It is more difficult to predict a concentration of ivermectin in undercage sediments. However, the NOEC for the amphipod is 50 $\mu g \cdot Kg^{-1}$ (dry weight sediment) and the food could be carrying 6000 $\mu g \cdot Kg^{-1}$.

EMAMECTIN BENZOATE (EB)

The optimum therapeutic dose for EB is 0.05 $mg \cdot Kg^{-1}$ $fish \cdot day^{-1}$ for seven consecutive days (Stone et al. 1999), which has been shown to be effective in removing sea lice of all developmental stages (Stone et al. 2000a, Stone et al. 2000b). This is also the dosage and administration approved for the product in Canada. EB was approved for use in Canada in 2010. DFO's latest use statistics show that EB was applied 120 times in 2018 with a total of 73.98 Kg of active ingredient used (Department of Fisheries and Oceans 2019).

EB also has low water solubility (5.5 $mg \cdot L^{-1}$) and high octanol-water partition coefficient (log K_{ow} = 5), indicating that it has the potential to be absorbed to particulate material and surfaces and that it will be tightly bound to marine sediments with little or no mobility (SEPA 1999). The half-life of EB is 193.4 days in aerobic soil and 427 days in anaerobic soil (SEPA 1999). In sediments, EB is persistent and a study by Benskin et al. (2016) suggests a minimum half-life of 404 days for this chemical.

In field trials, EB was not detected in water samples and only 4 of 59 sediment samples collected using a Van Veen sampler near a treated cage had detectable levels. Recently, however, SEPA (2018) reported the results of a monitoring program on 17 salmon farms in Scotland. Using chemical analytical techniques with much improved detection limits they looked at EB levels in sediments collected by Van Veen sampler at near field (0-100 m) and far field (>100 m but no more than 500 m). Results show that 3 of 17 sites have EB levels that exceed the current environmental quality standard (EQS) for near field concentrations (7.63 $\mu g \cdot Kg^{-1}$ WW). These samples were all taken at the cage edge. Two sites had at least one sample that failed the far field EQS (0.763 $\mu g \cdot Kg^{-1}$ WW) as established by SEPA for aquaculture operations in Scotland. Similarly, Langford et al. (2014) reported concentrations of EB in the upper 2 cm of sediments near cage sites in Norway that exceeded the far field EQS of 0.763 $\mu g \cdot Kg^{-1}$ WW but commented that the use of EB was greatly reduced due to loss of efficacy. Benskin et al. (2016) report the highest concentration of EB measured in sediments collected four months post-treatment was at 10 m from the cage.

In Canada, EB was not detected in sediment samples collected by grab sampler near an aquaculture site for the 10 weeks immediately after treatment with SLICE® (Parker and Mallory, 2003). Mussels were deployed and traps were set out to capture invertebrates near aquaculture sites undergoing treatment. While detectable levels of EB and metabolites were measured in mussels (9 of 18 sites) one week after treatment, no positive results were observed after 4 months (Scottish Environmental Protection Agency (SEPA) 1999). EB was found in crustaceans during and immediately after treatment. Species showing detectable levels for several months after treatment are scavengers which are likely to consume faecal material and waste food (SEPA 1999). Telfer et al. (2006) reported concentrations of EB in sediment near a site treated with EB. They sampled sediments immediately after treatment using traps and grab samplers and reported that the concentration of EB was 366 $\mu g \cdot Kg^{-1}$ dry weight (DW) of sediment. More recently Tucca et al. (2016) have reported concentrations of EB in sediment collected 0-100 m from cage site ranging from 5.29 – 9.97 $\mu g \cdot Kg^{-1}$ DW. Unfortunately, these authors do not report timing of sampling relative to timing of treatment. Ikonomou (2011) reported measurable quantities of EB in sediments collected using a Van Veen sampler directly under an aquaculture site in British Columbia, Canada. This work showed that EB can remain in sediments at detectable levels for >1.5 years. The maximum concentration measured was 35 $\mu g \cdot Kg^{-1}$ on a wet weight basis. He also reported that EB could be detected and measured in muscle tissue from spot prawn captured near an aquaculture site. Levels were from 0 to 3.1 $\mu g \cdot Kg^{-1}$ during sampling up to 100 days post-EB treatment.

Stomperudhaugen et al. (2014) showed that increased organic material resulted in an increase of the release of EB from contaminated sediments. In addition, the presence of a bioturbative invertebrate also increased the release of EB from sediments. The authors hypothesize that these processes could result in EB being redistributed over a larger area with time and consequently be subject to “dilution” in the environment. hey did not comment or speculate whether the wider distribution of a lower concentration of EB would have any consequence with respect to biological effects.

Biological Effects of Emamectin Benzoate

The treatment concentrations in salmon feed range from 1 to 25 $\mu g \cdot Kg^{-1}$ (Roy et al. 2000). Feeding EB to Atlantic salmon and Rainbow trout at up to ten times the recommended treatment dose resulted in no mortality. However, signs of toxicity, lethargy, dark colouration and lack of appetite were observed at the highest treatment concentration (Roy et al. 2000).

The effects of EB-treated fish feed on non-target organisms has been reported by a number of authors (van Aggelen et al. 2002, Waddy et al. 2002, Willis and Ling 2003, Burridge et al. 2004).

The compound is not lethal to organisms tested to date at recommended treatment concentrations. Waddy et al. (2002) reported that ingestion of EB induced premature moulting of adult American lobsters. This moulting response of lobsters may involve an inter-relationship of a number of environmental (water temperature), physiological (moult and reproductive status) and chemical (concentration/dose) factors (Waddy et al. 2002). Further studies of this response suggest that the risk may be limited to a small number of individuals and that widespread population effects are unlikely (Waddy et al. 2007).

As EB is expected to become bound to sediment, several authors have reported effects of sediment-borne EB on aquatic invertebrates. Kuo et al. (2010) reported the 10-day LC_{50} for EB in sediment and the amphipod *Eohaustorius estuarius* to be 146 $\mu g \cdot Kg^{-1}$ DW (95% CI = 134-157. Tucca et al. (2014) also exposed an amphipod, *Monocorophium insidiosum,* to EB in sediment and reported a 10-day LC_{50} of 890 $\mu g \cdot Kg^{-1}$ DW (95% CI = 672-1171).

Environment and Climate Change Canada's (ECCC) Atlantic lab conducted effects studies using sediment spike with florfenicol and four marine species, the amphipod, *Eohaustorius estuaris*, the polychaete, *Polydura cornuta*, embryos of the sea urchin, *Lytechinus pictus*, and the bacterium, *Vibrio fischeri*. Exposures and determination of mortality and sublethal effects thresholds were determined according to standard (ECCC) protocols. Lethal thresholds were estimated for EB and two of the test organisms. The 10-day LC_{50} for *E. estuarus* was estimated to be 82.1 $\mu g \cdot Kg^{-1}$ (95% CI = 73.9-91.4). A 14-day LC_{50} for *P. cornuta* was estimated to be 207 $\mu g \cdot Kg^{-1}$ (95% CI = 164-258). EB did not affect growth of these worms in this study. EB had no effect on sea urchin embryos or the bacterium, *Vibrio fischeri* when exposed to a maximum concentration of 10 $mg \cdot Kg^{-1}$ (ECCC unpublished results). Sediment concentrations are reported on a wet weight basis.

McBriarty et al. (2017) exposed *N. virens* to EB in sediment at a nominal target concentration of 366 $\mu g \cdot Kg^{-1}$ DW, a concentration previously reported to have been found immediately after EB treatment near an operational cage site (Telfer et al 2006). The measured EB concentration during the experiment was approximately 45% of this value (165.6 $\mu g \cdot Kg^{-1}$). Exposure was for 30 days and the worms showed no treatment-related mortality; however, treated worms had lower specific growth rates than the control worms and also exhibited behavioural changes such as reduced burrowing compared to controls. Daoud et al. (2018) exposed stage IV American lobster (*Homarus americanus*) to EB in sediment and estimated a 10-day LC_{50} of 250.2 95% CI = 159.8-340.6) $\mu g \cdot Kg^{-1}$ wet weight. These authors suggest a moisture content of 20-30% thus producing an estimate on a dry weight basis of between 300 and 350 $\mu g \cdot Kg^{-1}$. These authors also report sublethal effects of chronic exposure, including delayed moulting and growth. These effects were noted at exposure concentrations greater than 34.0 $\mu g \cdot Kg^{-1}$. Their noted effect on moulting is interesting in that it seems to be opposite to the result reported by Waddy et al. (2002). The estimates of thresholds for these effects have wide confidence intervals which means no statistically significant differences were observed.

Veldhoen et al. (2012) exposed the spot prawn, a commercially important species in British Columbia to EB and observed some mortality over an eight-day exposure period. The deaths occurred at lower exposure concentrations and no thresholds were determined. The authors also report effects on gene expression in spot prawns exposed to EB at concentrations ranging from 0.1 to 4.8 $mg \cdot Kg^{-1}$ wet weight. However, they warn that the results are variable and this endpoint may not be suitable for risk assessment. Tucca et al. (2014) also report biochemical changes (induction of glutathione S transferase activity and lipid peroxidation) in an amphipod exposed to EB in sediment.

van Aggelen 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 nominal concentrations of 0, 1, 10, 100 and 500 $mg \cdot Kg^{-1}$ 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 (Bright and Dionne 2005).

Overuse or over-reliance on any single compound can lead to the development of resistance to the compound in the parasite. Whyte et al. (2019) report that double and triple dosing of EB and ivermectin occurs in New Brunswick, Canada and these treatments marginally improve efficacy of the drugs and reduce stress in sea louse-infested salmon. They also note that molecular and biochemical endpoints measured in sea lice after exposure to either of these drugs show that they may share a mode of action for resistance development and suggest further research in this area of study. These data are of environmental interest for (at least) two reasons: If ivermectin and emamectin must be applied at high concentrations (doses), it would seem that some level of resistance already exists. Whyte et al. (2019) acknowledge this. Double and triple dosing of fish to achieve acceptable efficacy completely changes any risk assessment of use of these drugs. The suggestion by Whyte et al. (2019) that sea lice are similarly resistant to ivermectin and EB on Canada's east coast further would raise concerns that use of other avermectins may be of limited value in treating against infestations of sea lice.

In the past, Canada limited the number of sea lice treatments with EB during a grow-out cycle to three (Burridge and Van Geest 2014). The authors have been unable to verify if this is still the case, although no treatment with EB is recommended within 60 days of harvesting market fish (Canadian Food Inspection Agency (CFIA) 2019). Up to five treatments may take place during the grow out cycle in Norway and the UK, and in Chile between four and eight treatments may take place.

Where thresholds have been estimated they are consistently higher than either the recommended concentration in medicated feed to reach the target dose to fish or the concentration of EB measured in sediments near operational cage sites.

SELAMECTIN

There is a wealth of information on the avermectin, selamectin, in relation to its use as a veterinary anti-parasitic drug. The only reference the authors could find with respect to selamectin and sea lice is the application for a US patent to use this active to combat sea lice infestations.

ABAMECTIN

Similarly, no published reports could be found regarding the use of the avermectin, abamectin, to treat fish against infestations of sea lice.

Neither abamectin nor selamectin are listed on DFO's list of substances specifically authorized for sale in Canada for use in aquaculture (DFO 2018).

LUFENURON

Lufenuron is a member of the benzoyl-phenyl-urea class of compounds and acts as a chitin synthesis inhibitor; it is classified as a growth regulator for animals with a chitin exoskeleton. As such it will not affect adult sea lice which no longer moult but should prevent sea lice from getting to the adult stage. Lufenuron also has low water solubility ($0.046\ mg \cdot L^{-1}$) and high octanol-water partition coefficient ($\log K_{ow} = 5.12$), indicating that it has the potential to be absorbed to particulate material and surfaces and that it will be tightly bound to marine

sediments with little or no mobility, i.e., it has the potential to persist and bioaccumulate (FDA 2016). Similarly, the product should be tightly bound within the fatty compartments of the salmon. The Chilean label, reported by the FDA, states that a withdrawal period of 2050 degrees days must be adhered to. At an average water temperature of 10°C this would mean 205 days withdrawal (FDA 2016).

The product is to be used only in freshwater (hatchery) prior to sea water introduction. The prescribed treatment is 5 mg (lufenuron)·Kg^{-1} BW until 35 mg·Kg^{-1} BW have been delivered. Treatment must last at least seven days and may be extended up to 14 days to ensure that the 35 mg·Kg^{-1} BW has been delivered. Poley et al. (2018) have shown the product to be highly efficacious (~90%) in controlling infestations and report that the product is the first new anti-sea louse product available for over 20 years.

A product containing lufenuron (IMVIXA) is approved for use in Chile to prevent sea lice infestations in salmon (FDA, 2016); however, it is not approved for sale in Canada. DFO lists this product as available for use in hatcheries under the Emergency Drug Release Program (DFO 2018). Limited IMVIXA use in Canada has occurred in BC when, according to a group called Clayoquot Action (2019), Health Canada granted an authorization for its use as an Emergency Drug Release (EDR). A newspaper published in British Columbia reported that lufenuron was used to combat sea lice at several sites on Vancouver Island in 2019 (The Tyee 2019). According to these articles the product was used at seawater sites in Clayoquot Sound, BC. The product label for Chile states that the product is only to be used in freshwater facilities with active effluent system treatment that allow the retention of suspended solids, according to valid regulatory requirements (FDA 2016).

There are very few publications regarding use of lufenuron in the aquatic environment. Soares et al. (2016) reported that lufenuron was acutely lethal to the freshwater fish, *Colossoma macropomum*. The fish were exposed to lufenuron in water and estimates of the 24- and 96-h LC_{50} was reported to be ~0.6 mg·L^{-1} (95% CI = 0.41-0.81). Brock et al. (2016) and Brock et al. (2018) performed sediment-spiking experiments with freshwater benthic invertebrates. They found that lufenuron was found in sediments but not in pore water and that effects were related to location and bio-turbative activity. Mayfly larvae were most sensitive with a 10-day NOEC of 0.97 µg (a.i) g^{-1} organic carbon. The amphipod, *Gammarus pulex*, had a 10-day NOEC of 3.32 µg (a.i)· g^{-1} organic carbon and the crustacean, *Asellus aquaticus*, had a NOEC of 31.7 µg (a.i)· g^{-1} organic carbon. No mention is made of moulting in these studies. When the same authors look at effects over a 28-day period The NOEC values change in relative order: Mayfly larvae 4.92 µg (a.i)·g^{-1} organic carbon, the crustacean 1.95 µg (a.i)·g^{-1} organic carbon and the amphipod 0.50 µg (a.i)·g^{-1} organic carbon. The most sensitive animal was the midge larvae, *Chironomus riparius* with a NOEC of 0.15 µg (a.i)·g^{-1} organic carbon (Brock et al. 2018).

Table 3. Thresholds of toxicity for anti-parasitic compounds and aquatic species determined after water exposures.

Species	**Endpoint**	**Result**	**EB (µg·L^{-1}) (95% CI)**	**HP (mg·L^{-1}) (95% CI)**	**AZ (µg·L^{-1}) (95% CI)**	**Reference**
Echinoderm (*Strongylocentrous purpuratus*)	Fertilization	IC_{25} (20 min)	2.1 (1.4-2.5)	2.8 (2.7-3.0)	>12.5	Strachan and Kennedy 2021*
Kelp (*Macrocystis pyrifera*)	Germination	EC_{50} (48 h)	>5	4.5 (4.1-4.8)	>12.5	Strachan and Kennedy 2021*
	Growth	IC_{50} (48 h)	> 5	3.7 (3.2-4.2)	>12.5	Strachan and Kennedy 2021*
Topsmelt (*Atherinops affinis*)	Survival	LC_{50} (96 h)	0.35 (0.29-0.42)	172 (140-211)	0.98 (0.8-1.2)	Strachan and Kennedy 2021*
Mysid (*Mysidopsis bahia*)	Survival	LC_{50} (96 h)	0.617 (0.48-0.78)	7.7 (5.8-10.1)	1.218 (1.08-1.37)	Strachan and Kennedy 2021*
Indigenous (NB) copepods	Feeding	EC_{50} (6 h)	-	4.2 (3.4-5.2)	>500	Van Geest et al. 2014****
Indigenous (NB) copepods	Survival	LC_{50} (6 h)	-	68 (58-82)	-	Van Geest et al. 2014
Bivalve (*Mytilus galloprovincialis*)	Proportion Normal	EC_{50} (48 h)	1.03 (1.01-1.05)	2.02 (1.97-2.06)	6.01 (5.80-6.22)	Strachan and Kennedy 2021*
	Survival	LC_{50} (48 h)	1.605 (1.58-1.63)	2.9 (2.8-2.92)	>12.5	Strachan and Kennedy 2021*
Crab (*Metacarcinus edwardsii*)	Survival	LC_{50} (24 h)***	-	-	2.84 (2.45-3.23)	Gebauer et al. 2017****
Shrimp (*Pandalus borealis*)	Survival	LC_{50} (24 h)	-	-	>0.1	Bechmann et al. 2019
Shrimp (*Pandalus borealis*)	Feeding/ Swimming	EC_{50} (24 h)	-	-	>0.1	Bechmann et al. 2019
Lobster (*Homarus gammarus*) Stage I	Survival	LC_{50} (1 h)***	-	-	43.1 (22.2-81.5)	Parsons et al. 2020****
Lobster (*Homarus gammarus*) Stage II	Survival	LC_{50} (1 h)***	-	-	20.5 (12.7-31.8)	Parsons et al. 2020****
Lobster (*Homarus gammarus*) Stage I	Survival plus immobility	EC_{50} (1 h)***	-	-	15.5 (9.3-24.5)	Parsons et al. 2020****
Lobster (*Homarus gammarus*) Stage II	Survival plus immobility	EC_{50} (1 h)***	-	-	9.2 (5.5-14.6)	Parsons et al. 2020****
Lobster (*Homarus americanus*) Stage I	Survival	LC_{50} (1 h)**	-	1637 (1358-2004)	> 86.5	Burridge et al. 2014
Lobster (*Homarus americanus*) Adult	Survival	LC_{50} (1 h)**	-	>3750	24.8 (21.7-27.9)	Burridge et al. 2014
Shrimp (*Crangon septemspinosa*)	Survival	LC_{50} (1 h)**	-	3182 (2539-5368)	>85.5	Burridge et al. 2014
Mysid sp.	Survival	LC_{50} (1 h)**	-	973 (668-1427)	>85.5	Burridge et al. 2014

Species	Endpoint	Result	EB (µg·L^{-1}) (95% CI)	HP (mg·L^{-1}) (95% CI)	AZ (µg·L^{-1}) (95% CI)	Reference
Lobster (*Homarus americanus*) Stage I	Survival	LC_{50} (48 h)	-	-	3.57 (1.76-5.37	Burridge et al. 1999
Lobster (*Homarus americanus*) Stage II	Survival	LC_{50} (48 h)	-	-	1.03 (0-4.28)	Burridge et al. 1999
Lobster (*Homarus americanus*) Stage III	Survival	LC_{50} (48 h)	-	-	2.29 (0.72-3.88)	Burridge et al. 1999
Lobster (*Homarus americanus*) Stage IV	Survival	LC_{50} (48 h)	-	-	2.12 (1.06-2.02)	Burridge et al. 1999
Lobster (*Homarus americanus*) Adult	Survival	LC_{50} (48 h)	-	-	1.39 (0.78-2.02)	Burridge et al. 1999
Juvenile Spot prawn (*Pandalus platyceros*)	Survival	LC_{50} (24 h)	482 (370-616)	34.1 (24.0-46.3)	3.39 (2.19-5.24)	Strachan and Kennedy 2021*
Adult Spot prawn (*Pandalus platyceros*)	Survival	LC_{50} (24 h)	893 (674-1187)	107 (84.5-140)	106 (80.8-140)	Strachan and Kennedy 2021*
Juvenile Dock prawn (*Pandalus danae*)	Survival	LC_{50} (24 h)	577 (476-669)	40.4 (29.3-53.7)	4.39 (2.68-7.19)	Strachan and Kennedy 2021*
Adult dock prawn (*Pandalus danae*)	Survival	LC_{50} (24 h)	738 (567-964)	104 (62.3-171)	129 (101-168)	Strachan and Kennedy 2021*
Juvenile Pink prawn (*Pandalus borealis*)	Survival	LC_{50} (24 h)	670 (530-884)	41.2 (30.3-54.1)	7.63 (4.64-12.6)	Strachan and Kennedy 2021*
Adult Pink prawn (*Pandalus borealis*)	Survival	LC_{50} (24 h)	927 (725-1210)	()	81.0 (61.6-106)	Strachan and Kennedy 2021*
Adult Ghost shrimp (*Neotrypaea californiensis*)	Survival	LC_{50} (24 h)	385 (286-524)	70.0 (56.4-83.6)	63.0 (48.2-81.4)	Strachan and Kennedy 2021*
Juvenile Sand shrimp (unidentified)	Survival	LC_{50} (24 h)	389 (296-505)	125 (95.3-165)	68.0 (50.4-90.6)	Strachan and Kennedy 2021*
Juvenile Starry flounder (*Platichthys stellatus*)	Survival	LC_{50} (24 h)	1208 (956-1580)	173 (134-226)	7.90 (4.63-13.5)	Strachan and Kennedy 2021*
Adult Threespine stickleback (*Gasterosteus aculeatus*)	Survival	LC_{50} (24 h)	1310 (1020-1720)	158 (122-207)	11.1 (6.69-18.3)	Strachan and Kennedy 2021*
Adult Tidepool sculpin (*Oligocottus maculosus*)	Survival	LC_{50} (24 h)	1307 (1024-1717)	252 (188-338)	4.72 (2.95-7.55)	Strachan and Kennedy 2021*

*The work by Strachan and Kennedy was conducted with technical compounds not formulated products

**1 h exposures followed by monitoring for 95 h

***1 h exposures followed by monitoring for 23 h

****Estimates based on nominal concentrations

Table 4. Thresholds of toxicity for anti-parasitic compounds and aquatic species determined after sediment or in-feed exposures

Species	**Endpoint**	**Result**	**AZ (μg·Kg^{-1}) (95% CI)**	**EB (μg·Kg^{-1}) (95% CI)**	**IVM (μg·Kg^{-1}) (95% CI)**	**Reference**
Amphipod (*Corophium volutator*)	Survival	LC_{50} (10 day)	182 (152-217)	-	-	Mayor et al. 2008**
Juvenile Spot prawn (*Pandalus platyceros*)	Survival	LC_{50} (10 day)	-	332 (237-448)	-	Strachan and Kennedy 2021*
Juvenile Pink prawn (*Pandalus platyceros*)	Survival	LC_{50} (10 day)	-	599 (434-878)	-	Strachan and Kennedy 2021*
Amphipod (*Eohaustarius estuarius*)	Survival	LC_{50} (10 day)	-	156 (100-231)	-	Strachan and Kennedy 2021*
Amphipod (*Eohaustarius estuarius*)	Survival	LC_{50} (10 day)	-	146 (134-157)	-	Kuo et al. 2010
Amphipod (*Eohaustarius estuarius*)	Survival	LC_{50} (10 day)	-	82.1 (73.9-91.4) wet weight	-	ECCC unpublished
Polychaete (*Atilla virens*)	Survival	LC_{50} (10 day)	-	376 (269-478)	-	Strachan and Kennedy 2021*
Adult Tidepool sculpin (*Oligocottus maculosus*)	Survival	LC_{50} (10 day)	-	1980 (1249-3750)	-	Strachan and Kennedy 2021*
Shrimp (*Crangon septemspinosa*)	Survival	LC_{50} (10 day)	-	-	8500 (6200-10800)	Burridge and Haya 1993
Amphipod (*Corophium volutator)*	Survival	LC_{50} (10 day)	-	-	180 (130-240)	Davies et al. 1998
Lugworm (*Arenicola marina*)	Survival	LC_{50} (10 day)	-	-	23 (18-27)	Thain et al. 1997
Starfish (*Asterius rubens*)	Survival	LC_{50} (10 day)	-	-	23600 (20300-27300)	Davies et al. 1998
Lobster (*Homarus americanus*) Stage IV	Survival	LC_{50} (10 day)	-	250.2 (159.8-340.6) wet weight	212.4 (19.8-415) wet weight	Daoud et al. 2018
Lobster (*Homarus americanus*) adults	Survival	LC_{50} (7 day) feeding	-	644 μg·g^{-1} on food (428-1275)	-	Burridge et al. 2004

Species	Endpoint	Result	AZ (µg·Kg^{-1}) (95% CI)	EB (µg·Kg^{-1}) (95% CI)	IVM (µg·Kg^{-1}) (95% CI)	Reference
Lobster (*Homarus americanus*) Stage V and VI	Survival	LC_{50} (7 day) feeding	-	>589 µg·g^{-1} on food	-	Burridge et al. 2004
Amphipod *Monocorophium insidiosum*	Survival	LC_{50} (10 day)	-	890 (672-1171)	-	Tucca et al. 2014
Polychaete worm (P*olydura cornuta*)	Survival	LC_{50} (14 day)	-	207 (164-258) Wet weight	-	ECCC unpublished
Polychaete worm (*Polydura cornuta*)	Growth	EC_{50} (14 day)	-	No Effect at 10,000 wet weight	-	ECCC unpublished
Polychaete worm (*Neries virens*)	Growth	30 day	-	reduced growth rate at 165.6	-	McBriarty et al. 2017
Sea urchin embryos (*Lytechinus pictus*)	Survival	LC_{50} (10 day)	-	No Effect at 10,000 wet weight	-	ECCC unpublished
Bacterium (*Vibrio fischeri*)	Survival	LC_{50} (10 day)	-	No Effect at 10,000 wet weight	-	ECCC unpublished

Dry weight unless otherwise identified

* The work by Strachan and Kennedy was conducted with technical compounds not formulated products

** Estimate based on nominal concentration

PESTICIDES

In Canada two pesticides are registered for use in combating sea lice infestations on Atlantic salmon, azamethiphos in the Salmosan® formulation and hydrogen peroxide in the Interox® Paramove 50® or Aquaparox®.

SALMOSAN® (AZAMETHIPHOS)

Efficacy and Mechanism of Action of Azamethiphos

Azamethiphos is an organophosphate insecticide and the active ingredient in the formulation Salmosan®. The formulation is currently fully registered by Health Canada, Pest Management Regulatory Agency with a registration expiry date of December 31, 2024. The formulation is a wettable powder consisting of 47.5% azamethiphos. It is used as a bath treatment at 100 $\mu g \cdot L^{-1}$ for 30-60 minutes in well boats and tarps. At water temperatures above 10°C a 30 min treatment is recommended (Salmosan® product label). The product is effective only against pre-adult and adult sea lice and has no effect on the larval stages. This results in a need to treat cages repeatedly during periods of high infestation. Application of Salmosan® is limited to two treatments per day per aquaculture site that use 150 m polar radius net pens, no more than 10 treatments during the normal grow-out cycle and no treatments allowed within 1 Km of a lobster holding facility (Health Canada 2018). DFO's latest use statistics show that Salmosan® was applied 69 times in 2018 with a total of 502.13 Kg of active ingredient used (Department of Fisheries and Oceans 2019).

Azamethiphos has neuro-toxic action, acting as an acetylcholinesterase (AChE) inhibitor. In the absence of AChE activity nerves repetitively fire and the affected organisms eventually die. Azamethiphos has been shown to be mutagenic in several *in vitro* tests (EMEA 1999). DNA damage was induced in mammalian cell lines *in vitro* and azamethiphos induced an increase in revertant genes in the yeast *S. cerevisiae* D7, also *in vitro*. Zitko (2001) suggested that the high alkylating potency of azamethiphos could explain the mutagenic response and recommended that biological effects studies on non-target biota should include tests for delayed effects. However, *in vivo* studies with azamethiphos did not result in evidence of mutagenicity (EMEA 1999). The reason for this could be related to experimental protocols or to metabolism of the product *in vivo*.

Sea lice sensitivity to azamethiphos is variable, and some sea lice populations are more sensitive to this compound than others (Roth et al. 1996). Development of resistance to organophosphates is common and has been shown for azamethiphos (Levot and Hughes 1989). In sensitive sea lice populations, azamethiphos is effective in removing >85 % of adult and pre-adult sea lice but is not effective against the earlier life stages of the parasite (Roth et al. 1996).

Distribution and Fate of Azamethiphos

Azamethiphos is soluble in water (1.1 $g \cdot L^{-1}$) and has a low octanol-water partition coefficient (log K_{ow} = 1.05) (SEPA 2005). Azamethiphos is likely to remain in the aqueous phase on entering the environment. It is unlikely to accumulate in tissue or in sediment. Azamethiphos decomposes by hydrolysis in natural water with a half-life of 8.9 days. Dispersion studies indicated that after release of an experimental treatment (200 $\mu g \cdot L^{-1}$ as Salmosan®), the concentration of azamethiphos was below detection (0.1 $\mu g \cdot L^{-1}$) in a short period of time. It was not detected below 10 m depth and it was suggested that it is unlikely that azamethiphos would accumulate in sediment (SEPA 2005).

The bioaccumulation of azamethiphos by salmon is low and depletion of total azamethiphos in salmon is rapid and the pre-marketing withdrawal time is 24 h (EMEA 1999).

Biological Effects of Salmosan® (Azamethiphos)

Research commissioned by Ciba Geigy shows that azamethiphos is not lethal to several groups of invertebrates (bivalve molluscs and gastropods, amphipods, and echinoderms) unless the treatment concentrations are greater than the prescribed treatment concentration of 100 $\mu g \cdot L^{-1}$ (SEPA 2005 reported in Burridge and Van Geest 2014). The 24-h LC_{50} of azamethiphos to the copepod, *Temora longicornis*, is reported to be >10 $\mu g \cdot L^{-1}$. The 96-h LC_{50} for European lobster larvae, *Homarus gammarus*, is 0.5 $\mu g \cdot L^{-1}$ and is in general agreement with the 48-h LC_{50} for the American lobster, 1.39 $\mu g \cdot L^{-1}$ (Burridge et al. 1999, Table 3). Finally, the 96-h LC_{50} for the mysid shrimp, *Mysidopsis bahia*, is reported as 0.52 $\mu g \cdot L^{-1}$ (SEPA 2005).

Lobster and shrimp were the most susceptible species to azamethiphos in laboratory-based acute toxicity tests, while bivalves such as scallops and clams were unaffected (Burridge and Haya 1998). The 48-h LC_{50}'s estimated for the first four larval stages and adults of the American lobster (*Homarus americanus*) after exposure to Salmosan® are shown in Table 3. LC_{50}s are reported as the concentration of azamethiphos. There was no statistically significant difference between threshold values for each stage. There is a seasonal aspect to susceptibility of American lobsters to azamethiphos. Adult female lobsters are significantly more sensitive to azamethiphos in the summer than at any other time of year (Burridge et al. 2005). For adult and Stage IV lobsters exposed repeatedly for varying lengths of time to four concentrations of azamethiphos (Burridge et al. 2000), the No Observed Effect Concentration (NOEC) was nine exposures of 30 min each over three days to 1 $\mu g \cdot L^{-1}$of azamethiphos. In addition to observed lethality, many surviving lobsters showed significant behavioural responses, after repeated exposure to concentrations of 10 $\mu g \cdot L^{-1}$ (see description below).

In a similar experiment Daoud et al. (2016) exposed adult male lobsters to Salmosan® for one hour and repeated the exposure to a total of five exposures over 48 h. They determined the lethal threshold and monitored a number of sublethal endpoints. They reported a 48-h LC_{50} of 0.45 $\mu g \cdot L^{-1}$ (95% CI = 0.39-0.51). A NOEC was estimated to be 0.5 $\mu g \cdot L^{-1}$ and the third exposure (Daoud et al. 2016). The authors also reported neuromuscular dysfunction, hypoxia and metabolic disturbances.

In laboratory studies, adult American lobsters exposed to Salmosan® (5.0-10.0 µg (azamethiphos)· L^{-1}) became quite agitated, often 'flopping' erratically around the exposure tank (Burridge et al. 2000). They were also aggressive to other lobsters and reacted very quickly to any movement. They seemed to lose control of their claws and eventually flipped onto their backs and died within hours. Some affected lobsters remained moribund for periods of time ranging from hours to days. The consequences of behavioural responses such as these on organisms and populations in the natural environment are unknown.

Preovigerous female lobsters were exposed for 1 h biweekly to a nominal concentration of 10 $\mu g \cdot L^{-1}$ azamethiphos and monitored for spawning success and survival (Burridge et al. 2008). Surprisingly, even with such infrequent exposures, up to 100% of the animals exposed to this concentration died during the experiment: some expired after only three treatments. At lower concentrations a significant number of the surviving lobsters failed to spawn. A laboratory study indicated that shelter use behaviour could be affected by Salmosan® (Abgrall et al. 2000). However, exposure to concentrations of azamethiphos in water was greater than five times the recommended treatment concentration for periods of several hours. Ernst et al. (2001) measured the toxicity of Salmosan®, as azamethiphos, to a number of species including: the bacterium (*Vibrio fisheri);* the adult Green sea urchin (*Stongylocentrus droebrachiensus*), the

painted urchin (*Lytechinus pictus)* (fertilization); the Threespine stickleback (*Gasterosteus acualeatus*); three amphipods (*Amphiporeia virginiana, Gammarus* spp., and *Eohaustorius estuaries*); a polychaete (*Polydora cornuta*); Brine shrimp (*Artemia salina*); and a rotifer (*Brachionus plicatilis*). They determined that amphipods were most sensitive with *Eohausorius estuarius* having a 48-h EC_{50} (immobilization) of approximately 3 $\mu g \cdot L^{-1}$.

The response of mussels to stimuli was unaffected by exposures to 10.0 $\mu g \cdot L^{-1}$ for up to 24 h (SEPA 2005). The inhibition of AChE by azamethiphos is not cumulative in fish (Roth et al. 1993). However, cumulative inhibition of AChE occurred in lobster in studies to determine the effect of Salmosan® on spawning (Burridge et al. 2008). Mussel closure rate was affected at concentrations above 100 $\mu g \cdot L^{-1}$ and exposure to 46.0 $\mu g \cdot L^{-1}$ resulted in 50% inhibition of AChE activity (SEPA 2005). AChE activity in herring yolk sac larvae and post-yolk sac larvae was inhibited by 96-h exposure to azamethiphos at 33.4 and 26.6 $\mu g \cdot L^{-1}$, respectively. Herring larvae were reported to tolerate azamethiphos better than another organophosphate, DDVP (Roth et al. 1993).

Burridge et al. (2014) report results of studies to determine lethality after short (1-h) exposures followed by a 95-h monitoring period, see Table 3. Results show that no LC_{50} could be determined for Stage I lobster larvae, the mysid shrimp, *Mysis stenolepsis*, or the sand shrimp, *Crangon septemspinosa*, after a 1-h exposure to 85.5 µg azamethiphos$\cdot L^{-1}$ followed by 95 h in clean water. The LC_{50} for adult lobsters was estimated to be 24.8 µg azamethiphos$\cdot L^{-1}$ (95% CI = 21.7-27.9). When adult lobsters were exposed to Salmosan® continuously for 10 days the LC_{50} was estimated to be 0.216 µg azamethiphos L^{-1} (Burridge, unpublished results).

Van Geest et al. (2014) exposed indigenous (New Brunswick, Canada) copepods to a series of concentrations of Salmosan® for 1 h followed by 5 h in clean water, see Table 3. The proportion of copepods feeding was assessed by providing carmine particles to copepods for the final 2 h and lethality was assessed with a vital stain and visual observation at the end of the 5 h. No effects on mobility and mortality were observed at concentrations as high as 500 $\mu g \cdot L^{-1}$ (nominal concentration of azamethiphos).

In another experiment Stage IV-V post-larvae lobster were exposed to 12 or 57 $\mu g \cdot L^{-1}$ of azamethiphos in filtered seawater or raw seawater and sediment substrate for 1 h under static conditions, followed by a return to flow-through conditions with clean water for an additional 96 h. Effects were noted at both treatment concentrations including changes in behaviour, presence of moribund animals (non-responsive but respiring) and death (Dr. Andrew Cooper, DFO, St. Andrews, NB, unpublished results). Surprisingly, these responses were different between the two types of exposures suggesting that the presence of organic solids (raw seawater and sediment) increased the toxicity of azamethiphos under the conditions tested. These data are counterintuitive to what was expected and impossible to explain without further testing. They suggest either: 1) Additional exposure in organisms during raw seawater with sediment trials which may be more representative of the natural environment leading to increased respiration, contact with organic particles, other behaviour such as burrowing, swimming, and feeding, all of which might enhance uptake of the pesticide. 2) Alternatively, the presence of sediment and raw seawater and subsequent changes in environmental conditions may be an additional stressor to the juvenile lobsters and therefore may result in increased sensitivity (Dr. Andrew Cooper, DFO, St. Andrews, NB, personal communication).

Groups of adult lobsters (n = 20/group, with consistent proportions of males and females) were exposed 1, 2, 4, or 6 times to either 0.1 or 1 $\mu g \cdot L^{-1}$ of azamethiphos (nominal; representing “low” or “high” sublethal concentrations) for 30 minutes over three days. None of the lobsters displayed any behavioural and/or orientation problems after exposure, and survival in the treated (99%) and control lobsters (100%) was similar. Lobsters were held for several months to

determine whether molting and reproduction were affected by repeated exposure to azamethiphos. There was no detectable effect on incidence of molting, time to complete each of the premolt (D_1 to D_3) and postmolt (A to C_1) stages, molt success, size increase at molt, or recovery from molt. Female lobsters displayed normal mating behaviour and resumed cement gland development early in postmolt, reaching stage 1 or 2 by molt stage C_{1-2} (normal for that that time of year and stage of the molt cycle) (Burridge and Waddy, unpublished results).

Couillard and Burridge (2015) exposed adult male lobsters to 0.078 $\mu g \cdot L^{-1}$ of azamethiphos (in Salmosan® formulation) continuously for 10 days. At the end of the exposure one group of lobsters were sampled and dissected for morphometric and biochemical analysis. A second group of lobsters were transferred to clean seawater and held for 24 h then sampled. A third group was mixed, controls with treated lobsters, and packed with damp seaweed in an ice chest (33 cm x 41 cm x 23 cm, 0.031 m^3) to simulate commercial live transportation. These lobsters were kept in a cold room at 7°C for approximately 24 h before sampling and a suite of endpoints were measured.

A single treated lobster died on Day 10, while no other lobsters died during the 10-day treatment or during 24 h in running seawater post-treatment. However, >33% of the treated lobsters held under simulated shipping conditions were dead after 24 h compared to 2.6% of the control lobsters. As expected, treatment with azamethiphos significantly reduced acetycholinesterase activity and 24-h depuration or shipping did not change this result. Other biochemical endpoints were also affected. For example, the hepatosomatic index, the gonadosomatic index and percent lipid and water in the hepatopancreas were all affected by exposure to Salmosan®. Hemolymph protein was also elevated in lobsters after exposure; the effect was greater after simulated shipping. Shipping also affected condition factor and gill protein carbonyl concentrations (Couillard and Burridge 2015). The authors state that sublethal exposure to azamethiphos markedly increases the risk of mortality of adult lobsters during live transportation.

This study has shown that chronic exposure to low concentrations of the anti-sea lice pesticide azamethiphos induced sublethal effects in adult lobsters. Cholinesterase activity inhibition could lead to disturbance of critical behavioural functions (Domingues et al. 2010). Altered energy allocation could lead to delayed gonad maturation and impaired reproduction. These effects persist for at least 24 h after cessation of exposure, increasing the risk of cumulative impacts when lobsters are exposed to further chemical or non-chemical stress.

The work of Daoud et al. (2016) with Salmosan® showed (the expected) significant inhibition of acetylcholinesterase activity. They also report significant effects of the formulation on some hemolymph plasma biochemistry endpoints at the highest exposure concentration, 5 $\mu g \cdot L^{-1}$.

Mayor et al. (2008) exposed several marine invertebrates to sediment-borne therapeutants and determined lethal thresholds. They report a 10-day LC_{50} for azamethiphos and *Corophium volutator* of 182 $\mu g \cdot Kg^{-1}$ sediment (wet weight) (95% CI= =152-217). The results are based on nominal concentrations and no chemical characterisation of the sediment or water was conducted. It is unclear therefore if azamethiphos was indeed sediment bound.

Bechmann et al. (2019) exposed northern shrimp (*Pandalus borealis*) to Salmosan® at a concentration of 100 $ng \cdot L^{-1}$ (as azamethiphos) for 24 h or for 2-h pulses. There was no mortality, effects on swimming behaviour or effects on feeding in any exposure. However, they report tissue damage in the hepatopancreas.

Gebauer et al. (2017) exposed larvae of the crab *Metacarcinus edwardsii* to pulses of azamethiphos at nominal concentrations between 0.0625 and 0.5 $\mu g \cdot L^{-1}$. Their results are shown in Table 3. They report effects on survival but no effect on the development time of

Zoea I. The LC_{50} 24 after a 1 h exposure was estimated to be 2.84 $\mu g \cdot L^{-1}$ (95% CI = 2.45-3.23). No water chemistry is reported, and the authors suggest that the water-soluble nature of azamethiphos would lead to potential negative impacts of this product used at the recommended treatment concentration (Gebauer et al. 2017).

Parsons et al. (2020) reported the lethal threshold of azamethiphos to Stage I and Stage II European lobsters (*Homarus Gammarus*). The 1 h exposure was followed by 23 h in untreated water The LC_{50}s were 43.1 $\mu g \cdot L^{-1}$ (95% CI + 22.2-81.5) for Stage I and 20.5 $\mu g \cdot L^{-1}$ (95% CI = 12.731.8) for Stage II. The authors also combined dead and immobile larvae and estimated EC_{50} values. The 1 h EC_{50}s were 15.5 $\mu g \cdot L^{-1}$ (95% CI + 9.3-24.5) for Stage I and 9.2 $\mu g \cdot L^{-1}$ (95% CI = 5.5-14.6) for Stage II.

Bechmann et al. (2020) exposed Northern shrimp (*Pandalus borealis*) larvae to azamethiphos at 0.1 $\mu g \cdot L^{-1}$ for 2 h daily for 3 days. No effect on survival or swimming activity. At 13 d post-hatch swimming activity and feeding were lower than controls but there was no effect on survival or successful development to Stage II.

Field Studies with Salmosan®

During 1995, a study was conducted to determine the effects of single operational Salmosan® treatments on juvenile and adult American lobsters, shrimp, *(Pandalus montagui*), clams, (*Mya arenaria*), and scallops, (*Placopecten magellanicus*), suspended at two depth and varying distances from the treated cage. During two of the treatments, all lobsters held within the treatment tarpaulin died (Chang and McClelland, 1996). No other treatment-related mortalities were observed. In addition, no mortalities were observed with lobsters that were suspended at three depths at 20 sites surrounding a salmon cage site that was conducting operational treatments with Salmosan®. Mussels deployed during field trials in Scotland were unaffected (SEPA 2005). Mortality among lobster larvae was 27% but was not correlated to distance from the treatment cage.

The amphipod *Eohaustorius estuarius* was used as a test organism in a dye dispersion study designed to simulate net-pen releases. The study used a rhodamine dye as a tracer and found that 1/200 - 1/3000 the release concentration was not achieved until post-release times ranging from 2-5.5 h. Most samples from the plume were not toxic when azamethiphos was the test pesticide and none were toxic past 20-minute post release. Ernst et al. (2001) suggest that Salmosan® presents a lower environmental risk than the other pesticide they tested during that study, cypermethrin. In a similar study, Ernst et al. (2014) collected water samples from the effluent plume during a commercial cage treatment with Salmosan®. They used fluorescein dye to follow the plume. *Eohaustorius estuarius* was exposed to the water samples for either 1 h or 48 h. Exposure to water collected from within the cage during treatment was toxic (mortality and paralysis) but exposure for 1 h to water collected at the cage edge immediately post-treatment had no effect. If the exposure was extended to 48 h, effects were noted in water samples collected as far as 850 m away.

Finally, survival of American lobsters suspended at mid-depth and near bottom at four sites in the salmon farming area of Lime Kiln Bay, New Brunswick, Canada, plus a control site, was monitored for nine weeks during August-October 1996. There were no apparent differences in lobster survival between the experimental and control sites (Chang and McClelland 1997). No residues of azamethiphos were detected in water samples collected weekly from the five sites (Detection Limit = 50 $pg \cdot L^{-1}$). Diving surveys at a lobster nursery area located near a salmon farm in early August, September and late October of 1996 found no apparent changes in lobster populations over time, and the area was found to have a considerable population of juvenile lobsters.

Measurements of primary productivity and dissolved oxygen were made before, during and after chemical treatments at salmon farms in southwest New Brunswick in August-September 1996. There were no evident effects on dissolved oxygen and chlorophyll-*a* levels, indicating no impact on primary production (Dr. David Wildish, DFO St. Andrews Biological Station, St. Andrews, NB, unpublished data).

PARAMOVE 50® (HYDROGEN PEROXIDE)

Efficacy and Mechanism of Action of Hydrogen Peroxide

Hydrogen peroxide is a strong oxidizing agent that was first considered for the treatment of ecto-parasites of aquarium fish (Mitchell and Collins 1997). It is widely used for the treatment of fungal infections of fish and their eggs in hatcheries (Rach et al. 2000) and is registered in Canada by PMRA for that purpose. With the development of resistance of sea lice to organophosphates it was preferable to use of hydrogen peroxide to treat infestations of both *Lepeophtheirus salmonis* and *Caligus elongatus* (Jones et al. 1992). Hydrogen peroxide was used in salmon farms in Faroe Islands, Norway, Scotland and Canada in the 1990's (Treasurer and Grant 1997). Hydrogen peroxide (Paramove 50®) is fully registered for use in Canada with an expiry date on the registration of December 31, 2023. DFO's latest use statistics show that hydrogen peroxide was applied 65 times in 2018 with a total of 418747.08 Kg of active ingredient used (Department of Fisheries and Oceans 2019).

The suggested mechanisms of action of hydrogen peroxide are mechanical paralysis, peroxidation by hydroxyl radicals of lipid and cellular organelle membranes, and inactivation of enzymes and DNA replication (Cotran et al., 1989). Most evidence supports the induction of mechanical paralysis when bubbles form in the gut and haemolymph and cause the sea lice to release and float to the surface (Bruno and Raynard 1994).

The half-life in seawater (the time to reach 50% of the starting concentration) of hydrogen peroxide ranges from 7 to 28 days. Bruno and Raynard (1994) report a half life of seven days. These authors do not identify the formulation, if any used in their study. Recently Lyons et al. (2014) reported that the Paramove 50® formulation (50% hydrogen peroxide has a half life of 28 days under static conditions. Interestingly, they report that the half life is shorter in filtered seawater than in raw seawater. Regardless, a 28-day half life compared to a seven-day half life is significant.

Hydrogen peroxide is perceived as being of low risk as a sea lice treatment; however, there is very little information on the non-target effects of the use of this chemical. It is known to have toxic effects to Atlantic salmon at concentrations of 2.4 $g \cdot L^{-1}$, which is near the treatment concentrations of 1.2-1.8 $g \cdot L^{-1}$ (Haya et al. 2005).

The recommended dosage for bath treatments is 1.2-1.8 $g \cdot L^{-1}$ for 40 min but the effectiveness is temperature dependent. Treasurer et al. (2000) suggest the compound is not effective below 10°C; however, the product is used and is effective at temperatures below 6°C in British Columbia (Richard Opala, Mowi Canada West, personal communication). Hydrogen peroxide has little efficacy against larval sea lice and its effectiveness against pre-adult and adult stages has been inconsistent (Mitchell and Collins, 1992). Effectiveness can be difficult to determine on farms as the treatment concentration varies due to highly variable volumes of water enclosed in the tarpaulin. Temperature and duration also influence the efficacy. Ovigerous females are less sensitive than other mobile stages (Treasurer et al. 2000). It is possible that a proportion of the eggs on gravid female sea lice may not be viable after exposure to hydrogen peroxide (Johnson et al., 1993). Hydrogen peroxide was less efficacious when treating sea lice infestations on salmon in a cage that had been treated regularly for six years than in cages where the sea lice

were treated for the first time. This suggested that *Lepeophtheirus salmonis* had developed some resistance to hydrogen peroxide (Treasurer et al. 2000).

In a laboratory experiment, all adult and pre-adult sea lice exposed to 2.0 $g \cdot L^{-1}$ hydrogen peroxide for 20 min became immobilized, but half had recovered 2 h post-treatment (Bruno and Raynard 1994). The recovered sea lice swam normally and may have been able to reattach to the host salmon (Hodneland et al. 1993). Therefore, it was recommended that floating sea lice should be removed. However, re-infection has not been noticed in practice (Treasurer et al. 2000) as the removed sea lice generally show little swimming activity. Re-infection in the field is less likely because the free sea lice will be washed away with the tidal flow or eaten by predators. After treatment of a cage with approximately 1.5 $g \cdot L^{-1}$ hydrogen peroxide at 6.5°C, all the sea lice that were collected from surface water of treated cages were inactive, but recovery commenced within 30 minutes and 90-97% of the sea lice were active 12 h post-treatment (Treasurer and Grant 1997). In this study, a higher proportion of pre-adult sea lice was removed than of adult sea lice.

Distribution and Fate of Hydrogen Peroxide

Hydrogen peroxide is fully miscible in water and has a calculated K_{ow} of -1.5 and has little or no potential to persist or bioaccumulate (HERA project 2005). Hydrogen peroxide formulations are generally considered to be the treatment method of lowest environmental risk because it decomposes into oxygen and water. At 4°C and 15°C, 21% and 54% respectively of the hydrogen peroxide has decomposed after seven days in sea water. If the sea water is aerated the amount decomposed after seven days is 45% and 67%, respectively (Bruno and Raynard, 1994). These authors report that field observations suggest that decomposition in the field is more rapid, possibly due to reaction with organic matter in the water column, or decomposition catalyzed by other substances in the water, such as metals. The data from Lyons et al. (2014) contradict this assertion as they show the half life in filtered seawater to be shorter than in raw seawater. The half-life of the formulation(s) is obviously variable and dependent on a number of chemical (formulation, stabilizing agents) and environmental factors.

Biological Effects of Hydrogen Peroxide

Experimental exposure of Atlantic salmon to hydrogen peroxide at varying temperatures shows that there is a very narrow margin between the recommended treatment concentration identified by the authors (0.5 $g \cdot L^{-1}$) and that which causes gill damage and mortality (2.38 $g \cdot L^{-1}$) (Keimer and Black 1997). As can be expected, hydrogen peroxide is toxic to crustaceans with a 24-h LC_{50} to the Brine shrimp (*Artemia salina)* of 0.8 $g \cdot L^{-1}$ (Mathews 1995). Hydrogen peroxide has been shown to cause a decrease in aerobic metabolic rate and intracellular pH in the sand shrimp (*Crangon crangon)* at concentrations of 0.68 $g \cdot L^{-1}$ as a result of 5-h exposures (Abelel-Oesschger et al. 1997). Those concentrations are one-half to two-thirds of the prescribed Canadian treatment concentration (1200-1800 $mg \cdot L^{-1}$).

Toxicity to fish varies with temperature; for example, the 1-h LC_{50} to Rainbow trout at 7°C was 2.38 $g \cdot L^{-1}$, at 22°C was 0.218 $g \cdot L^{-1}$ (Mitchell and Collins, 1997) and for Atlantic salmon increased five-fold when the temperature was raised from 6°C to 14°C (Roth et al., 1993). There was 35% mortality in Atlantic salmon exposed to hydrogen peroxide at 13.5°C for 20 min. Bruno and Raynard (1994) reported that there was a rapid increase in respiration and loss of balance, but if the exposure was at 10°C there was no effect. There is evidence that the concentrations of hydrogen peroxide used in sea lice treatments can cause gill damage and reduced growth rates for two weeks post treatment (Carvajal et al. 2000).

Abelel-Oeschger et al. (1997) reported that hydrogen peroxide can affect the metabolism of the shrimp *Crangon*. These authors were discussing peroxide in episodic rainfall with relatively low concentrations (micro-molar). However, this could be representative of diluted effluent from a cage treatment. None of the authors referred to above state whether or not the hydrogen peroxide used was in a formulation licensed for aquaculture use.

Burridge et al. (2014) reported the acute lethality of Paramove 50®. As expected, this product is much less lethal to the aquatic invertebrates tested than Salmosan®, AlphaMax®, or Excis®. When experimental animals were exposed to Paramove 50® for 1 h then monitored for a further 95 h, the LC_{50} estimate for Stage I lobster larvae was 1637 $mg \cdot L^{-1}$, while adult lobsters survived exposure to 3750 $mg \cdot L^{-1}$, approximately three times the prescribed treatment concentration. The LC_{50} for Paramove 50® and *Mysid stenoplepsis* was estimated to be 973 $mg \cdot L^{-1}$. The LC_{50} for *Crangon septemspinosa* was estimated to be 3182 $mg \cdot L^{-1}$.

These authors also looked at the time to 50% mortality (LT_{50}) for several concentrations of hydrogen peroxide. The estimates were made from data collected during 1-h exposures followed by 95 h of monitoring. The data shows that death occurs quickly at or above the recommended treatment concentration especially with adult lobsters and mysids. At 750 $mg \cdot L^{-1}$ mysids are the only species where >50% of exposed animals die, which took > 80 h (Burridge et al. 2014).

Van Geest et al. (2014) have reported the acute toxicity of Paramove 50® to copepods collected routinely from the Passamaquoddy Bay area of New Brunswick. Copepods were exposed to a series of concentrations of the pesticide for 1 h and then transferred to clean water for 5 h. The proportion of copepods feeding was assessed by providing carmine particles for the final 2 h and lethality was assessed with a vital stain and visual observation at the end of the 5 h. Copepods exposed to 1200-120 or as low as 12 $mg \cdot L^{-1}$ were immobilized and sunk to the bottom of test beakers within 15 and 60 minutes of the 1-h exposure, respectively. In one of five bioassays, poor or no vital staining was observed in the two highest concentrations, indicative of mortality. The LC_{50} (95% C.I.) was 68 (58-82 $mg \cdot L^{-1}$) for this bioassay. Feeding behaviour was affected and a mean EC_{50} (95% C.I.) of 4.2 (3.4-5.2) $mg \cdot L^{-1}$ was determined based on five bioassays.

Bechmann et al. (2019) showed that northern shrimp (*Pandalus borealis*) were affected by exposure to 1.5 or 15 $mg \cdot L^{-1}$ hydrogen peroxide in the Paramove 50® formulation. Three 2-h pulses at 1.5 $mg \cdot L^{-1}$ resulted in increased mortality and reduced feeding rate. The same results were observed after one 2-h pulse at 15 $mg \cdot L^{-1}$. Gill damage was observed after one 1-h exposure to the same concentrations with the higher concentration resulting in more severe damage. Finally, the authors also saw evidence of sublethal effects at the biochemical level in the hepatopancreas of exposed shrimp (Bechmann et al. 2019).

McCurdy et al. (2013) reported results of studies to determine if sequential exposures to of Salmosan® and Paramove 50® were more, equal or less toxic to mysid shrimp than single pesticide exposures. These pesticides are the only registered formulations for bath treatments in Canada. In 2011, some well boat treatments were conducted wherein a treatment with Salmosan® was followed by a treatment with Paramove 50® while the fish remained in the boat (Dr. Michael Beattie, personal communication, 2013). Experiments were conducted in which shrimp were exposed, for 1 h, to Salmosan®, moved to clean water then exposed for 1 h to Paramove 50®. The results of these studies showed there was no additive toxicity. The LC_{50}s were the same as observed in previous experiments where mysids were exposed to Paramove 50® only. Salmosan® exposure did not result in > 50% of the exposed shrimp dying at any concentration.

McCurdy et al. (2013) also conducted an experiment in which mysid shrimp were exposed to true mixtures of Salmosan® and Paramove 50®. Results of these studies also show that the mixtures were no more, or less, toxic than the individual formulations. Paramove 50® appears to be driving any lethality and the thresholds are close to or above recommended treatment concentrations. Interestingly, when chemical measurements were made during this study the concentration of azamethiphos dropped significantly in the presence of hydrogen peroxide (McCurdy et al. 2013).

Gebauer et al. (2017) exposed larvae of the crab *Metacarcinus edwardsii* to hydrogen peroxide at up to 100-fold dilution of recommended treatment concentrations for the sea louse, *Caligus rogercresseyi.* The authors state that the recommended treatment concentration is 1500 $mg \cdot L^{-1}$. The authors state that formulations were used but do not state which formulations and all concentrations are nominal. The authors found hydrogen peroxide to be the least toxic of the four products tested (azamethiphos, deltamethrin and cypermethrin were also tested). However, repeated exposure to hydrogen peroxide (concentrations as low as 188 $mg \cdot L^{-1}$) resulted an increase in time to develop in the Zoea I stage. This was not observed with other compounds and the authors are unsure if the response is of biological significance.

Haugland et al. (2019) exposed juvenile sugar kelp, *Saccharina latissimi*, to hydrogen peroxide for 1 h then monitored survival and photosynthetic capacity and efficiency over 15 days. They report an LC_{50} of 80.7 (± 53 (95% CI)) $mg \cdot L^{-1}$ with a NOEC of 72.9 (±0.4 (95% CI)) $mg \cdot L^{-1}$. The EC_{50} for photosynthetic capacity is reported as 27.8 (±9.1 (95% CI)) $mg \cdot L^{-1}$ with a NOEC of 13.1(±11.2 (95% CI)) $mg \cdot L^{-1}$. The EC_{50} for photosynthetic efficiency is reported as 35.4 (±13.4 (95% CI)) $mg \cdot L^{-1}$ with a NOEC of 13.1(±11.2 (95% CI)) $mg \cdot L^{-1}$.

Hydrogen peroxide has been considered the most environmentally friendly anti-sea louse pesticide. However, the work by Van Geest et al. (2014) and by Bechmann et al. (2019) and Haugland et al. (2019) shows lethal and sublethal effects at concentrations well below treatment concentrations after as little as 1 h exposure.

ANTI-FUNGALS

Formalin is sometimes used as an antifungal agent and as a parasiticide. It is generally non-toxic at therapeutic doses and they are almost always diluted before or during release to the environment. It is considered as low risk for causing significant deleterious effects near aquaculture sites. The frequency of use and the spatial distribution of releases are also unknown making it impossible to confirm the assertion of low risk and to realistically assess the potential for effects to take place in the aquatic environment.

Formalin is a monoaldehyde that reacts with proteins, DNA and RNA in vitro (Bravo et al. 2005). It is recommended for controlling external fish parasites and for the control of fungi of the Saprolegniaceae family, and it has moderate to weak antibacterial activity. It is a 37% formaldehyde solution with a reported lethality (24-h LC_{50}) to Rainbow trout of 7.77 $mg \cdot L^{-1}$ (Scott 2004).

ANTIBIOTICS

Antibiotics in salmon aquaculture, as in other industrial husbandry of aquatic and terrestrial food animals including other fish, shrimp, cattle and poultry, are used as therapeutic agents in the treatment of infections (Alderman and Hastings 1998). While the Pest Management Regulatory Agency produces labels describing concentrations and treatment conditions for use of antiparasitic pesticides used in finfish aquaculture, the Veterinary Drugs Directorate does not have labels for drugs. In addition, recommended treatment doses, while considered by prescribing veterinarians, may be adjusted according to their knowledge of the product and its

intended use. For example, the recommended treatment dose of oxytetracycline is 75 $mg \cdot Kg^{-1}$ BW/day x 10 days but it is administered routinely at doses of at least 100 $mg \cdot Kg^{-1}$ (Dr. Michael Beattie, GIS Gas Infusion Systems, personal communication).

The use of antibiotics in salmon aquaculture has been reviewed by several authors (Burridge et al. 2010; Cabello et al. 2016; Bentley et al. (2019a, 2019b, in press)). The following is, therefore, a brief summary of information relating to prescribed dosages and potential hazards to non-targets associated with antibiotics use. Fisheries and Oceans Canada (2018) lists the following antibiotics as registered for use in Canada: florfenicol, oxytetracycline hydrochloride, sulfamethoxine-ormetoprim and trimethoprim/sulfadiazine powder. In addition, like ivermectin, erythromycin is/has been prescribed under Emergency Drug Registration. Antibiotics are designed to inhibit the growth (bacteriostatic activity) and kill pathogenic bacteria (bacteriocidal activity). Compounds with antibiotic activity are selected for use in human and veterinary medicine because of their selective inhibition of the synthesis of the cell wall and other membranes, macromolecular synthesis or enzyme activity in prokaryotic cells (Guardabassi and Courvalin 2006; Todar 2019). As a result of these selective traits they show low or very low toxicity in higher organisms (Guardabassi and Courvalin 2006; Todar 2019). The antibiotics registered for use in Canada all have water solubilities in excess of several hundred $mg \cdot L^{-1}$ and Log K_{ow} ranging from -0.9 to 2.37 (DrugBank, 2021). These properties suggest a potential to dissolve in water and not to accumulate in sediments. However, the products are all delivered in feed and therefore are formulated to remain in the food pellet. In these cases, the physical chemical properties of the active ingredient are unlikely to be predictive of environmental fate. The environmental concern with antibiotic use in salmon aquaculture is the potential for development of antibiotic resistant micro-organisms which may have negative consequences for fish, non-target organisms and even human health. Salmon aquaculture as a driver for development of antibiotic resistant organisms has been reviewed recently by Cabello et al. (2016). Antibiotic resistance is the topic of another paper (Murphy and Robinson, 2022, in press) and will not be addressed in this review.

The biological effects of antibiotics on marine biota are primarily assessed through toxicity, growth, and reproductive studies (Jessick 2010). The following is a brief summary of products that are currently registered for use or, in the case of erythromycin, being used in combatting bacterial infections in salmon aquaculture in Canada and data regarding their effects on non-target organisms. Table 5 shows these data in tabular form.

Table 5. Thresholds of toxicity for antibiotic compounds and aquatic species determined after exposure in sediment, in-feed or by injection.

Species	Endpoint	Result	Oxytetracycline	Florfenicol	Ometoprim	Trimethoprim	Erythromycin	Reference
Daphnia magna	survival	96 h LC_{50} (water exposure)	-	>330 $\mu g \cdot L^{-1}$	-	-	-	Armstrong et al. 2005
Daphnia magna	survival	7 d LC_{50} (water exposure)	-	>100 $\mu g \cdot L^{-1}$	-	-	-	Florêncio et al. 2014
Trout (*Onchorhyncus mykiss*)	survival	96 h LC_{50} (sediment exposure)	-	>780 $\mu g \cdot L^{-1}$	-	-	-	Armstrong et al. 2005
Polychaete worm (P*olydura cornuta*)	survival	96 h LC_{50} (sediment exposure)	>10 $m \cdot Kg^{-1}$ wet weight	10 $m \cdot Kg^{-1}$ wet weight	>100 $m \cdot Kg^{-1}$ wet weight	>100 $m \cdot Kg^{-1}$ wet weight	-	ECCC unpublished results
Polychaete worm (*Polydura cornuta*)	survival	96 h LC_{50} (sediment exposure)	10 $m \cdot Kg^{-1}$ wet weight	10 $m \cdot Kg^{-1}$ wet weight	>100 $m \cdot Kg^{-1}$ wet weight	>100 $m \cdot Kg^{-1}$ wet weight	-	ECCC unpublished results
Sea urchin embryos (*Lytechinus pictus*)	survival	96 h LC_{50} (sediment exposure)	10 $m \cdot Kg^{-1}$ wet weight	10 $m \cdot Kg^{-1}$ wet weight	>100 $m \cdot Kg^{-1}$ wet weight	>100 $m \cdot Kg^{-1}$ wet weight	-	ECCC unpublished results
Bacterium (*Vibrio fischeri*)	survival	96 h LC_{50} (sediment exposure)	10 $m \cdot Kg^{-1}$ wet weight	10 $m \cdot Kg^{-1}$ wet weight	>100 $m \cdot Kg^{-1}$ wet weight	>100 $m \cdot Kg^{-1}$ wet weight	-	ECCC unpublished results
Lobster (*Homarus americanus*)	Survival	Injection	-	>100 $mg \cdot Kg^{-1}$ body weight	-	-	-	Basti et al. 2011

Species	Endpoint	Result	Oxytetracycline	Florfenicol	Ometoprim	Trimethoprim	Erythromycin	Reference
Shrimp (*Litopenaeus vannamei*)	Survival after feeding	Biochemistry (EROD and GST)	-	Elevated enzyme activity at 200 $mg \cdot Kg^{-1}$ body weight	-	-	-	Ren et al. 2014
Brazilian fish (*Piaractus mesopotamicus*	Survival	48 h LC_{50} (water exposure)	7.6 $mg \cdot L^{-1}$	-	-	-	-	Carraschi et al. 2011
Juvenile goldfish (*Tilapia nilotica*)	Survival	96 h LC_{50} (water exposure)	-	-	-	-	242.7 $\mu g \cdot L^{-1}$	Yasser and Nabila 2015
Lake trout (*Salvelinus namaycush*)	Survival, growth	Water exposure, injection, feeding	No effect at up to 5 times treatment level	-	-	-	No effect at up to 5 times treatment level	Marking et al. 1988

Kemmerer (2009a, 2009b) states that antibiotic use in aquaculture is not considered to be a major contributor to overall quantities of antibiotics in the marine environment. The author states, however, that aquaculture usage may contribute to problems locally and that, despite many studies, the data are insufficient to do an adequate risk assessment.

Florfenicol is also a broad-spectrum antibiotic used to treat salmon against infections of furunculosis. It is part of the phenicol class of antibiotics which act by inhibiting protein synthesis (Guardabassi and Courvalin 2006; Todar 2019). Its water solubility is 219 $mg \cdot L^{-1}$ and its K_{ow} is 0.67-0.98 (DrugBank, 2021). The product is registered for use in aquaculture in Canada. There were 118 treatments using this antibiotic in Canada in 2018 with a total of 4120.58 Kg of active ingredient delivered (Department of Fisheries and Oceans 2019). The recommended treatment regime is 10 $mg \cdot Kg^{-1}$ for 10 days presented on medicated food. The withdrawal period for florfenicol is 12 days in Canada. The concentration (in water) which is expected to be lethal to 50% of an exposed population over 96 h (96-h LC_{50}) of florfenicol is >330 $mg \cdot L^{-1}$ (Daphnia) and >780 $mg \cdot L^{-1}$ (Rainbow trout). This product is not generally considered a problem for persistence in the environment as it degrades in sediment with a half-life of 4.5 days (Armstrong et al. 2005).

ECCC's Atlantic lab conducted effects studies using sediment spike with florfenicol and four marine species, the amphipod, *Eohaustorius estuaris*, the polychaete, *Polydura cornuta*, embryos of the sea urchin, *Lytechinus pictus*, and the bacterium, *Vibrio fischeri*. Exposures and determination of mortality and sublethal effects thresholds were determined according to standard (ECCC) protocols. No effects were observed at a maximum sediment concentration of 10 $mg \cdot Kg^{-1}$ wet weight (ECCC unpublished results).

Studies examining the biological effects of Aquaflor® (florfenicol) on the marine environment have predominantly focused on its toxicity to target organisms, primarily finfish, to obtain information required to establish dosing regimens for use in aquaculture (Bentley et al. (2019a, 2019b, in press)). Florêncio et al. (2014) reported LC_{50}s Brazilian fish, snails and microcrustaceans (*Daphnia*) and found 7-day threshold to be near or greater than 100 $mg \cdot L^{-1}$. Water exposure especially at this concentration is clearly not representative of an operational concentration. Ren et al. (2014) exposed white shrimp (*Litopenaeus vannamei*) to food-borne florfenicol (100 or 200 $mg \cdot Kg^{-1}$ body weight for six days) and found increased activity enzyme associated with elimination of contaminants (EROD and GST). The authors made no comment regarding the biological significance of these effects. Basti et al. (2011) injected the American lobster (*Homarus americanus*) with florfenicol at concentrations up to 100 $mg \cdot Kg^{-1}$ and report no deleterious effects. All these studies indicate that the toxicity of florfenicol to target species is generally low.

Potentiated sulfonamide antibiotics, Romet 30®, a combination of sulfadimethoxine and ormetoprim and Tribrissen®, a combination of sulphonamide and trimethoprim are broad spectrum antibacterial agents used to treat salmon infected with gram negative bacteria such as Furunculosis (Romet 30®) and Vibrios (*Vibrio anguillarum*, Tribrissen®). Ormetoprim has a water solubility of 1540 $mg \cdot L^{-1}$ and a Log K_{ow} of 1.2 (DrugBank 2021). Trimethoprim has a water solubility of 400-615 $mg \cdot L^{-1}$ and a Log K_{ow} of 0.9-1.28 (DrugBank 2021). They act by inhibiting folic acid metabolism at two different levels (Guardabassi and Courvalin 2006; Todar 2019). The recommended treatment regime for Tribrissen® is 30 $mg \cdot Kg^{-1}$ for 5-10 days delivered on medicated food (Scott 2004). The Health Canada approved label dose for Romet 30® is "Administer medicated feed daily for ten consecutive days to provide approximately 15 mg of active ingredients per Kg of live body weight of fish per day." At this dosage, the withdrawal period is 42 days after the latest treatment with the drug at temperatures >10°C (Drugs.com). There were no treatments with this antibiotic in Canada in 2018 (Department of Fisheries and Oceans 2019).

It is suggested that the low palatability of Romet 30® and Tribrissen® to salmon means very little is used in salmon aquaculture in Canada (Burridge et al. 2011). Two applications of Romet 30® were made in BC in 2018 (Department of Fisheries and Oceans 2019). Despite the product still being registered for use in fish by Health Canada, the drug sponsor for Tribrissen 40% Powder for use in finfish, cancelled their Drug Identification Number (DIN) in 2014. Therefore, this product is no longer available on the Canadian market (Health Canada). Despite this fact, one treatment with Tribrissen® was conducted in NB in 2018 (Department of Fisheries and Oceans 2019).

ECCC's Atlantic lab conducted effects studies using sediment spiked with ormetoprim (Romet 30®) or trimethoprim (Tribrissen®) and four marine species, the amphipod, *Eohaustorius estuaris*, the polychaete, *Polydura cornuta*, embryos of the sea urchin, *Lytechinus pictus*, and the bacterium, *Vibrio fischeri*. Exposures and determination of mortality and sublethal effects thresholds were determined according to standard (ECCC) protocols. No effects were observed for the amphipod, polychaete or sea urchin at a maximum sediment concentration of 100 $mg \cdot Kg^{-1}$ wet weight. IC_{50}s were observed in the microtox test in controls and at the highest exposure concentration (100 $mg \cdot Kg^{-1}$). However, there was no dose response and the effects did not meet ECCC's threshold guideline for toxicity (ECCC, unpublished results).

Hossain et al. (2017) have reported on the occurrence and distribution of antibiotics in water near finfish and shellfish aquaculture sites in Bangladesh. They report that measured concentrations of antibiotics, including potentiated sulfonamides, when compared to effects thresholds resulted in risk quotients of less than 1, meaning there was low potential for ecological or resistance risks (Hossain et al. 2017).

The environmental impact of use of these products is unknown but given their broad spectrum and the fact that may be degraded slowly, it may affect bacteria of the marine sediments and fish pathogens selecting for resistance (Armstrong et al. 2005).

Oxytetracycline is a broad spectrum antibiotic active against infections of Furunculosis and Vibrio (Powell 2000). In Canada the approved product is Terramycin Aqua®. Oxytetracycline has a water solubility of 313 $mg \cdot L^{-1}$ and a Log K_{ow} of -0.9 (DrugBank 2021). This product is delivered on medicated food at a dose of 75 $mg \cdot Kg^{-1}$ per day for 10 days. Tetracyclines act by inhibiting protein synthesis (Guardabassi and Courvalin 2006; Todar 2008). The withdrawal period is 40 days at 10°C or 80 days when water temperatures are below 10°C. There were 24 treatments with this antibiotic in Canada in 2017 resulting in 11692.69 Kg of active ingredient being delivered (Department of Fisheries and Oceans 2019).

The compound has a low toxicity (96-h LC_{50} for fish is >4 $g \cdot Kg^{-1}$). Carraschi et al. (2011), however, reported a 48-h LC_{50} of 7.6 $mg \cdot L^{-1}$ for oxytetracycline in water and the Brazilian fish (*Piaractus mesopotamicus*). These authors consider the product to be moderately toxic in these water exposures. Oxytetracycline has a relatively high-water solubility but since it delivered to salmon bound to food pellets it can become bound to sediments and may be remain there for several hundred days complexed to ions and with decreased antibacterial activity (Armstrong et al. 2005). Oxytetracycline has low toxicity to the commercially important lobster. In fact, it is safe and efficacious for treating bacterial infections in lobsters (Bayer and Daniel 1987). The target dose for lobsters is 2200 $mg \cdot Kg^{-1}$ (Drugs.com). Marking et al. (1988) treated Lake trout (*Salvelinus namaycush*) with oxytetracycline by water exposure, injection and by feeding and found no effects at doses and concentrations well above recommended treatment levels.

ECCC's Atlantic lab conducted effects studies using sediment spike with oxytetracycline and four marine species, the amphipod, *Eohaustorius estuaris*, the polychaete, *Polydura cornuta*, embryos of the sea urchin, *Lytechinus pictus*, and the bacterium, *Vibrio fischeri*. Exposures and determination of mortality and sublethal effects thresholds were determined according to

standard (ECCC) protocols. No effects were observed at a maximum sediment concentration of 10 $mg \cdot Kg^{-1}$ wet weight (ECCC unpublished results).

Rico et al. (2014) reported that high concentrations of oxytetracycline were found in sediments near tilapia farms (freshwater) in Thailand. They reported up to 6.91 $mg \cdot Kg^{-1}$ concentrations in sediment (dry wt) and 49 $\mu g \cdot L^{-1}$ concentrations in water. These concentrations are well below any effects thresholds and the authors contend that there is no risk to pelagic organisms while they don't mention benthic organisms.

Erythromycin is a macrolide antibiotic useful in combating gram positive and non-enteric gram-negative bacteria. It has a water solubility of 459 mg·L-1 and a Log K_{ow} of 2.37 (DrugBank 2021). It is presented on medicated food at dosages ranging from 50-100 $mg \cdot Kg^{-1}$ for 21 days. It is used to combat Bacterial Kidney Disease (BKD) (Powell 2000). Erythromycin inhibits genetic translation, therefore protein synthesis (Guardabassi and Courvalin 2006; Todar 2019). It can accumulate in sediments and organisms and is a concern in terms of antibiotic resistance. This antibiotic is not approved for salmon aquaculture use in countries which belong to International Council for the Exploration of the Seas (ICES). This includes Norway, Scotland and Canada (Armstrong et al. 2005). It is listed by DFO as being available for use in marine finfish aquaculture. One treatment was conducted in BC in 2018 (Department of Fisheries and Oceans 2019). A veterinary drug product – Erymicin 200 injectable is available through the EDR program at Health Canada as it has an Investigational New Animal Drug (INAD) approval in the US for controlling mortality caused by Bacterial Kidney Disease (BKD) and for controlling vertical transmission of BKD from BKD-positive female broodstock. Fish treated for controlling mortality can be treated a single time with Erymicin 200 Injection at 10–25 mg erythromycin per kilogram (Kg) fish body weight. Fish treated to control vertical transmission can be treated one to three times with Erymicin 200 at 10-25 mg erythromycin per kg fish body weight with a minimum injection interval of 21 days. The total dosage will not exceed 75 mg erythromycin per kilogram (Kg) fish body weight over three injections. Adherence to a 60-day investigational withdrawal period is required for all fish treated with Erymicin 200 under this INAD (US FDA).

Yassar and Nabil (2014) report a 96 h LC_{50} of erythromycin to Juvenile goldfish (*Tilapia nilotica*) of 242.7 $\mu g \cdot L^{-1}$. Marking et al. (1988) treated lake trout (*Salvelinus namaycush*) with erythromycin by water exposure, injection and by feeding and found no effects at doses and concentrations up to five times recommended treatment levels. Hossain et al. (2017) detected the risk quotients for antibiotics detected in surface water of aquaculture sites in Bangladesh. RQs were less than one for each antibiotic, including erythromycin, indicating that the ecological risk associated with the antibiotics at this site was low.

Similarly, in a study by Zheng et al. (2012), erythromycin was detected in 100% of water samples obtained from the Beibu Gulf, four rivers and from surface water near aquaculture sites in China. The concentrations for erythromycin ranged between 0.91 and 2.55 $ng \cdot L^{-1}$. When compared against erythromycin toxicity data for an aquatic plant, *Pseudokirchneriella subcapitata*, (EC_{50} = 0.02 $mg \cdot L^{-1}$) the authors suggested that erythromycin might pose a chronic environmental risk to this species.

Chen et al. (2015) examined bioaccumulation of antibiotics in marine organisms. The authors suggest that erythromycin was neither bioaccumulative nor potentially bioaccumulative under their experimental conditions.

Machado and Soares (2019) reported that two freshwater green algae species were affected by water-borne exposure to erythromycin one at 38 $\mu g \cdot L^{-1}$ and the other at a 1000-fold higher concentration (5750 $\mu g \cdot L^{-1}$).

While effects have been reported for antibiotics used in the salmon aquaculture industry, the data are fairly sparse and most have little relevance to real world treatments in Canada. Where effects are reported for water exposures, only a few relate to medicated food or sediments. Several reports of effects relate to freshwater organisms and several reports cited herein relate to tropical applications and subsequent chemical analysis.

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APPENDIX I: ABBREVIATION INDEX AND DEFINITIONS

BC - British Columbia

BW - Body Weight

CFIA - Canadian Food Inspection Agency EC_{50} median effective concentration, i.e., concentration of chemical in water or sediment that is expected to cause a specified effect (e.g., immobility) in 50% of test organisms.

DW - Dry Weight

IC_{50} - inhibiting concentration for a specified percent effect, i.e., concentration of chemical in water or sediment that is estimated to cause a 50% impairment in a quantitative biological function, such as growth or reproductive performance.

LOEC - lowest-observed-effect concentration, i.e., the lowest tested concentration of a chemical which has an effect that is different from the control, according to the statistical test used for analysis.

Log K_{ow} - Logarithm of the Octanol Water Partition Coefficient which is a partition coefficient for the two-phase system consisting of *n*-octanol and water. K_{ow} serves as a measure of the relationship between lipophilicity (fat solubility) and hydrophilicity (water solubility) of a substance. The value is greater than one if a substance is more soluble in fat-like solvents such as n-octanol, and less than one if it is more soluble in water.

LT_{50} - median lethal time, i.e., the exposure time that is estimated to be lethal to 50% of test organisms for a given concentration of chemical.

NB - New Brunswick

NL - Newfoundland and Labrador

NOEC - no-observed-effect concentration, i.e., the concentration that is the next lowest to the control, among those concentrations tested. (Almost always, the NOEC is also the highest tested concentration where the effect on test organisms is not different from the control, according to the statistical test used for analysis.)

NS - Nova Scotia

SEPA - Scottish Environmental Protection Agency

WW - wet weight