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A review of potential environmental risks associated with the use of pesticides to treat Atlantic salmon against infestations of sea lice in Canada

L.E. Burridge and J.L. Van Geest

Fisheries and Oceans Canada
St. Andrews Biological Station
531 Brandy Cove Road,
St. Andrews, NB, E5B 2L9

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Canada

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.

Research documents are produced in the official language in which they are provided to the Secretariat.

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ABSTRACT

This research document was one of three documents prepared as part of a DFO Canadian Science Advisory Secretariat (CSAS) process held 13-15 March, in Saint John, NB. The purpose of the process was to evaluate the current knowledge relating to the exposure and biological effects of pesticide bath treatments on non-target organisms in order to provide peer-reviewed science advice to DFO's Aquaculture Management Directorate and identify knowledge gaps and research needs. This advice is required to inform the development of regulations and policies under Section 36 of the Fisheries Act related to aquaculture pest and pathogen management and will also support Health Canada's environmental risk assessments related to the emergency registrations of pesticides. This paper reviewed the biological effects of four pesticide formulations, three that have been available via emergency registration: Salmosan® (active ingredient: azamethiphos), AlphaMax® (active ingredient: deltamethrin), and Paramove 50® (active ingredient: hydrogen peroxide) and one which is used in other jurisdictions and interest had been expressed in using the product in Canada, Excis® (active ingredient cypermethrin). Of these products only Salmosan® and Paramove 50® are currently being used as bath treatments to control sea lice in farmed salmon in Canada. In general, the effects on non-target organisms varied with the formulation being applied with lobster being the most sensitive species tested. The degree of toxicity was therapeutant specific with Paramove 50® being the least toxic of the three formulations tested, while AlphaMax® was the most toxic. Sublethal effects of repeated or long-term exposure of lobsters to Salmosan® are presented showing that repeated exposure may affect reproduction and shipping quality may be affected by long-term exposure. Data on the effects of these pesticides on Pacific non-target species is rare.

Examen des risques environnementaux potentiels liés à l'utilisation de pesticides pour traiter le saumon de l'Atlantique contre les infestations de pou du poisson au Canada

RESUME

Le présent document de recherche fait partie d'un ensemble de trois documents préparés dans le cadre du processus du Secrétariat canadien de consultation scientifique (SCCS) qui a eu lieu du 13 au 15 mars à Saint John, au Nouveau-Brunswick. Le processus visait à évaluer les connaissances actuelles sur l'exposition et les effets biologiques des bains thérapeutiques de pesticides sur les organismes non ciblés afin de formuler un avis scientifique examiné par les pairs à l'intention de la Direction générale de la gestion de l'aquaculture de Pêches et Océans Canada (MPO) et de déceler les lacunes dans les connaissances ainsi que les besoins en matière de recherche. Cet avis est nécessaire pour documenter l'élaboration de règlements et de politiques en vertu de l'article 36 de la *Loi sur les pêches* en ce qui concerne la gestion des parasites et des agents pathogènes en aquaculture. Il appuiera également les évaluations du risque environnemental menées par Santé Canada en lien avec l'homologation d'urgence des pesticides. Le présent document passe en revue les effets biologiques de quatre préparations de pesticides, dont trois sont devenues disponibles après avoir obtenu une homologation d'urgence : Salmosan® (ingrédient actif : azaméthiphos), AlphaMax® (ingrédient actif : deltaméthrine) et Paramove 50® (ingrédient actif : peroxyde d'hydrogène); l'autre préparation, Excis® (ingrédient actif : cyperméthrine), est utilisée dans d'autres juridictions et le Canada a démontré de l'intérêt pour ce produit. Parmi ces produits, seuls Salmosan® et Paramove 50® sont actuellement utilisés dans les bains thérapeutiques pour lutter contre les infestations de pou du poisson sur le saumon d'élevage au Canada. En général, les effets sur les organismes non ciblés variaient selon la formulation utilisée, le homard étant l'espèce la plus sensible parmi celles testées. Le degré de toxicité variait en fonction de l'agent thérapeutique : sur les trois formulations testées, Paramove 50® était la moins toxique et AlphaMax® la plus toxique. Les effets sublétaux de l'exposition répétée ou à long terme des homards au Salmosan® sont présentés et illustrent que l'exposition répétée pourrait avoir une incidence sur la reproduction et que l'exposition à long terme pourrait avoir un effet sur la qualité du produit lors de son transport. On dispose de très peu de données sur les effets de ces pesticides sur les espèces non ciblées dans le Pacifique.

ABBREVIATION INDEX AND DEFINITIONS

(As defined in Environment Canada (2005) Guidance Document on Statistical Methods for Environmental Toxicity Tests)

EC50 *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.

IC50 *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.

LC50 *median lethal concentration,* i.e., concentration of chemical in water or sediment that is estimated to be lethal to 50% of test organisms.

LC10 concentration of chemical in water of sediment that is estimated to be lethal to 10% of test organisms.

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.

LT50 *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.

NOEC *no-observed-effect concentration,* i.e., the concentration that is the next lowest from the LOEC, 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.)

INTRODUCTION

Cultured salmon in the crowded conditions of aquaculture are susceptible to epidemics of infectious bacterial, viral and parasitic diseases. Sea lice are ectoparasites of many species of fish and are a serious problem for salmon aquaculture industries (Roth et al., 1993; MacKinnon, 1997). The species that infest cultured Atlantic salmon are *Lepeophtheirus salmonis* and *Caligus elongatus*. Infestations result in skin erosion and sub-epidermal haemorrhage which, if left untreated, would result in significant fish losses, probably as a result of osmotic stress and other secondary infections (Wooten et al., 1982; Pike, 1989). The first severe epidemic of sea lice in Atlantic Canada occurred in 1994 (Hogans, 1995). Sea lice reproduce year round and the aim of a successful sea lice control strategy must be to pre-empt an internal infestation cycle from becoming established on a farm by exerting a reliable control on juvenile and preadult stages, thus preventing the development to gravid females (Treasurer and Grant, 1997). Effective control of sea lice infestations requires good husbandry and effective anti-parasitic chemicals (Rae, 2000; Eithun, 2004).

The types of therapeutants available for use and the treatment protocols are tightly regulated in Canada and therapeutants can only be used under prescription from a licensed veterinarian. Health Canada regulates chemotherapeutants used in the aquaculture industry, which are considered either a drug or a pesticide depending on the use and method of application. If the product is applied topically or directly into water, it is considered a pesticide; however, if a product is delivered through medicated feed or by injection, it is considered a drug. In order for pesticide formulations to be registered for use in aquaculture they must be shown to be efficacious (i.e., it will kill the target organism), it must be shown to be safe for the fish, and it must be shown to have an acceptable risk to non-target organisms (Peter Delorme, Pest Management Regulatory Agency, personal communication).

There are provisions for Emergency Release and 'off-label' use of drugs and pesticides. Pesticides are the responsibility of the Pest Management Regulatory Agency (PMRA) of Health Canada and are registered under the authority of the *Pest Control Products Act* (PCPA). The PCPA requires the registrant to submit environmental data as part of the registration process. Most data submitted to the regulatory agencies are proprietary and, as such, are not available to the general public, but may be obtained by researchers (with restrictions) from Health Canada.

Aquaculture, like all forms of intensive food production, may generate environmental costs. Chemicals used in the treatment of sea lice infestations are subsequently released to the aquatic environment and may impact other aquatic organisms and their habitat. This paper will review the chemical therapeutants applied as bath treatments that are available to control sea lice in Canada and assess their potential risks to the aquatic ecosystem. The review will be limited to three pesticides currently or recently, applied in eastern Canada: Salmosan® (active ingredient: azamethiphos), Paramove 50® (active ingredient: hydrogen peroxide) and AlphaMax® (active ingredient: deltamethrin) and one formulation for which interest has been expressed by the aquaculture industry for registration, Excis® (active ingredient: cypermethrin). The authors have relied heavily on summary papers prepared by Burridge (2003), Haya et al. (2005) and Burridge et al. (2010b) and Burridge et al. (2010a).

SEA LICE BIOLOGY

The life cycle of the sea louse *L. salmonis* is shown in Figure 1. Adult females of *L. salmonis* are 8 to 12 mm in length, while males are about half of this size. The sea lice on cultured fish tend to be a bit smaller than those on wild fish. Sea lice eggs hatch directly into the water from egg strings fastened to the genital segment of females. The larvae are free-swimming nauplii

through one moult and then become infective copepodids. These are about 0.7 mm long and 0.3 mm wide, and it is at this stage that the sea lice can recognize and become attached to a host fish. It is, however, observed that adult sea lice can transfer from fish to fish. The dispersion of the nauplii is primarily passive as the larvae drift in the water, but the vertical movements of the larvae (copepodids are positively phototaxic) will also influence their position in a water column. In total, the sea lice pass through 10 stages, with one moult between each stage (Rae, 1979).

Sea lice development rates are dependent on the sea temperature. It takes a male 42 days, and a female 50 days, to develop from egg to adult at 10°C. The sea lice can, however, tolerate a relatively large range of temperatures and can hatch and develop at as low as 2°C (Boxaspen and Naess, 2000).

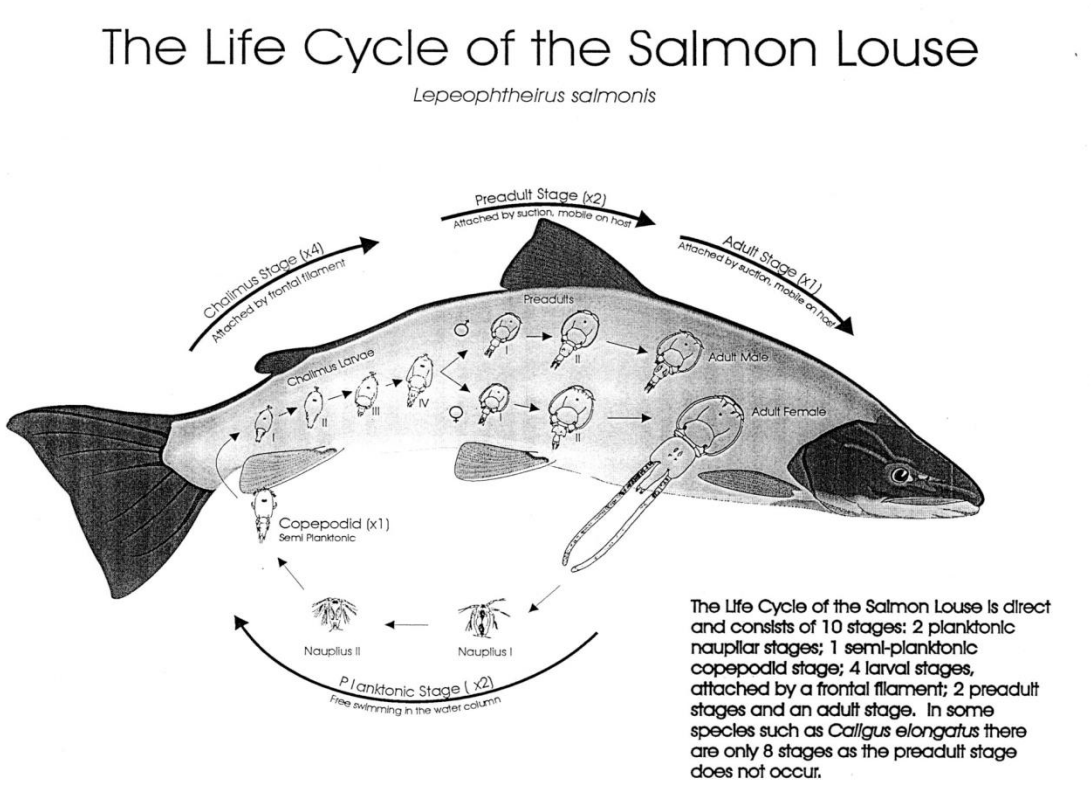

Figure 1. The life cycle of Lepeophtheirus salmonis (from Health Canada 2003).

The period during which a copepodid can infect a fish is called the infective window and is crucial in the control of sea lice. Larvae can infect fish from the first day after moulting, but they appear to be more infective after a few days. Longer than this and the copepodid exhausts its energy reserves, and becomes less successful in infecting susceptible host fish. Calculations based on empirical data indicate that the latest day that a larva can infect fish is 32.5 days after hatching at 6°C and 17 days at 12°C. Such long infective pelagic stages suggest that *L. salmonis* has a great potential for dispersion and that it can infect fish over a wide area away from the source. Thus, massive infection problems may be encountered by the salmon farming industry. This emphasizes the need for efficient husbandry strategies and chemical agents to control infections on fish farms and to reduce the potential for transfer of sea lice between farms (Haya et al., 2005). It also highlights the likelihood of this transfer in areas such as southwest New Brunswick where sites may be as close as 500 meters apart.

THERAPEUTANTS IN USE

A BRIEF HISTORY OF SEA LICE TREATMENT IN CANADA

Widespread and severe infestations of sea lice on farmed Atlantic salmon were first experienced in southwest New Brunswick in 1994. Previous to that a single cage site on Grand Manan was rumoured to have experienced a problem and the operators treated the affected fish with an organophosphate pesticide formulation called Nuvan® (active ingredient: chlorpyrifos) which had been used extensively in Europe.

During the infestation experienced in 1994, a series of products were granted emergency registration status from the PMRA and were used with varying levels of success. These included a pyrethrum formulation containing a mixture of natural pyrethrins, hydrogen peroxide in the Salartect® formulation, an in-feed product with ivermectin as the active ingredient, as well as Excis® and Salmosan®. The Excis® formulation was applied under a research permit in 1995. It was never registered by PMRA for emergency release due, in part, to objections from other government departments (Environment Canada (EC) and Fisheries and Oceans Canada (DFO)). During the period from 1995 through 2000 Salmosan® and Salartect® had full registration status with PMRA. However, Salartect® was considered a less efficacious product than Salmosan® and with reliance on only one product resistance to the product developed. An in-feed product, SLICE® (active ingredient: emamectin benzoate) became available under an emergency registration in 1999. This product works against all stages of the parasite, and is easy to use. This became the product of choice for salmon farmers throughout Canada, and was fully registered in 2009. As a result of universal use of SLICE® and poor efficacy of Salmosan® and Salartect® the manufacturer and distributors of these bath treatments did not apply to have their registration with PMRA renewed and the Canadian salmon aquaculture industry had only one product available as an anti-sea louse treatment option. An in-feed product, Calicide® was registered for use against sea lice infestations, but the active ingredient, teflubenzuron, is only effective against adult sea lice and for a number of years there was no manufacturer of the formulation.

SLICE® remained the only product used in Canada until 2009 when sea lice started to develop a resistance to the active ingredient. Poor efficacy of SLICE® resulted in a crisis situation in the salmon aquaculture industry in southwest New Brunswick in 2009 and 2010 and emergency registration was granted by PMRA for Salmosan®, Paramove® 50 and AlphaMax®. In the fall of 2010 Environment Canada issued a directive regarding the use of AlphaMax® and that product was no longer applied. Currently Salmosan® and Paramove® 50 are used in New Brunswick, Nova Scotia, and Newfoundland and SLICE® remains the only product used in British Columbia.

OVERVIEW OF BATH TREATMENTS

As outlined, the chemicals currently authorized for treating sea lice infestations are classified into two groups based on their route of administration, bath treatments or in-feed additives (Haya et al., 2005). This review will focus only on bath treatments.

In Canada, bath treatments are conducted in one of three ways: skirting, tarping, and well boats. Skirt and tarp treatments involve reducing the depth of the net in the salmon cage, thus reducing the volume of water. The net-pen and enclosed salmon are either completely surrounded by an impervious tarpaulin (tarping) or a skirt is hung around the cage to a depth exceeding that of the enclosed salmon (skirting) and the chemical is added to meet the recommended treatment concentration. The salmon are maintained in the bath for a specified period (usually 30-60 minutes) and aeration/oxygenation may be provided. After treatment, the tarpaulin is removed and the treatment chemical is allowed to disperse into the surrounding

water. Bath treatments are considered a topical application as the therapeutant is absorbed by the sea lice from the water.

Well boat treatments are conducted by pumping salmon into wells or treatment chambers on specially designed ships. Well boats used in Atlantic Canada typically have two wells each capable of holding ~300-350 m^3 of water. Fish are pumped into these wells, allowed to acclimate for a short period of time and then the pesticide is added to the appropriate concentration. Aeration/oxygenation may be provided. At the end of the prescribed treatment period (30-60 minutes) the wells are flushed by pumping in "clean" seawater. The fish are then pumped back into net-pens.

The use of well boats generally leads to smaller quantities of pesticides being used in comparison with tarp or skirt bath treatments. A "typical" 100 m net-pen, fully tarped at 3 m depth has a treatment volume of ~ 2250 m^3 while a skirt treatment of the same cage with a skirt depth of 4 m would result in a treatment volume of ~3000 m^3. Using a well boat the same net-pen would require four wells for treatment, but with a maximum treatment volume of 1400 m^3. As such, well boats require only 46% of a skirted treatment and 62% of a full tarp treatment and a concomitant reduction in pesticide use.

It is important to clearly distinguish between active ingredients and formulations. Active ingredients are the chemical compounds (pesticides) designed to kill the target organism (sea lice). The active ingredients are applied as part of pesticide formulations to optimise delivery, exposure and efficacy. The ingredients in the formulation will affect how the active compound behaves in the environment. While the PMRA requires data on the physical-chemical properties of the active ingredients as well as information on the constituents of the formulations, registration of a formulation is often completed without physical-chemical data specific to the formulation, such as solubility and partition coefficients for example.

Pesticide formulations are prepared to optimise the probability that the active ingredient reaches and affects the target organism, in this case sea lice. The formulation, therefore, is prepared with a number of chemicals in addition to the active ingredient, which may include solvents, surfactants, and stabilisers. These chemical additions to pesticide formulations are proprietary and therefore not known to the researcher. In work conducted at our laboratory at the St. Andrews Biological Station (SABS) we tested formulations of the product used commercially. Recommended treatment concentrations and chemical measurements are reported or prescribed on the basis of the active ingredient. In the absence of information on the constituents of the formulations it is impossible to quantify their concentration and estimate thresholds.

Pesticide formulations have a defined therapeutic index defining the difference in effectiveness for killing sea lice and the level that will negatively affect salmon. Infestations of sea lice cause stress to salmon making them more susceptible to disease and further infestation by sea lice. Other treatment-related factors are also known to cause stress in salmon. Handling, crowding and short-term exposure to pesticide formulations may all result in a generalised stress response in salmon (Wendelaar Bonga, 1997). In a sea lice treatment context these stressors are applied over short time periods (<1 h) and stress responses are also of short duration. Fish are known to recover quickly to acute, short-term stressors (Wendelaar Bonga, 1997). In addition, developers and suppliers of anti-sea louse formulations report wide safety margins for their products and salmon, i.e., the recommended treatment concentration is well below thresholds of effects for salmon. Hydrogen peroxide is the lone exception, see Paramove 50® section below.

This review focuses on four pesticide formulations that are either currently or have been used to combat infestations of sea lice in eastern Canada. While no bath treatments to combat sea lice

have taken place on Canada's west coast and none of the formulations are registered for use on that coast we will endeavour to consider potential consequences of use of pesticides on non-target organisms. Each formulation has a different active ingredient. The four formulations are Salmosan®, AlphaMax®, Excis®, and Paramove 50® and the four active ingredients are: azamethiphos, deltamethrin, cypermethrin, and hydrogen peroxide, respectively. The active ingredients in AlphaMax® and Excis® have the same mode of action. The active ingredients in Salmosan® and Paramove 50® have specific modes of action that are different from each other and from that for AlphaMax® and Excis®. All effects data presented in this review are reported as the concentration of the active ingredient.

Of the four pesticide formulations being considered for this review (Salmosan®, AlphaMax®, Excis® and Paramove 50®), only Paramove 50® is fully registered for use in finfish aquaculture in Canada; however, its registration is for use in hatcheries and not for use as a bath treatment to control sea lice. Both hydrogen peroxide, as the product Salartect®, and azamethiphos, as the product Salmosan®, were previously registered as anti-sea louse treatments for use in finfish aquaculture in Canada. Recently, both pesticides have been given emergency registration (ER) status and are being used to combat sea lice infestations in southwest New Brunswick (Health Canada, PMRA, 2012). Azamethiphos is still applied as Salmosan®; however, hydrogen peroxide is now applied as Paramove 50®. AlphaMax® was used under an ER from Health Canada in the fall of 2009 and the summer of 2010. Excis® was applied experimentally in southwest New Brunswick the mid 1990's and is used extensively in other jurisdictions (Chang and McLelland, 1996; 1997).

The information presented in this paper represents the relevant toxicity studies published in the literature for the therapeutants included in the scope of the paper and a considerable amount of unpublished data from recent DFO research (for which there is the intention to publish formally). As this is a review paper, detailed information on the experimental design, protocols, and rational for procedures are not included for the various studies discussed. Where possible, we present data that are most relevant in terms of realistic environmental exposures; however, data are scant in some circumstances.

SALMOSAN®

Efficacy and Mechanism of Action of Azamethiphos

Azamethiphos is an organophosphate insecticide and the active ingredient in the formulation Salmosan®. The formulation is a wettable powder consisting of 47.5% azamethiphos. It is used as a bath treatment at 100 µg L^{-1} for 30-60 minutes in well boats and tarps and at 150 µg L^{-1} if applied as a skirt treatment. At water temperatures below 10°C, treatments can last up to 60 min at the discretion of the attending veterinarian. 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. The PMRA's Emergency Registration limits the application of Salmosan® to two treatments per day per aquaculture site. Azamethiphos has a therapeutic index for salmon of near 10 (Haya et al., 2005).

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).

The use of Salmosan® was discontinued in Canada in 2002. The product had ceased to be effective, in-feed products were available and the registrant did not request a renewal of the registration through PMRA. Burridge et al. (2010b) noted that after several years of no sales, Salmosan® was re-introduced as an anti-sea louse treatment in Europe in 2008. It was given an Emergency Registration for use in New Brunswick in 2009.

Distribution and Fate of Azamethiphos

Azamethiphos is soluble in water (1.1 g L^{-1}) and has a low octanol-water partition coefficient (log K_{ow} = 1.05) (SEPA, 2005). The log K_{ow} is the logarithm of the octanol-water partition coefficient. 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 et al., 2000). Consequently, 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 µg L^{-1}) the concentration of azamethiphos was below detection (0.1 µg 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)

Laboratory Studies (published data)

Lobster and shrimp were the most susceptible species to azamethiphos (in Salmosan® formulation) 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: Stage I 3.57 µg L^{-1}, Stage II 1.03 µg L^{-1}, Stage III 2.29 µg L^{-1}, Stage IV 2.12 µg L^{-1}, and adults 1.39 µg L^{-1} (Burridge et al., 1999). LC_{50}s are reported as the concentration of azamethiphos. There was no statistically significant difference between these values. There is a seasonal aspect to susceptibility of American lobsters to azamethiphos. 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., 2000a), the No Observed Effect Concentration (NOEC) was nine exposures of 30 min each over three days to 1 µg L^{-1} of azamethiphos. In addition to observed lethality, many surviving lobsters showed significant behavioural responses, after repeated exposure to concentrations of 10 µg L^{-1} (see description below).

Research commissioned by Ciba Geigy on Salmosan® shows that azamethiphos is only lethal to several groups of invertebrates (bivalve molluscs and gastropods, amphipods, and echinoderms) at concentrations greater than the prescribed treatment concentration of 100 µg L^{-1} (SEPA, 2005). The 24-h LC_{50} of azamethiphos to the copepod, *Temora longicornis*, is reported to be >10 µg L^{-1}. The 96-h LC_{50} for European lobster larvae, *Homarus gammarus*, is 0.5 µg L^{-1} and is in general agreement with the 48-h LC_{50} for the American lobster, 1.39 µg L^{-1} (Burridge et al., 1999). Finally, the 96-h LC_{50} for the mysid shrimp, *Mysidopsis bahia*, is reported as 0.52 µg L^{-1} (SEPA, 2005).

In laboratory studies, 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., 2000a). 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.

Laboratory studies were conducted to investigate possible sublethal effects of Salmosan® exposure on American lobster. Preovigerous females were exposed for 1 h biweekly to 10 µg 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 (*Stongylocentrotus droebrachiensus*), the white sea urchin (*Lytechinus pictus)* (fertilization); the Three spine stickleback (*Gasterosteus acualeatus*); three amphipods (*Amphiporeia virginiana, Gammarus spp,* and *Eohaustorius estuarius*); 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 µg L^{-1}.

The response of mussels to stimuli was unaffected by exposures to 10.0 µg 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 µg L^{-1} and exposure to 46.0 µg 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 µg L^{-1}, respectively. Herring larvae were reported to tolerate azamethiphos better than another organophosphate, dichlorvos (Trade Name DDVP) (Roth et al., 1993).

Biological Effects of Salmosan® (unpublished results)

In 2011-2012 staff at SABS conducted a series of bioassays to determine the acute response of several invertebrate species to Salmosan® (Table 1). Preliminary 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 L^{-1} followed by 95 h in clean water. The LC_{50} for adult lobsters was estimated to be 24.8 µg azamethiphos L^{-1}. Table 2 shows the LT_{50} estimates for the two concentrations of azamethiphos that resulted in

>50% mortality of exposed organisms following 1-h exposures and 95 h of monitoring. Under these conditions only adult lobsters were killed with >50% mortality occurring very quickly or not at all. When adult lobsters were exposed to Salmosan® continuously for 10 days the LC_{50} was estimated to be 0.216 µg azamethiphos L^{-1} (Table 6).

Table 1. The 1-h LC_{50} of Salmosan® (as azamethiphos) to several crustacean species. Organisms were exposed for 1 h then monitored for a further 95 h. Estimates are calculated as the mean of several replicate bioassays (N) and are based on measured concentrations of azamethiphos.

Species/Life Stage	**LC_{50} (µg L^{-1})**	**95% CI**	**N**	**Dilution factor[a]**
Lobster Stage I	> 86.5	ND	4	<1.2
Lobster Adult	24.8	21.7-27.9	3	4.0
Crangon septemspinosa	>85.5	ND	3	<1.2
Mysid sp.	>85.5	ND	3	<1.2

[a] Dilution factors are based on prescribed treatment concentrations of 100 µg L^{-1} as azamethiphos.
ND – not determined

Table 2. LT_{50} (h) of Salmosan® (as azamethiphos) to several crustacean species for measured concentrations of azamethiphos in water. Organisms were exposed for 1 h then monitored for a further 95 h.

Concentration µg L^{-1}	**Stage I Lobster**	**Adult Lobster**	**Mysids**	**Crangon**
85.5	>95	0.75	>95	>95
27.7	>95	2.5	>95	>95

In 2012-2013, staff at SABS conducted laboratory bioassays to determine the acute toxicity of Salmosan® 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 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 µg L^{-1} (nominal concentration of azamethiphos). A consistent concentration-response of feeding was not observed in four bioassays and an EC_{50} could not be calculated.

As noted previously, azamethiphos has a low probability of binding to organics or sediment. In 2011 and 2012 staff at SABS compared the toxicity of Salmosan® to Stage IV-V post-larvae lobster when exposed in filtered seawater only versus raw seawater with a sediment substrate. Lobsters were exposed to 12 or 57 µg L^{-1} of azamethiphos for 1 h under static conditions, with a return to flow-through conditions with clean water for an additional 96 h. Affects were noted at both treatment concentrations including changes in behaviour and animals moribund (non-responsive but respiring) or dead (Dr. Andrew Cooper, DFO, pers. comm.). 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; or 2) 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.

In May 2012, staff at SABS conducted a study to determine the response of adult lobsters to repeated exposure to sublethal concentrations of azamethiphos, as Salmosan®. Groups of 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 µg L^{-1} of azamethiphos (nominal; representing "low" or "high" sublethal concentrations) for 30 minutes over 3 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 time of year and stage of the molt cycle).

In the fall of 2012, SABS staff in collaboration with DFO staff from l'Institut Maurice Lamontagne (Dr. C.M. Couillard and B. Légaré), exposed adult male lobsters to 0.078 µg L^{-1} of azamethiphos (in Salmosan® formulation) continuously for 10 days, in order to simulate exposure to Salmosan® at a distance from farm sites with multiple treatments over a 10 day period. In addition to the direct effects of sublethal exposure to Salmosan®, effects on the ability of adult lobster to cope with simulated live transport and the persistence of the effects after a 24 h depuration period in clean seawater were also assessed.

At the end of the 10 day exposure period, one group of controls and treated lobsters were dissected and sampled. A second group of controls and treated lobsters was exposed to simulated commercial live transportation, in a cold room at 7°C for approximately 24 h before sampling. One last group of controls and treated lobsters was transferred to clean seawater and held for 24 h before sampling. In addition to the classical biomarker of organophosphate neurotoxic activity, cholinesterase (ChE) activity in muscle, indicators of stress (haemolymph protein) and altered energy allocation (hepatosomatic and gonadosomatic indices) were evaluated.

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 shipped control lobsters. Treatment with azamethiphos significantly reduced acetylcholinesterase activity. Hepatosomatic index and hepatopancreas lipid content were increased and gonadosomatic index was reduced in male lobster exposed to azamethiphos.These effects persisted after 24-h depuration or shipping. Haemolymph protein concentration was also elevated in treated lobsters after exposure; the effect was greater after simulated shipping.

Thus, preliminary results of this study indicate 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 lobster are exposed to further chemical or non-chemical stress.

Preliminary results also indicate that sublethal exposure to azamethiphos markedly increases the risk of mortality of adult lobsters during simulated live transportation. The mechanism for this indirect effect of sublethal exposure to azamethiphos is not known but is under investigation. Further studies are needed on the interactions between aquaculture pesticides and other stress such as hypoxia, emersion, high temperature, and handling since these interactions could lead to lethal impacts on crustaceans exposed to sublethal concentrations of pesticides.

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 depths 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 the 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 were not achieved until post-release times ranging from 2-5.5 h. Most samples from the plume were not toxic when azamethiphos as Salmosan® 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.

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 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, St. Andrews Biological Station, St. Andrews, NB, unpublished data).

ALPHAMAX® AND EXCIS®

Efficacy and Mechanism of Action of Deltamethrin and Cypermethrin

Pyrethrins are the active constituents of an extract from flower heads of *Chrysanthemum cinerariaefolium*. This mixture of chemically related compounds has been used for their insecticidal activity since the late 19th century (Davies, 1985). The pyrethrins decompose readily as they are susceptible to catabolic enzymes and sunlight. In the early 1960s, synthetic

analogues that were more persistent than the natural pyrethrins were developed and referred to as pyrethroids (Barthel, 1961). It was their high degradability, low toxicity to mammals and high toxicity to crustaceans that led to the initial interest in pyrethrins and pyrethroids as treatments for sea lice infestations.

The mechanism of action of the pyrethroids involves interference with nerve membrane function, primarily by their interaction with sodium (Na^+) channels (Miller and Adams, 1982), which results in depolarization of the nerve ending. In the case of the synthetic pyrethroids cypermethrin and deltamethrin, this interaction results in repetitive firing of the nerve ending resulting in eventual paralysis and death (Crane et al., 2011; Haya et al., 2005).

Deltamethrin is the active ingredient in the formulation AlphaMax® and cypermethrin is the active ingredient in the formulation Excis®. Each pyrethroid makes up 1% of their respective formulations, the remaining solvents, surfactants, and other formulation products are not publicly known. AlphaMax® and Excis® are registered or approved for use in a number of salmon producing nations. While an application for registration of Excis® in Canada in the late 1990s was refused, AlphaMax® was given an emergency registration for use in southwest New Brunswick in 2009 and 2010.

The recommended treatment of salmon against sea lice is a 40-minute bath with AlphaMax® with a target concentration of 2.0 µg deltamethrin L^{-1} (SEPA, 2008) or a 1-h bath with Excis® with a target concentration of 5.0 µg cypermethrin L^{-1} in tarped cages (SEPA, 1998). The pyrethroids, cypermethrin and deltamethrin, are effective against all attached stages of sea lice including adults, and therefore, less frequent treatments should be required than with organophosphates; 5-6 week intervals rather then 2-3 week intervals, respectively for these classes of pesticide (SEPA, 1998; Haya et al., 2005).

In one of five Norwegian salmon sites that used deltamethrin for the treatment of sea lice, there was a significant decrease in effectiveness of the treatment with an increase in the number of treatments (Sevatadal and Horsberg, 2003). Bioassays using pre-adult stage II sea lice under laboratory conditions verified that resistance contributed to treatment failure, and that the EC_{50} was 25-times higher than at an area previously unexposed.

Distribution and Fate of Deltamethrin and Cypermethrin

Synthetic pyrethroids are unlikely to accumulate to a significant degree in fish and aquatic food chains since they are rapidly metabolized (Kahn, 1983). Deltamethrin and cypermethrin have very low water solubility (<2 and 4 µg L^{-1}, respectively) and a log K_{ow} of 4.6 and 4.5, respectively (Tomlin, 1994; Vershueren, 1996). While not expected to persist in the aqueous phase, these pyrethroids can persist in sediments and may be desorbed and affect benthic invertebrates (Haya et al., 2005). Much of the available information on deltamethrin and cypermethrin comes from the freshwater literature, although several recent publications have addressed deltamethrin use in marine waters (Gross et al., 2008; Fairchild et al., 2010; Crane et al., 2011).

Deltamethrin's high toxicity and rapidity of action could cause significant harm to limnic ecosystems after direct treatment (Thybaud, 1990). The adsorption of pyrethroids onto suspended solids can produce dramatic reductions in the apparent toxicity of the compound. The 96-h LC_{50} value for Rainbow trout is 0.1-0.5 µg deltamethrin L^{-1} (NRCC, 1986). When trout were caged in a pond containing 14-22 mg L^{-1} suspended solids, the 96-h LC_{50} was 2.5 µg deltamethrin L^{-1}. In a pond sprayed with deltamethrin containing 11 and 23 mg L^{-1} suspended solids, deltamethrin partitioned rapidly to suspended solids, plants, sediment, and air with a half-life of 2-4 h in water (Muir et al., 1985). Because pyrethroids tend to adsorb onto particulate matter, chronic aqueous exposures may not occur other than in laboratory studies. However, the presence of surfactants and stabilizers in aquaculture formulations may affect the rate of

adsorption of these pyrethroids to particulate matter, keeping them in solution longer than expected.

Biological Effects of AlphaMax® and Deltamethrin

Laboratory Studies (published)

The impact of pyrethroids on non-target aquatic animals, especially invertebrates has been reviewed (Mian and Mulla, 1992). In general pyrethroids are more toxic to non-target insects and crustaceans than to other phylogenetically distant invertebrates. Among arthropods, however, crustaceans are phylogenetically closer to insects than molluscs and showed noticeable sensitivity (Hill, 1985; Haya et al., 2005).

Deltamethrin is extremely toxic to crustaceans. The 96-h LC_{50} for adult lobsters was determined to be 0.0014 µg L^{-1} (1.4 ng L^{-1}) for deltamethrin in the agricultural formulation Decis® (Zitko et al., 1979). Fairchild et al. (2010) reported the 96-h LC_{50} for deltamethrin in the AlphaMax® formulation was 3.7 – 4.9 ng L^{-1} for Stage III lobster larvae and 28.2 ng L^{-1} for Stage IV post-larvae. The 96-h LC_{50} for the amphipod, *Eohaustorius estuarius,* was between 1.7 and 8.0 ng L^{-1}. The Sand shrimp, *Crangon septemspinosa*, was less sensitive to AlphaMax® with a 96-h LC_{50} of 45.3 ng L^{-1} (Fairchild et al., 2010). These authors exposed these invertebrates to various formulations of deltamethrin and for various lengths of time including 1-h exposures followed by 95 h or 16 days in clean water. The LC_{50}s determined after only 1-h exposure were 36.5, 13.1, and 142 ng L^{-1} for Stage III lobster larvae, *E. estuarius*, and *C. septemspinosa*, respectively. Irreversible immobilization was also observed for *E. estuarius* with an EC_{50} of 5.5 ng L^{-1}.

There are some data which suggest that deltamethrin may have a sublethal effect on the immune function of fish (Pimpão et al., 2007; 2008); however, the exposure to pesticide was by injection and the environmental relevance is unclear.

Biological Effects of AlphaMax® (unpublished results)

In 2010 and 2011 staff at SABS conducted a series of bioassays to determine the acute response of several invertebrate species to AlphaMax®. These studies consisted of 1- or 24-h exposure to AlphaMax® followed by 95 or 72 h in clean water (respectively), for monitoring of delayed toxicity, with the objective of being more representative of environmentally relevant durations of exposure. These data are summarised in Tables 3 and 4. Briefly, estimates of the 24-h LC_{50}s for Stage I, II and IV lobster larvae are 0.8, 0.6, and 1.7 ng L^{-1}, respectively. These estimates are based on average measured concentrations of deltamethrin in exposure tanks. The 24-h LC_{50} for adult lobsters was estimated to be 15 ng L^{-1}. The mysid shrimp *Mysis sp.* were equally sensitive to AlphaMax® as lobster larvae with a 24-h LC_{50} of 1.4 ng deltamethrin L^{-1}. *Crangon septemspinosa* was less sensitive with a 24-h LC_{50} of 27 ng deltamethrin L^{-1}.

The same species were exposed to AlphaMax® for 1 h followed by holding for 95 h in clean seawater (Table 4). The 1-h LC_{50} estimates based on measured concentrations of deltamethrin are as follows: Stage I lobster larvae 3.4 ng L^{-1}, adult lobsters 18.8 ng L^{-1}, *Mysis sp.* 13.9 ng L^{-1}. As would be expected, these lethal thresholds are higher than those calculated for 24-h exposures, except in the case of adult lobsters where the 1- and 24-h LC_{50}s are essentially the same. Tables 3 and 4 also include estimates of the dilution factors based on prescribed treatment concentrations. For the most sensitive species tested, larval lobsters, the LC_{50}s over 1 or 24 h are an approximate 600-or 3000-fold dilution of the recommended treatment concentration, respectively.

Table 3. The 24-h LC_{50} of AlphaMax® (as deltamethrin) to several crustacean species. Organisms were exposed for 24 h then monitored for a further 72 h. Estimates are calculated as the mean of several replicate bioassays (N) and are based on measured concentrations of deltamethrin.

Species/Life Stage	LC_{50} (ng L^{-1})	95% CI	N	Dilution factor[a]
Lobster Stage I	0.8	0.6-1.0	12	2500
Lobster Stage II	0.6	0.3-1.0	5	3300
Lobster Stage IV	1.7	0-4.8	3	1200
Lobster Adult	15	11-19	3	130
Mysis sp.	1.4	0-3.6	3	1400
Crangon septemspinosa	27	14-40	3	75

[a] Dilution factors are based on prescribed treatment concentrations of 2000 ng L^{-1} as deltamethrin.

Table 4. The 1-h LC_{50} of AlphaMax® (as deltamethrin) to several crustacean species. Organisms were exposed for 1 h then monitored for a further 95 h. Estimates are calculated as the mean of several replicate bioassays (N) and are based on measured concentrations of deltamethrin (except where noted).

Species/Life Stage	LC_{50} (ng L^{-1})	95% CI	N	Dilution factor[a]
Lobster Stage I	3.4	1.5-6.0	2	590
Lobster Stage III[b]	36.5	25.0 – 53.3	1	55
Lobster Adult	18.8	3.9-33.6	3	110
Mysis sp.	13.9	10.9-17.7	3	140
Eohaustorius estuarius[c]	13.1	4.77 – 35.8	1	150
Crangon septemspinosa[c]	142	104 – 194	1	14

[a] Dilution factors are based on prescribed treatment concentrations of 2000 ng L^{-1} as deltamethrin.
[b] 1-h exposure followed by 16 days in "clean" water from Fairchild et al. (2010) at 20°C, based on nominal concentrations
[c] From Fairchild et al. (2010) at 15-16°C, based on nominal concentrations

Additionally, LT_{50} estimates were derived for a number of concentrations of deltamethrin found to result in >50% mortality of exposed organisms during 1-h exposures followed by 95 h of monitoring (Table 5). High concentrations of deltamethrin are necessary to kill >50% of exposed *Crangon*, but this threshold is met quite quickly (<5 h). Interestingly, and possibly of concern, is the observation that a 1-h exposure of Stage I lobster larvae to AlphaMax® can result in >50% mortality of exposed animals several days later (Table 5).

Table 5. LT_{50}(h) of AlphaMax® to various invertebrate species. Organisms were exposed for 1 h then monitored for a further 95 h. Water concentrations are ranges of measured concentrations of deltamethrin from several bioassays (except where noted).

Concentration ng L^{-1}	Lobster Stage I	Lobster Stage III[a]	Lobster Adults	Mysids	Crangon[a]
1000	ND	4.9	ND	ND	4.9
320	ND	ND	ND	ND	4.9
75-148	50	ND	5.5	20	ND
22-48	55	ND	5	>95	ND
7.6-8.3	42	ND	>95	>95	ND
2.5-6.7	37	>384	>95	>95	ND
1.0-2.1	42	>384	>95	>95	ND

[a] From Fairchild et al. (2010), based on nominal concentrations
ND - not determined

A 10-day constant exposure of adult lobsters to AlphaMax® resulted in an LC_{50} estimate of 14.7 ng L^{-1} based on measured concentrations of deltamethrin (Table 6). This is similar to the 1-h and 24-h LC_{50}s of 15 and 18.8 ng L^{-1}, respectively. This indicates that this product acts quickly, at low concentrations, and acute exposures can have lasting effects. There are examples of this with other species as well. A 48-h (+ 48 h recovery) LC_{50} of 16 ng L^{-1} and EC_{50} (immobility) of 4.2 ng L^{-1} recently determined for *E. estuarius* (Environment Canada Atlantic Laboratory for Environmental Testing (ALET) unpublished data) was similar to the 1-h (+ 95 h recovery) values previously reported (13.1 ng L^{-1} and 5.5 ng L^{-1}, respectively, in Fairchild et al., 2010). For *C. septemspinosa*, the 14-day LC_{50} of 23.8 ng L^{-1} (Fairchild et al., 2010) was similar to the 24-h (+ 72 h recovery) of 27 ng L^{-1}. The 16-day LC_{50} of 4.5 ng L^{-1} for Stage III lobster larvae was similar to the 96-h LC_{50} of 3.7-4.7 (Fairchild et al., 2010). The exact reasons for these patterns are not known, as they seem counterintuitive to basic principles of toxicology, but should warrant further investigation of the mechanisms of toxicity.

Table 6. The 10-day LC_{50} of AlphaMax® and Salmosan® to adult lobsters (as measured deltamethrin and azamethiphos, respectively). Dilution factors are based on recommended treatment concentrations of 2 µg L^{-1} and 100 µg L^{-1} for deltamethrin and azamethiphos, respectively. Organisms were exposed continuously for 10 days.

Compound	LC_{50} (ng L^{-1})	(95% C.I.)	N	Dilution factor
Deltamethrin	14.7	7.70-21.6	3	140
Azamethiphos	216	157-273	3	460

In May 2012, staff at SABS conducted a study to determine the response of adult lobsters to repeated exposure to sublethal concentrations of deltamethrin, as AlphaMax®. Groups of lobsters (n = 20/group, with consistent proportions of males and females) were exposed 1, 2, 4,

or 6 times to either 2 or 20 ng L^{-1} of deltamethrin (nominal; representing "low" or "high" sublethal concentrations) for 30 minutes over 3 days. After each exposure, each lobster was scored as to the degree of paralysis and/or disorientation. The lobsters that displayed problems were all in the 4- and 6-exposure "high" concentration groups (40% of each group). One lobster in each group (5%) was severely affected and death occurred within 18 hours. A lobster in the 4-exposure group that was moderately affected, recovered within 36 h. The 6 other "affected" lobsters in the 4-exposure group had only minor paralysis in a couple of their walking legs and recovered within an hour. Recovery from minor affects after 6 exposures took ~24 h. Although mortality was greatest in the 20 ng L^{-1} treatment (average 7.5% versus 2% in the 2 ng L^{-1} group and 0% in the control group), differences between groups were not statistically significant and repeated exposure did not increase mortality (Table 7). Nor were there any detectable effects on molting, growth, female mating behaviour, or cement gland development.

Table 7. Mortality in groups of 20 adult lobsters exposed to AlphaMax® (as deltamethrin) multiple times for 30 minutes over 3 days. Data courtesy of Susan Waddy (DFO).

Nominal Concentration	**Number of exposures**	**Mortality %**
Control	0	0
Low (2 ng L^{-1})	1	0
	2	0
	4	5
	6	5
High (20 ng L^{-1})	1	10
	2	0
	4	9.5
	6	10.5

In 2012-2013, staff at SABS, conducted laboratory bioassays (at 9-13°C) to determine the acute toxicity of AlphaMax® 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 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. Copepods exposed to 2000 or 200 ng L^{-1} of deltamethrin (nominal concentration) were immobilized and sank to the bottom of test beakers within 15-45 and 15-60 minutes of the 1-h exposure, respectively. At the end of the 5 h recovery, immobilized organisms were stained and exhibited little to no movement other than occasional twitching of antennae, indicating they were alive, although moribund, instead of dead. Lethality was not observed at concentrations as high as 20000 ng L^{-1} of deltamethrin (nominal). Feeding behaviour was affected and a mean EC_{50} (range of EC_{50}) of 35 (17-67) ng L^{-1} was determined based on measured deltamethrin concentrations in five bioassays.

As noted previously, the hydrophobic properties of pyrethroids can result in adsorption onto suspended solids and reduce the apparent toxicity of the compound. To examine this, staff at SABS compared the toxicity of AlphaMax® to Stage IV-V post-larvae lobster when exposed in filtered seawater only versus raw seawater with a sediment substrate. Lobsters were exposed to ~30 or 90 ng L^{-1} of deltamethrin for 1 h under static conditions, with 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 and animals moribund (non-responsive but respiring) or dead (Dr. Andrew Cooper, DFO, personal communication). However, these responses were not significantly different between the two types of exposures suggesting that the presence of organic solids (raw seawater and sediment) did not reduce the toxicity of deltamethrin under the conditions tested.

Biological Effects of AlphaMax® in Sediment

Due to its lack of water solubility, high lipophilicity, and high adsorption coefficients deltamethrin is predicted to absorb preferentially to particles, particularly those with high organic content, and to sequester to bottom sediments (Muir et al., 1985). The half-life for deltamethrin in marine sediments has been estimated at approximately 140 days, indicating that multiple treatments may result in accumulation of this compound in sediments near cage sites (Gross et al., 2008). Studies to examine the effects of deltamethrin in sediment on benthic invertebrates were conducted at the Environment Canada Atlantic Laboratory for Environmental Testing (ALET) and SABS in 2011-2012. No data in the published literature exist on the sediment toxicity of deltamethrin to marine species.

Eohaustorius estuarius and *Crangon septemspinosa* were exposed to AlphaMax® spiked into field-collected natural sediment relatively free of anthropogenic contamination. The 10-day LC_{50} for *E. estuarius* was 0.47-0.54 µg kg^{-1}. For *C. Septemspinosa* the 14-day LC_{50} was 8.6 µg kg^{-1} and IC_{50} for growth inhibition was 1.2 µg kg^{-1}. Similar to water-only exposures, *C. septemspinosa* was much less sensitive than *E. estuarius*. These estimates are based on nominal concentration of deltamethrin (ALET unpublished data).

The commercially valuable polycheate worm, *Nereis virens*, was exposed to AlphaMax® spiked into commercial sand or field-collected natural sediment. In 7-day tests, significant mortality was not observed in concentrations up to 700 µg kg^{-1}. Sublethal effects (i.e., burrowing avoidance or emergence onto the sand surface, hindered mobility or broken/damaged sections) were observed in the majority of worms exposed to concentrations ≥100 µg kg^{-1} in sand. The same extent of effects were only observed at 400 µg kg^{-1} in natural sediment, indicating its slightly higher content of total organic carbon likely reduced the bioavailability (and hence toxicity) of deltamethrin to the worms. No adverse effects were observed in a 30-day test with spiked sediment at concentrations up to the highest test concentration of ~150 µg kg^{-1}. Additionally, worms exposed to AlphaMax® directly in water only exhibited signs of mortality and impaired mobility at concentrations 5-times the recommended treatment concentration. Reported concentrations are measured concentrations of deltamethrin.

Environmental concentrations of deltamethrin in sediment related to use in aquaculture is scant. Chemical monitoring for therapeutants in sediment around aquaculture sites is not a requirement in Canada and deltamethrin has not been analyzed in SEPA's annual screening surveys of sediments around marine fish farms. The 10-d LC_{50}s determined for *E. estuarius* of 0.47-0.54 µg kg^{-1} are just slightly above the PNEC of 0.33 µg kg^{-1} proposed by SEPA (2008), suggesting that this value is not sufficiently protective of sensitive benthic species.

Biological Effects of Excis® and Cypermethrin

Published Studies

Cypermethrin is also very toxic to crustaceans, but to a lesser extent than deltamethrin. Burridge et al. (2000b) reported the 48-h LC_{50}s for Stage I, II, and III lobster larvae and Stage IV post-larvae were 0.18, 0.12, 0.06, and 0.12 µg L^{-1}, respectively, and the 24-h LC_{50} for adult lobster was 0.14 µg L^{-1}, as measured concentrations of cypermethrin in Excis®. The amphipod *Amphiporeia virginiana* was less sensitive to Excis® with a 48-h LC_{50} of 6.9-7.2 µg L^{-1}; however, when held for another 48 h post-exposure additional mortality occurred and the LC_{50} was 0.012

µg L^{-1}. Immobility was an observed effect for this amphipod with a 48-h EC_{50} of 0.0034 µg L^{-1} (Ernst et al., 2001). Ernst et al. (2001) used another amphipod *E. estuarius* to test the toxicity of water samples collected from a net-pen following treatment with Excis®. They reported 48-h LC_{50}s ranging from >0.004 to 3.6 µg L^{-1} and EC_{50}s (immobility) from 0.007 to 0.04 µg L^{-1}, based on measured concentrations. The effects of technical grade cypermethrin on a number of copepod species (non-parasitic) have also been investigated in 48-h studies. With *Acartia tonsa*, the 48-h LC_{50} for eggs was 0.129 µg L^{-1} and 48-h EC_{50} for feeding rate of copepodids was 0.065 µg L^{-1} (Barata et al., 2002). Willis and Ling (2004) reported 48-h EC_{50}s for immobility ranging from 0.12 to >5 µg L^{-1} in the nauplii, copepodid, and adult stages of four species of copepods (*Oithona similis, Acartia clausi, Pseudocalanus elongatus, Temora longicornis*).

McLeese et al. (1980) reported 96-h LC_{50}s for technical grade cypermethrin of 0.04 µg L^{-1} for adult lobsters and 0.01 µg L^{-1} for *Crangon septemspinosa*. Gross et al. (2008) also reported toxicity to brown shrimp, *Crangon crangon*, of 0.140 µg L^{-1} for 6 h exposures. Those values represent a 35-500 dilution of the recommended treatment concentration. According to the data from Ernst et al. (2001), these dilutions could occur up to 4 h post-release.

A variety of taxa have been tested for their sensitivity to cypermethrin (see Ernst et al., 2001) with LC_{50}s as much as three orders of magnitude below the recommended treatment concentration (5 µg L^{-1}). However, these tests have predominantly involved a standard 96-h exposure, which is not representative of the short exposures (i.e., minutes to hours) expected to occur in the scenario of aquaculture treatment. Even the 48-h toxicity data previously discussed may overestimate risks to non-target species due to the disparity in duration of exposure. Only a few studies have examined more acute exposures to cypermethrin, these are discussed below.

Burridge et al. (2000a) conducted repeated short-term exposures (e.g., 15-120 minutes) with lobsters to simulate actual treatment conditions where a cage-site operator would be treating multiple salmon cages per day for several days. They demonstrated that Stage IV lobsters could survive repeated short-term exposures to high concentrations of Excis® (i.e., 25% of the recommended treatment concentration of 5 µg L^{-1} as cypermethrin), but became inactive and in some cases moribund. Lethality in adult lobsters occurred quickly, after as few as two exposures of 30 minutes to the highest test concentration (LT_{50} of 25.5 h at 1.8 µg L^{-1} for 30 minutes). Adult lobsters were able to survive repeated exposures to <0.5% of the recommended treatment concentration, but showed signs of distress when exposed to 1% of the recommended treatment concentration. The estimated 48-h LC_{50} was 0.081 µg L^{-1} based on the mean test concentration of cypermethrin. Pahl and Opitz (1999) reported LC_{50}s for Stage II lobsters were 0.058-1.69 µg L^{-1} for exposures to Excis® ranging from 5 minutes to 12 h, with 12 h in clean water. Morbidity or twitching was observed at concentrations as low of 0.005 µg L^{-1}.

Medina et al. (2004a) monitored survival of the adult copepod *Acartia tonsa* for 144 h following 1- or 24-h pulse exposures to technical grade cypermethrin. Survival was significantly affected after 24-h exposure to 0.2 µg L^{-1} (lowest test concentration) and mortality increased with time. They noted the responses following the 24-h pulse were similar to those throughout 96 h of continuous exposures (Medina et al., 2002; 96-h LC_{50} of 0.142 µg L^{-1}) and suggested that damage caused in the first 24 h of exposure could explain long-term toxicity responses in *A. tonsa*. In addition, delayed toxicity following a 1-h pulse impaired male survival at 0.7 µg L^{-1} and changed copepod sex ratios.

Medina et al. (2004b) examined the impact of a single application of technical grade cypermethrin (5 µg L^{-1}) to marine zooplankton in mesocosm studies. Zooplankton density and biodiversity were reduced 2 days after treatment. Zooplankton density recovered, but

biodiversity remained altered, as copepod populations were permanently affected in the 14 days post-treatment.

Unpublished Studies

In 2012, staff at SABS conducted a series of bioassays (in triplicate at ~14°C) to determine the acute lethality of Excis® to the mysid shrimp, *Praunus flexuosus*. Shrimp were exposed for 1 or 24 h followed by transfer to clean water for monitoring of latent effects to a total duration of 96 h. A 1-h LC_{50} could not be determined as a maximum mortality of 22% was observed at the highest concentration tested of 0.142 µg L^{-1}. Sublethal effects of impaired mobility (swimming/balance) were only observed for the 1-h exposure at this highest test concentration and some recovery occurred with time. The average 24-h LC_{50} was 0.033 µg L^{-1}. With these 24-h exposures, impaired mobility was observed in concentrations as low as 0.016 µg L^{-1}. Reported concentrations are the average measured concentration of cypermethrin.

In 2012-2013, staff at SABS conducted laboratory bioassays (at 9-13°C) to determine the acute toxicity of Excis® 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 5 or 0.5 µg L^{-1} as cypermethrin (nominal) were immobilized and sank to the bottom of test beakers within 15 and 45-60 minutes of the 1-h exposure, respectively. At the end of the 5 h recovery, immobilized organisms were stained and exhibited little to no movement other than occasional twitching of antennae, indicating they were alive, although moribund, instead of dead. Lethality was not observed at concentrations as high as 50 µg L^{-1} cypermethrin (nominal). Feeding behaviour was affected and a mean EC_{50} (range of EC_{50}) of 0.26 (0.098-0.36 µg L^{-1} was determined based on measured cypermethrin concentrations in five bioassays.

Table 8 summarizes the acute LC_{50}s of cypermethrin for several crustacean species and also includes estimates of the dilution factors based on prescribed treatment concentration. For the most sensitive species tested, larval lobsters and *P. flexuosus*, their respective 12- or 24-LC_{50}s are an approximate 86- or 150-fold dilution of the recommended treatment concentration.

Table 8. Estimated acute LC_{50} of Excis® (as cypermethrin) to several crustacean species. Estimates are calculated as the mean of several replicate bioassays (N) and are based on measured concentrations of cypermethrin (except where noted).

Species/Life Stage/Exposure	LC_{50} (µg L^{-1})	95% CI	N	Dilution factor[a]
Lobster Stage II – 5 min[b]	0.66-1.69	ND	1	3-7.5
Acartia tonsa males – 1 h[c]	~0.7-2.2	ND	1	2-7
Praunus flexuous – 1 h	>0.142	ND	3	<35
Lobster Stage II – 12 h[b]	0.058-0.365	ND	1	14-86
Acartia tonsa – 24 h[c]	~0.2-0.8	ND	1	6-25
Praunus flexuous – 24 h	0.033	0.025-0.044	3	150
Lobster adult – 24 h[d]	0.14	ND	1	35

[a] Dilution factors are based on a prescribed treatment concentrations of 5 µg L^{-1} as cypermethrin.
[b] 5-min or 12-h exposure followed by 12 h in clean water from Pahl and Opitz (1999) at 10-12°C, based on nominal concentrations.
[c] 1- or 24-h exposure to technical grade cypermethrin followed by 144 h in clean water from Medina et al. (2004a) at 20°C, estimated values.
[d] From Burridge *Biological Effects of Excis® and cypermethrin (unpublished)*

Biological Effects of Excis® and Cypermethrin in Sediment

Cypermethrin will also adsorb to particles and potentially persist in sediments, with a half-life in sediment estimated at approximately 35 to 80 days, in high and low organic sediment, respectively, under aerobic conditions (SEPA, 1998). Little data in the published literature exist on the sediment toxicity of cypermethrin to marine species and we are only aware of following three studies discussed below.

Clark et al. (1987) spiked technical grade cypermethrin into sediment and reported 96-h LC_{50}s of 175-270 µg kg^{-1} for the Grass shrimp, *Palaemonetes pugio*. They noted cypermethrin was not acutely toxic until concentrations in sediment were great enough where partitioning into the overlying water reached lethal concentrations (0.016 µg L^{-1}). Shrimp were noted to tolerate concentrations of up to 10 µg kg^{-1} for 10 days (estimates based on measured concentrations). *Corophium volutator* was exposed to Excis® spiked in sediment and the 10-day LC_{50} was 8 µg kg^{-1} (Mayor et al., 2008). *Eohaustorius estuarius* was exposed to cypermethrin (undisclosed pesticide formulation) spiked in sediment and was found to be more sensitive with a 10-day LC_{50} of 1.58 µg kg^{-1} (ALET unpublished data). Estimated sediment thresholds are based on nominal concentrations of cypermethrin.

Environmental concentrations of cypermethrin in sediment related to use in aquaculture is somewhat scant. Chemical monitoring of therapeutants in sediment around aquaculture sites is not a requirement in Canada, but data does exist from four SEPA annual screening surveys of sediments around marine fish farms (from 2003-2006). These surveys reported concentrations of cypermethrin in sediment ranging from 0.03 to 7.19 µg kg^{-1} (see SEPA, 2004-2007). It was noted that most sites sampled were below the predicted no effects concentration (PNEC) of 2.2 µg kg^{-1} (SEPA, 1998), suggesting there would be little environmental impact. The 10-d LC_{50}

determined for *E. estuarius* is below the PNEC of 2.2 µg kg^{-1} proposed by SEPA, suggesting that this value is not sufficiently protective of sensitive benthic species.

PARAMOVE 50®

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 hydrogen peroxide to treat infestations of both *L. salmonis* and *C. 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®, Salartect®) is still authorized for use in Canada but its specific use as an anti-sea louse pesticide requires an Emergency Registration from PMRA. From 2000-2010 it was not used, or used sparingly, for the treatment of sea lice infestations in Canada.

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).

Hydrogen peroxide has a half-life in seawater of about 7 days and it degrades to oxygen and water (Haya et al., 2005). Hydrogen peroxide is perceived as being of relatively 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 L^{-1}, which is near the treatment concentrations of 1.2 - 1.8 g L^{-1} (Haya et al., 2005).

The recommended dosage for bath treatments is 1.2 - 1.8 g L^{-1} for 40 min but the effectiveness is temperature dependent and Treasurer et al. (2000) suggest that treatment with these concentrations may not be effective below 10°C. In southwest New Brunswick treatments are the norm at these temperatures and use of hydrogen peroxide is monitored carefully at temperatures above 10°C due to a low therapeutic index for salmon and hydrogen peroxide is not recommended as a treatment for sea lice infestations at water temperatures above 14°C. (Dr. Michael Beattie, Province of New Brunswick, personal communication). Treasurer et al. (2000) also state that treatments are rarely fully effective, but 85-100% of mobile stages may be removed. 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 6 years than in cages where the sea lice were treated for the first time. This suggested that *L. 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 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 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 less than 1 (K_{ow} = -1.5) indicating no potential for persistence or bioaccumulation (HERA project, 2005). Hydrogen peroxide is 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 7 days in sea water. If the sea water is aerated the amount decomposed after 7 days is 45% and 67%, respectively (Bruno and Raynard, 1994). 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.

Biological Effects of Hydrogen Peroxide

Published Studies

There is little information on the toxicity of hydrogen peroxide to marine organisms. Most toxicity data are related to the potential effects on salmonids during treatment of sea lice infestations. 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 L^{-1}) and that which causes gill damage and mortality (2.38 g L^{-1}) (Kiemer 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 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 L^{-1} as a result of 5-h exposures (Abele-Oesschger et al., 1997). Those concentrations are one-half to two-thirds of the prescribed Canadian treatment concentration (1200-1800 mg 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 L^{-1}, at 22°C was 0.218 g 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 *C. 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.

Unpublished Results

In 2011, staff at SABS conducted a series of bioassays to determine the acute response of several invertebrate species to Paramove 50® (Table 9). 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 L^{-1}, while adult lobsters survived exposure to 3750 mg L^{-1}, approximately three times the prescribed treatment concentration. The LC_{50} for Paramove 50® and *M. stenoplepsis* was estimated to be 973 mg L^{-1}. The LC_{50} for *C. septemspinosa* was estimated to be 3182 mg L^{-1}.

Table 10 shows estimates of the 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 L^{-1} mysids are the only species where >50% of exposed animals die, which took > 80 h for this to occur. The 50% lethal threshold was not met for other species exposed to this concentration.

Table 9. The 1-h LC_{50} of Paramove 50® (as hydrogen peroxide) to several crustacean species. Organisms were exposed for 1 h then monitored for a further 95 h. Estimates are calculated as the mean of several replicate bioassays (N) and are based on measured concentrations of hydrogen peroxide.

Species/Life Stage	**LC_{50} (mg L^{-1})**	**95% C.I.**	**N**	**Dilution factor[a]**
Lobster Stage I	1637	1358-2004	1	NA
Lobster Adult	>3750	ND	1	NA
Mysis sp.	973	668-1427	1	1.2
Crangon septemspinosa	3182	2539-5368	1	NA

[a] Dilution factors are based on a prescribed treatment concentrations of 1200 mg L^{-1} as hydrogen peroxide.
ND – not determined
NA – threshold is greater than the treatment concentration, no dilution

Table 10. LT_{50}(h) of Paramove 50® to several crustacean species. Organisms were exposed for 1 h then monitored for a further 95 h. Water concentrations are measured concentrations of hydrogen peroxide.

Concentration (mg L^{-1})	Stage I Lobster	Adult Lobster	Mysids	Crangon
3700	12	0.75	0.3	1.4
1800	>95	2.5	1.4	>95
950	>95	>95	83	>95

In 2012-2013, staff at SABS conducted laboratory bioassays (at 9-13°C) to determine 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 L^{-1} hydrogen peroxide (nominal concentrations) were immobilized and sunk to the bottom of test beakers within 15 and 60 minutes of the 1-h exposure, respectively. In two of four bioassays, poor or no vital staining was observed in the two highest concentrations, indicative of mortality. The LC_{50}s (95% C.I.) were 42 (34-51) and 75 (64-91) mg L^{-1} hydrogen peroxide (nominal) for these bioassays. Feeding behaviour was affected and a mean EC_{50} (range of EC_{50}) of 5.3 (2.6-10) mg L^{-1} was determined based on measured hydrogen peroxide concentrations in five bioassays. In 2012 staff at SABS conducted laboratory bioassays (at 9-11°C) to determine if mixtures of hydrogen peroxide and azamethiphos were more or less toxic to mysid shrimp than single pesticide exposures. These pesticides are the only products being applied as 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, Province of New Brunswick, personal communication). Experiments were conducted in which shrimp were exposed, for 1 h, to Salmosan®, moved to clean water then exposed 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 only one pesticide, i.e., no lethal threshold could be determined for Salmosan® (azamethiphos) and the lethal thresholds for Paramove 50® (hydrogen peroxide) were the no different.

Another experiment was conducted 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 (Quinn McCurdy, Mount Allison University, personal communication).

Summary of Lethal and Below Threshold Effects

Figure 4 is a summary of the acute LC_{50}s reported in this paper for crustaceans exposed to AlphaMax®, Excis®, Salmosan®, and Paramove 50®. It visually demonstrates the difference in toxicity between the four pesticides, as well as the difference in sensitivity of acute test endpoints (e.g., 1- vs 24-h LC_{50}s), and sensitivity of test organisms.

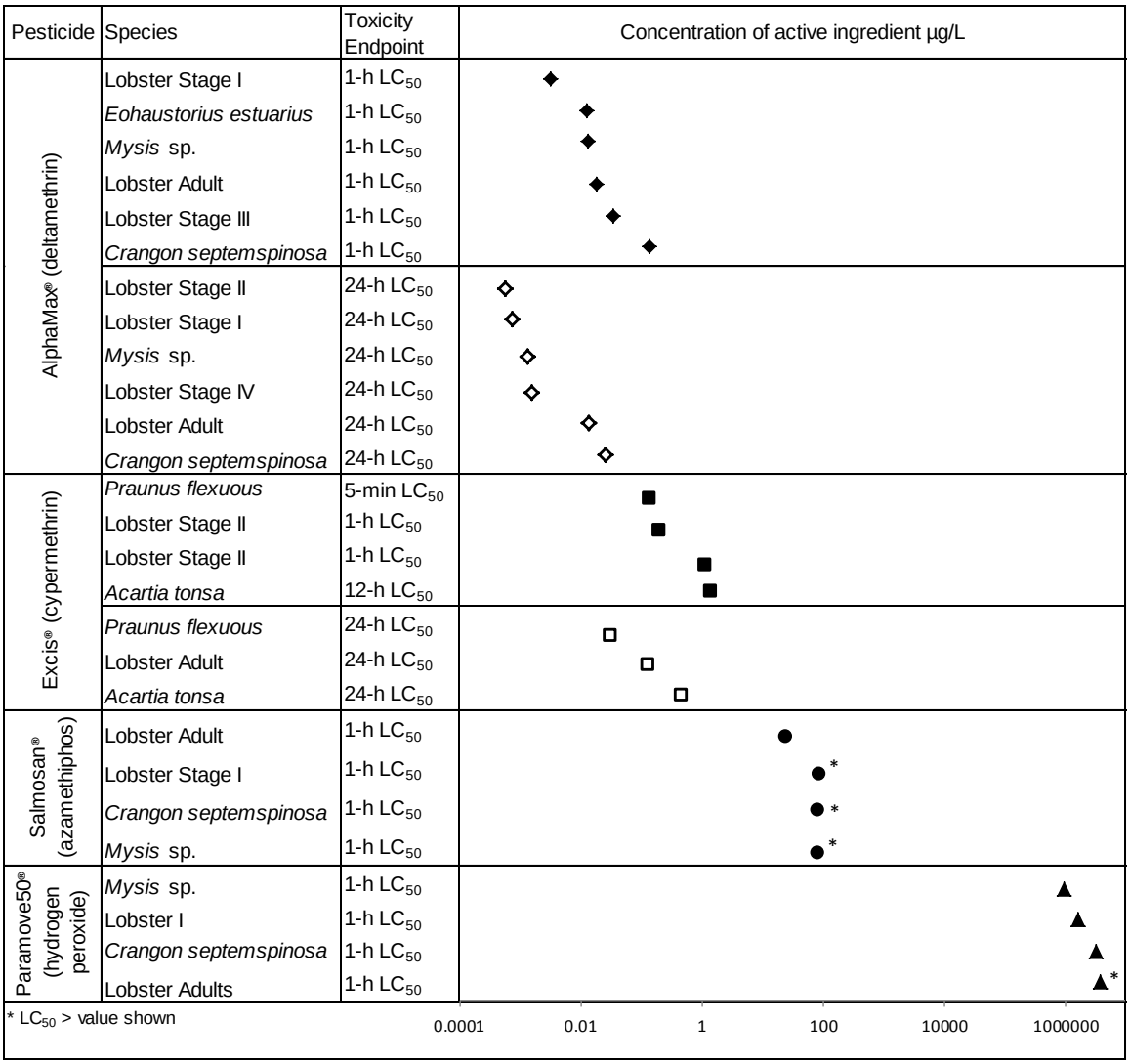

Figure 2. Summary of acute LC_{50}s for several crustaceans for the four pesticides reported in this review.

Table 11 is a summary of Lowest Observable Effects Concentrations (LOEC) observed in all acute lethality bioassays conducted at SABS in 2011-2012. These data show that in a number of cases, either lethal or sub-lethal effects were observed at the lowest concentration tested and in all cases effects were observed well below the LC_{50} threshold concentration. The intention of presenting this data is to introduce a level of caution or precaution against focusing risk estimates solely on lethality or LC_{50} values. However, in many cases these points fall into the category of anecdotal observations, i.e., experiments were not designed to find the LOEC. These data highlight the need for conservative estimates of toxicity, but cannot be considered as robust as the LC_{50}s, which the studies were designed to determine.

Table 11. Lowest concentration of active ingredient for four anti-sea louse pesticide formulations that resulted in at least one death or noted behavioural responses during lethality studies.

Species/Life Stage	**Salmosan®** (azamethiphos µg L^{-1})		**AlphaMax®** (deltamethrin µg L^{-1})		**Excis®** (cypermethrin µg L^{-1})		**Paramove 50®** (hydrogen peroxide mg L^{-1})	
	1 hr	**24 h**	**1 h**	**24 h**	**1 h**	**24 h**	**1 h**	**24 h**
Lobster Stage I	11.5	NA	0.0006	0.00008*	NA	NA	186	NA
Lobster Stage II	NA	NA	NA	0.00008*	NA	NA	NA	NA
Lobster Stage IV	NA	NA	NA	0.00008*	NA	NA	NA	NA
Lobster Adult	2.9	NA	0.006	0.0048	NA	NA	794	NA
Crangon septemspinosa	0.97*	71*	NA	0.005	NA	NA	223*	NA
Mysis sp.	3.3	18.5*	0.0006	0.0002*	0.018	0.010	245*	NA

* lowest exposure concentration in bioassay.

DISCUSSION

The terms of reference for this paper ask a simple question: What are the known biological effects of azamethiphos, deltamethrin, cypermethrin, and hydrogen peroxide, on key non-target organisms? The authors believe it is also of use to briefly discuss the relative toxicities of these compounds.

Table 12 shows a pesticide ranking system based on acute lethality. This ranking system is currently in use by the Pesticide Action Network. Using this rating system and our most sensitive species, azamethiphos, deltamethrin, and cypermethrin can be considered very highly toxic. Hydrogen peroxide would be considered practically non-toxic. Despite sharing a very highly toxic rating, deltamethrin (in AlphaMax®) is up to five orders of magnitude more lethal than azamethiphos (in Salmosan®) to Stage I lobster larvae.

Table 12. Acute toxicity ratings (ppb or μg^{-1}) based LC_{50}, Kamrin (1997).

Toxicity Category	**LC_{50} (µg L^{-1})**
Very highly toxic	<100
Highly toxic	100-1,000
Moderately toxic	1,000-10,000
Slightly toxic	10,000-100,000
Practically nontoxic	>100,000

Data presented in Table 3 shows the lethality of AlphaMax® to a number of indigenous species. It should be noted that some of the data were provided by collaborators from DFO and EC labs in Moncton. Their experiments were conducted at temperatures a few degrees warmer than

exposure temperatures at SABS. Pyrethroids have been shown to be more toxic at lower temperatures (Sparks et al., 1983). It is unlikely that the temperature difference will affect relative lethality in general; however, it may affect comparison of absolute LC_{50} estimates. It is also noteworthy that the estimated LC_{50} for AlphaMax® to adult lobsters was not significantly different between 24-h and 10-day exposures. There was a difference between 1-h and 24-h exposures with adults however. Fairchild et al. (2010) have shown a similar result with Stage III lobster larvae. A 1-h exposure with this stage followed by 16 days post-treatment monitoring provided the same LC_{50} estimate for 24 h and 16 days.

AlphaMax® is also an order of magnitude more lethal than Excis®, which is currently used in Europe and the UK. In 24-h studies with adult lobsters, the LC_{50} is reported as 15 ng deltamethrin L^{-1} compared to 140 ng cypermethrin L^{-1}. In a recently published paper, Palmquist et al. (2011) suggest that use of very sensitive organisms, in their case *Hyalella azteca*, should be discouraged when assessing the risk of deltamethrin. While they correctly suggest that laboratory-based studies may not fully reflect routes of exposure in the field, ignoring or downplaying data indicating that any product is lethal in the ng L^{-1} range seems ill-advised. The argument may be moot with respect to the data presented herein as treatment concentrations appear to be environmentally relevant and the species tested are not only indigenous but, as with lobsters, are also commercially important.

Crane et al. (2011) suggest environmental concentrations as low as 1.4 ng L^{-1} should be sufficiently protective for sensitive saltwater species exposed to AlphaMax® for 48 h. These authors based their assessment on LC_{10} values calculated using toxicity data from a number of studies and a number of species. Data presented here (see Table 13 for example) clearly show that concentrations of that magnitude for that time period would be lethal to lobster larvae and likely lethal to mysid shrimp. A similar approach to developing environmental quality guidelines could be attempted for species native to Canada and for the pesticides of interest: azamethiphos, deltamethrin, cypermethrin, and hydrogen peroxide.

The Atlantic Veterinary College has conducted bioassays with sea lice and Salmosan® or AlphaMax®. The response of adult females is somewhat variable with EC_{50} estimates including values below and above recommended treatment concentrations. The data show that, in some cases, sea lice are not sensitive to the active ingredients even at concentrations above the recommended treatment concentration (Table 13). One statistical outlier was identified in bioassays with AlphaMax® with an EC_{50} of 8.6 µg L^{-1}, four times the recommended treatment concentration (Dr. Larry Hammell, Atlantic Veterinary College, personal communication). This variability may be of concern from an efficacy perspective. The concern is magnified by the fact the results are for EC_{50}, i.e., effects for 50% of the population. To expect a reduction in sea lice and a reduction in the necessity to treat sea lice, nearly 100% efficacy must be attained. Therefore, the potential for development of resistance at current treatment doses is a concern. The bioassays used indigenous populations of sea lice and are difficult and expensive to plan and perform. Consequently, the number of assays performed is limited and this may contribute to the variability of responses. These bioassays are vitally important in assessing efficacy trends and a program should be established to routinely conduct bioassays on the target organism (sea lice) to ensure the various therapeutants are effective.

Table 13 shows the range of treatment concentrations prescribed for the four formulations discussed in this paper, the sensitivity of three non-target organisms, and data on the sensitivity of the target organism, sea lice. Noteworthy is the contrasting degrees of toxicity of the therapeutants (toxicity of deltamethrin > cypermethrin > azamethiphos >> hydrogen peroxide). In addition, the acute 1-h thresholds determined for these non-target species may be considerably lower than concentrations required to elicit effects on sea lice.

Table 13. LC_{50} (µg L^{-1} of active ingredient) of bath treatments used in southwest New Brunswick during 2009-2011. Exposures of lobsters and mysids were conducted at St. Andrews Biological Station and were of 1-h duration followed by 95-h monitoring. The threshold concentrations are measured concentrations of the active ingredient. General estimates of toxicity to Atlantic Salmon are also indicated.

Formulation (active ingredient)	Treatment Concentration (µg L^{-1} active ingredient)	Mode of Action	1-h LC_{50} (µg L^{-1})			Sea Lice[a]	Atlantic Salmon
			Stage I Lobsters	Adult Lobsters	Mysids		
AlphaMax® (deltamethrin)	2	CNS; chloride channels	0.0034	0.0188	0.0139	0.6-3.0[b] (n=4)	53-96[c]
Excis® (cypermethrin)	5		NA	NA	>0.142		2[d]
Salmosan® (azamethiphos)	100	CNS; AChE inhibition	>86.5	24.8	>85.5	15-460 (n=11)	1000[e]
Paramove 50® (hydrogen peroxide)	1,200,000 – 1,800,000	Mechanical paralysis	1,637,000	3,750,000	973,000		2,400,000[f]

[a] Data courtesy of Dr. Larry Hammell, Atlantic Veterinary College. Bioassays were conducted with adult female sea lice and were of 30-minute duration followed by 24-h monitoring.
[b] Range of EC_{50}s does not include one statistical outlier (8.6 µg L^{-1}).
[c] 30 min LC_{50} at 10 and 20°C (Gross et al., 2008)
[d] 96-h LC_{50} (McLesse et al., 1980)
[e] mortality observed after 1-h exposure (Roth et al., 1993)
[f] gill damage and mortality (Haya et al., 2005)

It remains unclear if operational treatments could have impacts on local populations of invertebrates. The risk associated with the use of hydrogen peroxide is low although very little work has been done to assess sublethal effects on non-target organisms. Repeated short term exposure to Salmosan® (10 µg L^{-1} a.i.) has been shown to affect survival and reproduction in female American lobsters in a cumulative manner but the risk has not been assessed. While 1 µg L^{-1} a.i. has been shown to have no effect, longer term exposure of lobsters to a low level (0.78 µg L^{-1}a.i.) of Salmosan® resulted in a number of sublethal effects including, at least the suggestion, that treated lobsters may not ship as well as untreated lobsters. Again the risk associated with this effect has not been assessed.

The chemical properties of hydrogen peroxide and azamethiphos (Salmosan®) indicate they should not be of concern for toxicity via sediment. AlphaMax® has been shown to be extremely toxic in laboratory studies employing waterborne exposures. Deltamethrin and cypermethrin, however, should bind to sediment and studies to determine if sediment-borne AlphaMax® and Excis® are lethal to benthic invertebrates are just beginning to yield data. In a previously published State of Knowledge document prepared for DFO, the author has stressed that most of the conclusions regarding risk are based on single-species, lab-based studies (Burridge, 2003). While lab-based studies still represent the best way of comparing toxicities of compounds and the standard methods employed give some confidence in making these comparisons, they lack the complexity of the real world. For example:

- Some sensitive life stages may be present for relatively short periods of time that may or may not coincide with sea lice treatments at farm sites (Table 14).

- The duration of exposure is likely to be quite variable in the field depending on tides, winds and currents, for example.
- Some life stages have been shown to be more sensitive than others and timing of exposure may be important. The physiological status of organisms can affect response as well. Female lobsters appear to be more sensitive to azamethiphos just before and while they are moulting (Burridge et al., 2005), for example. Incorporating these responses in a comprehensive risk assessment is difficult at best.

Table 14. Location and seasonal distribution of invertebrate species native to southwest New Brunswick that have been tested for their sensitivity to anti-sea louse bath treatments. The bulk of sea lice treatments take place from May to November.

	Larval lobsters Stages I-III	**Juvenile lobsters Stage IV +**	**Adult lobsters**	**Mysids**	**Crangon**
Position in water column*	Pelagic	Subtidal, benthic	Subtidal, benthic, epi-benthic	Intertidal, subtidal, pelagic or epi-benthic, depending on species, habitat, and time of year	Intertidal, subtidal, epi-benthic
Presence in Bay of Fundy*	June - Sept	July – October or Year-round	Mobile, but present year round	Seasonal	Year-round

* Dr. Andrew Cooper, DFO, personal communication

The complexity described in the previous statements is magnified by the potential for different formulations to be used at different, yet neighbouring, sites. Each of the three compounds discussed has a different mode of action (Table 13). Sessile or immobile individuals could be exposed to several formulations either in naturally produced mixtures or from sequential releases. As stated earlier, exposure may be short lived or of long duration. The consequences of this are not fully understood and extremely difficult to assess or model. Hartwell (2011) and Leight et al. (2005) monitored invertebrate populations in the southeast United States and related the trends in those populations to input of anthropogenic compounds including pesticides. They found lower numbers of Blue crab and Grass shrimp in areas of heavy agricultural runoff. Since bath treatments are applied directly to water, it is not unreasonable to expect that indigenous invertebrate populations could be affected by the pesticides. Finally, much of what is known about the biological effects of these pesticide formulations relates to lethality at varying lengths of exposure. While these data are vital for proper risk assessments, research into sublethal endpoints, especially those related to reproduction, must continue. Subtle effects (behavioural, reproductive, etc.) are often not revealed by lab-based acute exposures, nor are they necessarily captured by short term caging studies *in situ*. For example, Waddy et al. (2002) reported that lobsters treated with an in-feed anti-sea louse therapeutant (emamectin benzoate) molted much earlier than would have been predicted. This serendipitous finding clearly supports the suggestion that pesticides may affect non-target organisms over longer periods of time and in ways not predicted by looking only at the mode of action. Due to a number of factors, particularly cost and space, these types of studies are rarely conducted or reported. A prime example of this can be found in the results of the repeated exposures of adult lobsters to either Salmosan® or AlphaMax® described above. This study was designed to determine effects on reproduction or molting, however, some lobsters died. While no statistical

difference is observed when mortality rates are compared, the only lobsters that died during the experiment were treated lobster (AlphaMax®). Power analysis shows that should the experiment be repeated treatment groups of greater than 55 lobsters per treatment would be required.

To return to the question posed in the terms of reference:

What are the known biological effects (lethal, sub-lethal and/or behavioural) of azamethiphos, deltamethrin, cypermethrin, and hydrogen peroxide on key non-target organisms?

There are a number of considerations from the information presented in the current paper that would need to be considered in risk assessment, particularly as they relate to uncertainty associated with estimates of toxicity. This paper related acute toxicity values (LC_{50}s) to the required dilution of treatment concentrations to reach those toxicity values as a means of indicating the potential for effects. However, there is uncertainty around these toxicity values as presented with the 95% confidence intervals. Additionally, these toxicity values are calculated on the basis of what is considered the "active" ingredient. There is uncertainty about the potential toxicity of "non-active" ingredients, which are proprietary and known only to the manufacturers and regulators, but have in the case of other pesticides, been shown to be more toxic than the "active" ingredients. It is also possible that product formulations could be changed and this uncertainty could limit comparisons between different studies. However, the testing of formulations, over only technical grade components of the pesticides, still represents the more environmentally relevant exposure. For some of the pesticides, there is disparity between measured and nominal (expected) values and it unknown whether the reasons for this are chemical degradation, sorption, or analytical error. This is a source of uncertainty not only in estimates of toxicity, but in practical application and measures of chemicals in the field. While lethality was the main endpoint in many of the studies reported herein and there is good confidence in the estimates of LC_{50}s, the consequence of sublethal effects is not something that should be overlooked in a risk context. Sublethal effects observed in the laboratory such as molting success, orientation problems and even temporary paralysis affecting individuals can leave them vulnerable to predation and other stressors in the field, which many scientists consider equivalent to "ecological death". Sublethal effects and biochemical indicators of stress have not been investigated or identified for all the pesticides considered in this review paper and data sets for some pesticides are not as strong as for others, which represents areas of uncertainty especially when trying to compare or use common approaches to determine potential risks of these pesticides. Finally, translation of laboratory data to what may be expected in the field is a large area of uncertainty as very few and albeit crude field studies have been conducted to examine effects of these pesticides on non-target organisms.

Authors were asked to discuss the biological effects in a pan-Canadian context. Unfortunately, there are no data available regarding the effects of the "registered" products on species indigenous to the west coast of Canada, apart from the amphipod *Eohaustorius estuarius*. The pesticide formulations are not registered for use in the Pacific and consequently are not applied. However, history has shown that wherever pesticides and drugs have been used to treat against infestations of sea lice some level of resistance has developed. To date salmon aquaculture in British Columbia has bucked this trend and the drug emamectin benzoate remains the only compound applied in that jurisdiction. Data presented in this paper show a wide range of effects across all pesticide formulations. Most of these data have been generated in an atmosphere of urgency, if not crisis. Much more work must be carried out to adequately determine what is happening in field situations and therefore to inform risk assessment decisions. The fact that nothing is known of the effects of pesticides on west coast species is, or should be, troubling. An evaluation would need to be conducted as to whether or not the

current data using Atlantic species can be considered a surrogate for commercially relevant and potentially sensitive Pacific species and whether other considerations of oceanographic differences between regions influence exposure and toxicity.

FUTURE WORK/KNOWLEDGE GAPS

1. Within the Program for Aquaculture Regulatory Research funded studies, we focused, for the most part, on live or die responses. Research similar to that described above (molting, shipping, biochemistry) should continue especially for commercially important organisms, sensitive organisms, sensitive life stages, and crucial “activities” such as reproduction and moulting. Behavioural responses of non-target organisms, including avoidance, needs to be studied. Long term holding and assessment of treated non-targets may be required in order to assess some sublethal endpoints.
2. Work within DFO has largely focused on acute exposure and response. Previous work with Salmosan® showed short-term repeated exposure could result in delayed spawning in female lobsters. Similar studies should be designed to look at latent or delayed responses following single, or multiple, sublethal exposures.
3. DFO research continues to focus mainly on non-target crustaceans. Work on potential effects on zooplankton, initiated in 2012 should continue. Other classes of organisms, at various levels of the food chain, should also be considered in planning research.
4. Physico-chemical characteristics dictate the likely fate of chemicals. The Kow of deltamethrin and cypermethrin indicates that they are the only bath treatments likely to bind to sediment. Research should continue into the toxicity of these compounds in sediment and the concentrations surrounding aquaculture sites if treatments occur. Particularly, as low level, long-term exposure could have impacts on organisms from accumulation of chemicals in tissue. Similarly, the potential for bioaccumulation of these chemicals in tissue is unknown and whether this could represent a dietary source of exposure to other organisms. The potential for this would be greatly influenced by chemical properties and the extent of chemical loading to the environment.
5. PMRA registers pesticides based, at least in part, on physical–chemical characteristics of the active ingredient(s). It is clear that the formulation ingredients enhance factors such as solubility meaning that the physical–chemical data derived using active ingredients may not be appropriate when predicting fate and persistence of the formulation. In the authors’ opinion this is a serious short fall in the registration system.
6. While some mesocosm studies are being conducted to compare laboratory and field responses to the bath treatments, monitoring should be conducted to assess the effects of multiple treatments in small geographic areas and to determine the extent (spatially and temporally) of effects. In situ studies are recommended. The presence of dye ensures that exposure has occurred and strengthens interpretation of data. Laboratory work will be necessary to confirm that dye does not affect toxicity.
7. Research is needed to assess the cumulative effects of multiple exposures to single compounds and/or effects of multiple stressors. Multiple stressors can include exposure to several pesticides, effects of water temperature on responses and effects of water quality on responses, for example.
8. Classes of pesticides are defined by specific modes of action. Organophosphate compounds such as Salmosan® act by inhibiting enzyme activity and, as such, its effects can be monitored biochemically. Measuring the effects of deltamethrin, cypermethrin, and hydrogen peroxide are not as easy. There will be value in

determining the extent to which some responses are chemical specific. It is possible, even likely, that the pesticides may elicit a generalised stress response independent of the mode of action of the compound. There are some standard methods for assessing generalised stress. These could be integrated into a suite of endpoint assessments to determine if use of chemicals, in general, affects non-target organisms.

9. Research has shown that exposure to azamethiphos may affect lobsters to the extent that they may not ship well. While this is a significant commercial consideration, it also indicates that exposed lobster may be less tolerant of other stressors. Field monitoring (reference vs. aquaculture sites) of lobster behaviour, energy allocation, gonad maturation and ability to cope with environmental stressors is recommended as these studies will show whether laboratory-observed consequences occur in the field.
10. Further investigations of the mechanism of action of chemicals released by aquaculture facilities are required for the development of suites of biomarkers providing indications on the nature of toxicant involved in the observed environmental effects and discrimination between effects of environmental stressors (e.g., temperature, hypoxia) and xenobiotics. New genomic and proteomic tools are being developed in crustaceans for that purpose. Further studies on the relationship between molecular tools (e.g., ChE inhibition, protein carbonyls) and ecologically significant responses are also needed.
11. Sublethal effects, other than immobility/reduced feeding in copepods, have not been observed in laboratory studies with Paramove 50®, which represents a considerable data gap for this pesticide.
12. Extent of temperature effects were only slightly touched upon in this paper, and while many of the toxicity studies were conducted at environmentally relevant temperatures for periods during which sea lice treatment occurs, this remains an unknown and source of uncertainty in terms of effects on toxicity and whether temperature effects are also an additional stressor.

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