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Toxicity of two pyrethroid-based anti-sea lice pesticides, AlphaMax® and Excis®, to a marine amphipod in aqueous and sediment exposures

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ABSTRACT

Pyrethroid pesticides used to control ectoparasitic sea lice in salmon aquaculture are released in effluent plumes from cage sites and have the potential to adversely affect non-target organisms. Pyrethroids have been shown to be highly toxic to crustaceans, but the toxicity of the pyrethroid-based anti-sea lice pesticides AlphaMax® (active ingredient (a.i.) deltamethrin) and Excis® (a.i. cypermethrin) has not been thoroughly studied for non-target, marine benthic crustaceans such as amphipods. The amphipod *Echinogammarus finmarchicus*, which is ubiquitous in near-shore environments in the northern Atlantic Ocean, was collected from the field for use in laboratory toxicity tests. Amphipods were exposed to the two pesticides in 1- and 24-h water-only, single-pulsed exposures and 10-day spiked sediment tests. In water-only tests, immobility occurred within 1- or 24-h of exposure to the highest test concentrations and delayed mortality and immobility were observed following exposure to lower concentrations as well. Effect thresholds ranged from 6.7–70 ng/L for deltamethrin and 20–220 ng/L for cypermethrin. Organisms exposed to sediment were affected within 2–4 days; resulting 10-d LC50s were 16 and 80 ng/g (dry weight) for deltamethrin and cypermethrin, respectively. Results suggest that amphipods in an effluent plume may be exposed to aqueous concentrations of pyrethroids sufficient to cause adverse effects, including delayed toxicity. In contrast, the 10-day sediment LC50s were much higher than (limited) reported environmental concentrations in sediment. Overall, these results suggest a low potential for risk from sediment exposures for a northern Atlantic species of amphipod that inhabits the near-shore environment where cage aquaculture sites are located.

1. Introduction

Infestation of ectoparasitic sea lice in salmon aquaculture requires the treatment of fish, often by immersion in a pesticide bath. The pesticide is then released from the aquaculture site (i.e., from a tarped net pen or specialized well boat) into the marine environment where non-target organisms may also be exposed. Studies of pesticide dispersion from aquaculture sites have found that effluent plumes are detectable 2 to 5.5 h post-release at distances 0.9 to 3 km from the cage site, with pesticide concentrations that represent 1/1000 to 1/2000 of the pre-release concentrations (Ernst et al., 2001, 2014). Pesticides dispersed into the intertidal zone can also partition into bottom sediments, where they could impact benthic species (Ernst et al., 2001). Pyrethroids are some of the most toxic pesticides known (Smith and Stratton, 1986), and it was their low toxicity to mammals and high toxicity to crustaceans that led to their use as treatments for sea lice infestations (Haya et al., 2005). Pyrethroids affect nerve transmission by interfering with sodium (Na^+) channels (Miller and Adams, 1982), resulting in depolarization and repetitive firing of nerve endings, leading to eventual paralysis and death (Crane et al., 2011; Haya et al., 2005). Among the pyrethroids, deltamethrin is considered to be the most toxic to non-target organisms (Haya, 1989).

The anti-sea lice pesticide formulations AlphaMax® and Excis® are emulsifiable concentrates containing 1% of the synthetic pyrethroids deltamethrin or cypermethrin as the active ingredient, respectively. Both pesticides are effective against all attached stages of sea lice including adults (Burridge et al., 2010; Haya et al., 2005). Treatment of salmon is either a 40-minute bath with AlphaMax® at a target concentration of 2.0 μg/L deltamethrin (SEPA, 2008) or a 1-h bath with Excis® at a target concentration of 5.0 μg/L cypermethrin (SEPA, 1998). Both pesticides are registered or approved for use in a number of salmon-producing nations (Burridge et al., 2010). In Canada, however, Excis® was only applied under a research permit in the mid 1990s and was never approved for emergency use (Chang and McClelland, 1996, 1997), while AlphaMax® was registered for use in southwest New Brunswick in 2009 and 2010 (HC-PMRA, 2010).

Deltamethrin and cypermethrin have very low water solubility (<2 and 4 μg/L, respectively) and a log Kow of 4.6 and 4.5, respectively (Tomlin, 1994; Vershueren, 1996). Both chemicals are not expected to persist in the aqueous phase, but are known to absorb to particles and accumulate in sediments. The half-lives for deltamethrin and

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cypermethrin in marine sediments have been estimated at approximately 140 days (Gross et al., 2008) and 35 to 80 days (SEPA, 1998), respectively. As a result, multiple anti-sea lice treatments may result in accumulation of these chemicals in sediments near cage sites, where they may affect benthic invertebrates (Haya et al., 2005). The commercial formulations AlphaMax® and Excis® are prepared with emulsifiers to retain the active ingredients deltamethrin and cypermethrin in solution during the bath treatment. As a result, the formulation may affect the distribution of pesticide between water and sediment and, depending on the rate of breakdown of emulsifiers, non-target benthic organisms may be exposed to these pyrethroids via a number of phases, including water, sediment, or ingestion of contaminated organic particles. In addition, the presence of emulsifiers has been shown to have varying influence on the toxicity of pyrethroids compared to technical-grade forms (cf. Coats et al., 1989; Haya, 1989; Osterberg et al., 2012).

Sensitivity to these pyrethroid pesticides varies among non-target species. Amphipods were found to be the most sensitive species to AlphaMax® in water in both standard 96-h exposures and 1-h single-pulse exposures, when compared to stage III and IV lobster and *Crangon* shrimp (Fairchild et al., 2010). These tests used a standard test organism (Environment Canada, 1998) *Eohaustorius estuarius*, a species of amphipod found in the Pacific Ocean. However, the toxicity of AlphaMax® has not been investigated for an amphipod species from the Atlantic region of Canada, where various treatments of sea lice infestations in salmon aquaculture have occurred since the mid-1990s (Jones et al., 2012). Toxicity data are also limited for Excis® and for exposures to either chemical in sediment. The objective of the present study is to examine the toxicity of the anti-sea lice pesticides AlphaMax® and Excis® to an indigenous (Atlantic) species of amphipod using aqueous and sediment exposures.

2. Materials and methods

2.1. Organism collection and holding

Amphipods and sediment were collected in June, 2013 from tide pools at Sam Orr's Pond along Passamaquoddy Bay, in the Bay of Fundy region of New Brunswick (NB), Canada. During the outgoing tide, amphipods were found among partially exposed rockweed, which was shaken above sieves, and the organisms were then transferred to pails containing seawater. The species was identified as *Echinogammarus finmarchicus* (by R. Milne, Atlantic Reference Centre, St. Andrews, NB; based on Bousfield, 1973), an amphi-Atlantic (i.e., both sides of the northern Atlantic), shallow-water gammaridean amphipod, that is considered a dominant species of tide pools along bedrock surf coasts and salt marsh pools. Intertidal surface sediment was collected from exposed depositional areas by collecting the top ~5 cm of sediment with a scoop or shovel and was stored in the laboratory at ~4 °C until use.

Once at the laboratory (Department of Fisheries and Oceans Canada — DFO, St. Andrews Biological Station in St. Andrews, NB), organisms were sorted using a 2-mm sieve and held in 10-L plastic pails to which mesh substrates, fresh seawater, and air stones were added. Organisms retained on the 2-mm sieve were used in testing; these amphipods were 10–20 mm in length, which was within the size range listed for mature adults of this species (Bousfield, 1973). All water used for holding the organisms and for the toxicity tests was the main laboratory supply of sand-filtered (0.2 μm) seawater (~30 parts per thousand salinity, source Passamaquoddy Bay, NB). Organisms were provided a 12:12 hour light:dark photoperiod with a maximum light intensity of 2 lx and all holding/testing vessels were kept in a 10 ± 2 °C water bath. Amphipods were held for a minimum of 2 days (d) and maximum of 10 d before use in testing (Environment Canada, 1998).

2.2. Water-only toxicity tests

To examine the toxicity of AlphaMax® and Excis® from acute exposure in water, amphipods were exposed to 1- or 24-h single pulses of either pesticide. Stock solutions of each pesticide formulation were prepared in seawater and spiked into large volumes of seawater, which were mixed and distributed in 900-ml volumes to 1-L glass jars. Tests for the 1- and 24-h single pulses were run concurrently using the same batch of test solutions with exposure concentrations ranging from 6–200 ng deltamethrin/L and 32–10,000 ng cypermethrin/L (nominal), plus seawater-only controls. At test initiation, water from one replicate jar/test concentration was collected for water quality (temperature, pH, dissolved oxygen, salinity) and for chemical analysis (deltamethrin or cypermethrin) using pre-cleaned glass sample jars. Ten amphipods were added to each replicate, with three replicates per test concentration per single-pulsed exposure. The tests were static, with no aeration of water and no substrate. After 1- or 24-h exposure, organisms were transferred to clean water and then monitored again at 96 h. After the 1-h exposure, water was pooled from the three replicates for chemistry. Concurrently with the 24-h exposure, chemistry-only replicates (without organisms) were sampled at 3, 6, 12, and 24 h for two treatment concentrations/pesticide (12.5 and 50 ng/L deltamethrin and 100 and 1000 ng/L cypermethrin, nominal) to examine chemical loss over time. Water samples (0.5–0.9 L volumes) were preserved with dichloromethane (DCM; ~5% v/v in sample), shaken for 15 min, and refrigerated at ~4 °C until chemical analysis. Amphipod survival was determined after 96 h and immobilized organisms (i.e., unable to swim) were examined under a dissecting microscope to confirm mortality.

2.3. Sediment toxicity tests

The toxicity of sediment-borne AlphaMax® and Excis® to amphipods was examined in 10-d tests. The concentration series for these tests were selected based on preliminary range finding tests and ranged from 6–200 ng deltamethrin/g and 9–300 ng cypermethrin/g (dry weight, nominal concentration), plus un-spiked controls. On the day before the start of the test (Day −1), 150 ml of sediment (~4 cm depth) and 600 ml seawater were added to 1-L glass jars. Sediment had the following characteristics: density of 0.44 g dry weight/ml, 67% moisture, 87% sand, 13% silt/clay, and 3% total organic carbon (TOC). Stock solutions of each pesticide formulation prepared in seawater were spiked into jars containing sediment and water, rather than into sediment, as this is more reflective of field conditions where the chemical is added to the water. Jars were sealed and placed on a reciprocating shaker on high for 5 min, then in a water bath overnight with overlying water in each test jar aerated via a Pasteur pipette through a hole in a loosely fitted lid. At test initiation (Day 0), overlying water and settled sediment were collected from three replicates/test concentration for measurement of water quality and pesticides. Twenty amphipods were added to each replicate, with three replicates per test concentration. General observations of amphipod condition (mobile or immobile) were noted daily, but could not be accurately quantified due to difficulty in seeing all organisms in the test jars. Affected organisms were not removed and examined until the end of the test. At test termination, overlying water was carefully decanted from the test jars and collected for chemical analysis. Organisms were sieved from the surface layer of sediment, rinsed, and transferred to a shallow pan with water to assess survival. Sediment was then collected for chemistry and frozen until chemical analysis.

All concentrations reported herein are as the active ingredient deltamethrin or cypermethrin and expressed on a dry weight basis in sediment, with target nominal concentrations based on the assumption of 100% adsorption to sediment.

2.4. Chemical analyses

Samples were analyzed for deltamethrin, cypermethrin, sediment particle size, and sediment TOC at the University of New Brunswick (Saint John, NB, Canada) following standard methods. For water samples, a liquid–liquid extraction was completed using a separatory funnel and DCM (US EPA, 1996a). PCB 30 and PCB 204 were added to samples as surrogates prior to extraction. Each sample was extracted two times with DCM, which was then collected and combined for analysis. The extract was concentrated using a Büchi Rotavapor R200 and further concentrated with an N-Evap™ 112 nitrogen evaporator to a final volume in isooctane.

Sediment samples were freeze dried, and the percent moistures were determined gravimetrically. PCB 30 and PCB 204 were added to samples as surrogates prior to extraction. The freeze-dried samples were extracted using an Accelerated Solvent Extractor (ASE 300; Dionex), with 50:50 DCM:hexane (US EPA, 1996b). Extracts were concentrated using a Büchi Rotavapor R200 and an N-Evap™ 112 nitrogen evaporator to a final volume in 50:50 DCM:hexane. Extracts for sediment were run through a J2 Scientific Automated Gel Permeation Column to remove interfering compounds (US EPA, 1996c), re-concentrated, and then added to a Florisil column and eluted with a series of non-polar to polar solutions. Separate fractions containing PCB surrogates and deltamethrin or cypermethrin were solvent transferred to isooctane, and then further concentrated to a final volume.

PCB 103 and PCB 198 were added as internal standards to final extracts just prior to analysis. Extracts were analyzed by gas chromatography with electron capture detection (GC-ECD) and quantification using an internal standard calibration and standard methods (Hladik et al., 2009; US EPA, 1995). Quality assurance/quality control procedures performed on each set of 10 samples included: surrogates, calibration checks, a method blank, and a method spike. The method detection limit (MDL) for deltamethrin and cypermethrin in water was 1–2 ng/L (based on 1- and 0.5-L sample volumes) and reporting limits ranged from 7–14 ng/L based on the MDL, method blanks, and individual sample volume. The MDL for deltamethrin and cypermethrin in sediment was 2 ng/g.

2.5. Test endpoints

An LC50 or EC50 (i.e., concentration causing lethality or other effect in 50% of organisms) with 95% Confidence Intervals (C.I.) was determined for each test according to Stephan (1977) using the computer program Toxstats. These endpoints were calculated based on the mean response in each treatment and mean measured concentrations of deltamethrin and cypermethrin. Concentrations of pesticide reported as "less than reported value" were close to detection limits and were set equal to the reported value for the purpose of calculating average exposure concentrations. Measured concentrations for 1-h water-only exposures were considered to be the average of the concentrations measured at time (T) = 0 and 1 h. Average measured concentrations for the 24-h water-only exposures were determined according to Zitko et al. (1977) as follows. For treatments for which time-point sampling occurred (12.5 and 50 ng/L deltamethrin and 100 and 1000 ng/L cypermethrin, nominal), an average concentration for that treatment was calculated using the area under the exponential degradation curve fit to the measured data. The ratio between this time-weighted average concentration and the T = 0 h measured concentration was determined and averaged for the two treatment time-series per pesticide. All T = 0 h measured data were multiplied by this average ratio to calculate 24-h time-weighted average water concentrations for all treatments. For sediment, the concentrations measured at the beginning and end of each test were averaged for each treatment (n = 6) and used to calculate endpoints.

Table 1
Measured concentrations of deltamethrin from AlphaMax® in water from 1- and 24-h tests with amphipods (n = 1/concentration).

Nominal concentration ng/L	T = 0 h ng/L	% of nominal	T = 1 h ng/L	1-h average ng/L	24-h average[a] ng/L
0	<7	–	nm	–	–
6.25	<13	–	<14	<14	<6.6
12.5	<12	<96	<14	<13	<6.1
25	19	76	18	19	9.6
50	31	62	29	30	16
100	54	54	39	47	27
200	240	120	140	190	na

nm — not measured.
na — not applicable, treatment not included in 24-h exposure.
< Less than reported value, close to detection limits.
[a] Time-weighted average based on Zitko et al. (1977) using average area under the exponential degradation curve of 12.5 and 50 ng/L measured data. Average adjustment factor of two treatments was 0.51.

3. Results

3.1. Chemical concentrations

Measured concentrations of deltamethrin at T = 0 h in the water-only tests ranged from 54 to 120% of nominal concentrations (Table 1). The 12.5 and 50 ng/L (nominal) treatments were sampled over time, and concentrations of deltamethrin decreased exponentially, with an average loss of 77% of the initial concentration by 24 h (Fig. 1).

Measured concentrations of cypermethrin at T = 0 h in the water-only tests ranged from 47 to 65% of nominal concentrations (Table 2). The 100 and 1000 ng/L (nominal) treatments were sampled over time, and concentrations of cypermethrin decreased, with an average loss of 36% of the initial concentration by 24 h (Fig. 2). Loss of cypermethrin over time did not fit an exponential degradation curve as well as data for deltamethrin; however, these data were used to calculate an average concentration of cypermethrin over the 24-h period.

In AlphaMax®- and Excis®-spiked sediment, average measured concentrations of deltamethrin and cypermethrin on Day 0 ranged from 21–47% and 35–237% of nominal concentrations in sediment, respectively (Table 3). On average, sediment concentrations across all treatments decreased by 33% between Days 0 and 10. Deltamethrin and cypermethrin measured in overlying water of sediment tests on Day 0 represented ≤4% of the amount added to the test jars (data not shown). Therefore, the disparity between nominal and measured sediment concentrations was not attributed to the active ingredient remaining in solution due to the pesticide formulation.

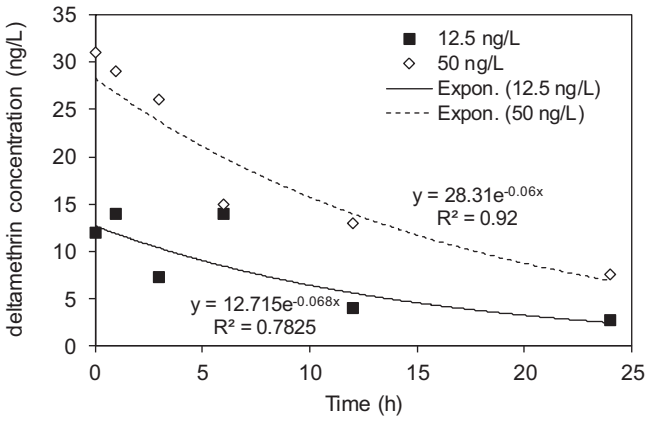

Fig. 1. Loss of deltamethrin from AlphaMax® formulation in water during 24-h test with amphipods. Nominal water concentrations at T = 0 were 12.5 and 50 ng/L (n = 1/time).

Table 2
Measured concentrations of cypermethrin from Excis® in water from 1- and 24-h tests with amphipods (n = 1/concentration).

Nominal concentration ng/L	T = 0 h ng/L	% of nominal	T = 1 h ng/L	1-h average ng/L	24-h average[a] ng/L
0	<7	–	–	–	–
32	15	47	15	15	12
100	65	65	50	58	54
320	160	50	150	160	130
1000	570	57	460	520	470
3200	1900	59	1700	1800	1600
10,000	5000	50	6200	5600	4100

nm — not measured.
< Less than reported value.
[a] Time-weighted average based on Zitko et al. (1977) using average area under the exponential degradation curve of 100 and 1000 ng/L measured data. Average adjustment factor of two treatments was 0.83.

3.2. Biological effects

3.2.1. Toxicity of AlphaMax® in water

Within 1-h of exposure to AlphaMax®, all amphipods in the highest test concentration (190 ng/L measured) were immobilized on the bottom of the test jars. At 96 h, 97 ± 3% mortality (mean ± SE) was observed in this treatment. Delayed toxicity, which included mortality and immobility, was observed in the two next highest treatments (47 and 30 ng/L), with a total of 47 ± 23% and 20 ± 10% of organisms being affected (mortality + immobility) at 96 h (Fig. 3A). Less than 10% of amphipods were dead or immobile in the three lowest test concentrations. Mortality in control organisms was 4 ± 4% and immobility was not observed.

After the 24-h exposure to AlphaMax®, all amphipods in the highest test concentration (27 ng/L measured) showed no movement and all organisms in the two next highest treatments (16 and 9.6 ng/L) appeared immobilized and were on their backs with their legs twitching. At 96 h, 100% mortality was observed in the highest treatment. Some of the organisms that were immobilized after 24 h in the 16 and 9.6 ng/L treatments subsequently died after 96 h, while others remained immobilized or recovered sufficiently to swim, and a total of 63 ± 3% and 37 ± 9%, respectively, remained affected (mortality + immobility) at 96 h (Fig. 3A). Delayed toxicity, which included mortality and immobility, was observed in the two lowest test concentrations with 31–45% of organisms being affected. Mortality in control organisms was 3 ± 3% and immobility was not observed.

Estimates of LC50s (mortality) and EC50s (total organisms affected; mortality + immobility) based on the average measured concentration of deltamethrin and mean response at 96 h were calculated for both 1- and 24-h single-pulse exposures (Table 4). As would be expected, thresholds for the 1-h exposure were higher than for the 24-h exposure, by approximately 7 times. For both exposure durations, LC50s were approximately 1.5-times higher than EC50s.

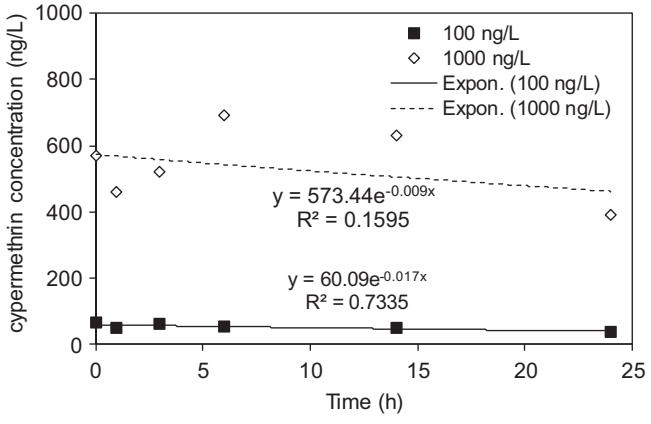

Fig. 2. Loss of cypermethrin from Excis® formulation in water during 24-h test with amphipods. Nominal water concentrations at T = 0 were 100 and 1000 ng/L (n = 1/time).

Table 3
Measured concentrations of deltamethrin and cypermethrin in sediment in 10-d tests of AlphaMax®- or Excis®-spiked sediment with amphipods (n = 3/concentration).

Nominal sediment concentration ng/g	Day 0			Day 10		Day 0 and 10	
	Average ng/g	SE	% of nominal	Average ng/g	SE	Average ng/g	SE
AlphaMax®							
0	<2.4	0.4	–	<1.3	0.4	<1.8	0.3
6	<2.8	0.8	<47	<2.1	1.2	<2.5	0.7
13	4.4	1.4	34	<2.6	1.6	<3.5	1.1
25	7.8	2.5	31	4.1	1.8	5.9	1.6
50	11	4.0	21	6.5	1.4	8.6	2.1
100	30	14	30	24	15	27	9.4
200	54	9.5	27	42	6.4	48	5.7
Excis®							
0	<2.0	0	–	<2.0	0	<2.0	0
9	21	1.2	237	15	4.0	18	2.4
19	33	1.5	174	24	3.0	29	2.5
38	47	4.9	124	35	4.9	41	4.2
75	48	13	64	26	5.2	37	7.8
150	66	14	44	47	8.4	57	8.4
300	106	9	35	65	9.0	86	11

< Less than reported value.

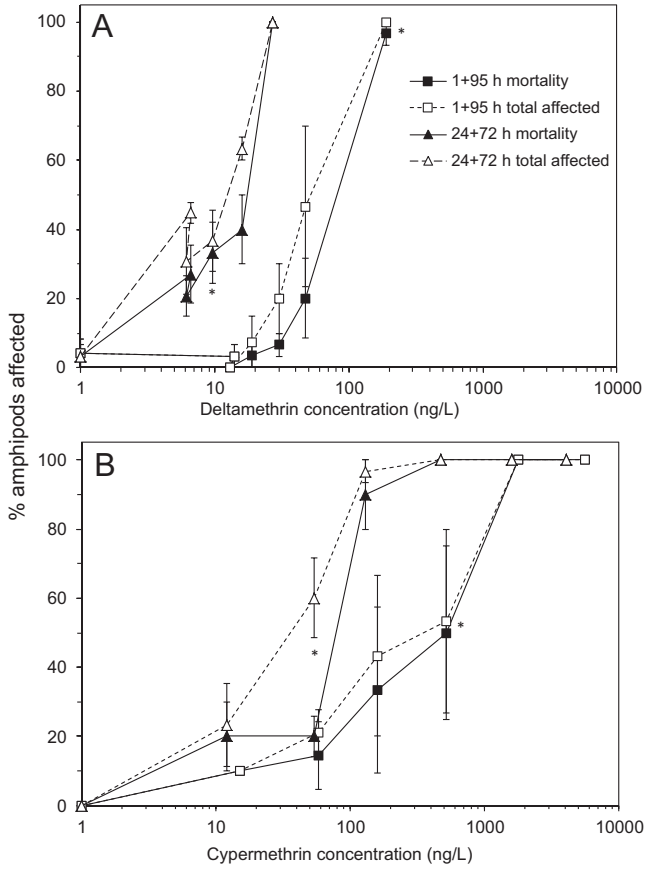

Fig. 3. Acute toxicity of A) AlphaMax® and B) Excis® in water to the amphipod *Echinogammarus finmarchicus*. Organisms were exposed for 1 or 24 h and then monitored at 96 h. (*) indicates the lowest concentration at which organisms were immobilized after 1- or 24 h exposure. Total affected includes mortality + immobility at 96 h. Toxicity data are means ± SE of n = 3 replicates (10 organisms/replicate). Concentrations are average measured concentrations of active ingredient.

Table 4
Estimates of LC50s and EC50s for *Echinogammarus finmarchicus* exposed to AlphaMax® and Excis® in water (95% confidence intervals in brackets). Organisms were exposed for 1 or 24 h and then monitored at 96 h. Thresholds are derived from average measured concentrations of active ingredient.

Pesticide (active ingredient)	1 h exposure + 95 h		24 h exposure + 72 h	
	LC50 ng/L	EC50[a] ng/L	LC50 ng/L	EC50[a] ng/L
AlphaMax® (deltamethrin)	70 (63–80)	47 (43–53)	9.4 (8.1–11)	6.7 (2.6–18)
Excis® (cypermethrin)	220 (130–390)	180 (140–220)	77 (70–83)	20 (17–24)

[a] Total affected includes mortality + immobility.

3.2.2. Toxicity of Excis® in water

Within a 1-h exposure to Excis®, all amphipods in the two highest test concentrations (5600 and 1800 ng/L measured) showed no movement and all organisms in the next highest treatment (520 ng/L) appeared immobilized and were on their backs with their legs twitching. At 96 h, 100% mortality was observed in the two highest treatments and 50 ± 25% mortality was observed in the 520 ng/L treatment in which organisms had been immobilized after the 1-h exposure (Fig. 3B). Delayed toxicity was also observed in other exposure concentrations (15 to 160 ng/L), with 10 to 43% of organisms being affected (mortality + immobility) at 96 h. No effects were observed in control organisms.

After the 24-h exposure to Excis®, all amphipods in the three highest test concentrations (4100, 1600, and 470 ng/L nominal) showed no movement and all organisms in the two next highest treatments (130 and 54 ng/L) appeared immobilized and were on their backs with their legs twitching. At 96 h, 90–100% mortality was observed in the four highest test concentrations (Fig. 3B). Immobilized organisms in the 54 ng/L treatment did not appear to recover, as 60 ± 12% of organisms remained affected (mortality + immobility) at 96 h. Delayed mortality (20 ± 10%) was observed in the lowest test concentration (12 ng/L). No effects were observed in control organisms.

As would be expected, thresholds for the 1-h exposure were higher than for the 24-h exposure, by approximately 3 and 9 times, respectively, for the LC50s and EC50s (Table 4). The 1-h LC50 was 1.2-times higher than the EC50 and the 24-h LC50 was approximately 4-times higher than the associated EC50. This latter difference between the 24-h thresholds is attributed to one treatment (54 ng/L) in which a larger proportion of organisms were immobilized (40 ± 6%) rather than dead (20 ± 6%), which was not typically observed in other treatments for either pesticide.

3.2.3. Toxicity of AlphaMax® and Excis® in sediment

Amphipods stayed mainly at the sediment–water interface throughout the 10-d sediment tests and their swimming caused some resuspension of sediment into the overlying water and oxidation of the surficial layer of sediment, as indicated by its color (personal observation, J. Van Geest). Organisms exposed to the highest test concentration (48 ng/g measured) of AlphaMax®-spiked sediment appeared immobilized by Day 2 (data not shown). At 10 d, 95 ± 3% mortality (mean ± SE) was observed in this treatment and 52 ± 6% and 37 ± 9% mortality was observed in the two next highest treatments (27 and 8.6 ng/g; Fig. 4). Overlying water in replicates from the three highest test concentrations was clear by the end of the test, likely because of reduced activity of the amphipods. Mortality ranged from 12–22% in the other test concentrations (2.5–5.9 ng/g) and was 10 ± 3% in the controls. The estimated 10-d LC50 (95% C.I.) was 16 (14–19) ng/g based on average measured concentrations of deltamethrin in sediment.

Some of the organisms exposed to the highest test concentration (86 ng/g measured) of Excis®-spiked sediment appeared immobilized by Day 4 (data not shown). At 10 d, 57 ± 3% mortality was observed in this treatment and 25 ± 3% mortality in the next highest treatment (57 ng/g; Fig. 4). Overlying water in replicates from the two highest test concentrations was clear by the end of the test, indicative of reduced organism activity. Mortality ranged from 5–13% in the other test concentrations (18–37 ng/g) and was 12 ± 6% in the controls. The estimated 10-d LC50 (95% C.I.) was 80 (72–94) ng/g based on average measured concentrations of cypermethrin in sediment.

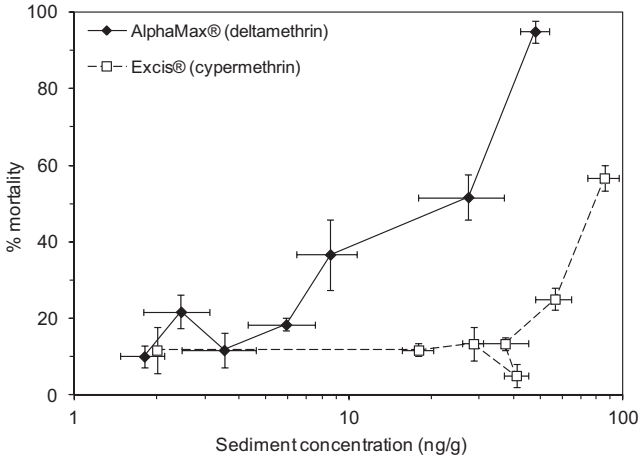

Fig. 4. Toxicity of AlphaMax® and Excis® in 10-d sediment tests to the amphipod *Echinogammarus finmarchicus*. Toxicity data are means ± SE of n = 3 replicates (20 organisms/replicate). Sediment data are means ± SE of measured concentrations of active ingredient on Days 0 and 10 (n = 6).

4. Discussion

In the present study, amphipods were exposed to AlphaMax® and Excis® in water in single-pulse exposures to reflect the acute nature of pesticide effluent plumes from aquaculture sites. With both pesticides, an increased response was observed with the longer exposure duration, as would be expected. This was reflected by the 3- to 9-fold difference between 1-h and 24-h thresholds, which is comparable to similar studies of other marine crustaceans exposed to these pesticides (cf. Burridge et al., 2014 and DFO unpublished data in Table 5). Amphipods that received a 1-h single-pulse exposure showed greater variability in response at the end of the test in treatments where partial effects were observed, compared with the 24-h exposures. For AlphaMax®, the response curve from the 24-h single-pulse exposure was shifted to lower concentrations because loss of deltamethrin over time was accounted for in the exposure estimates. The 1- and 24-h response curves for Excis® were at similar concentrations because loss of cypermethrin over time was minimal. Despite some of these noted differences between the 1-h and 24-h single-pulse exposures, the general shapes of the response curves were similar for each of the two pesticides. Thresholds for tests with Excis® were 3- to 8-times higher than those with AlphaMax®, which is consistent with other research indicating that deltamethrin is more toxic than cypermethrin.

Distinguishing between lethality and immobility is necessary to report absolute effects, and in the present study, as much as a 4-fold difference was observed between LC50 and EC50 thresholds. However, immobility is an equally important endpoint, equivalent to ecological death, because in the field an immobilized organism is unable to feed, seek shelter, or avoid predation. Other studies have found irreversible immobility to be a sensitive endpoint in the amphipod *E. estuarius* exposed to AlphaMax® and have reported EC50s that are 2.4- to 3.8-times lower than associated LC50s (cf. Fairchild et al., 2010 and EC-ALET unpublished data in Table 5).

Delayed toxicity, rather than recovery, was typically observed following single-pulse exposures to AlphaMax® and Excis®. Organisms immobilized after 1- or 24-h exposures to higher test concentrations typically did not recover by 96 h and organisms unaffected right after the pesticide exposure were later immobilized or dead. In the present study, delayed effects were observed in up to 50% of organisms in a test treatment following pesticide exposure. Delayed toxicity has been

Table 5
Summary of acute toxicity of AlphaMax® and Excis® in water to marine crustaceans. Thresholds are concentrations of active ingredient (95% confidence intervals in brackets).

Species — life stage	Exposure (+recovery)	LC50 ng/L	EC50[a] ng/L	Temperature °C	Reference
AlphaMax® (deltamethrin)					
Lobster stage I	1 h + 95 h	3.4 (1.5–6)		10	Burridge et al. (2014)
Eohaustorius estuarius	1 h + 95 h	13 (4.8–36)	5.5 (5.1–6)	15	Fairchild et al. (2010)
Mysis sp.	1 h + 95 h	14 (11–18)		13	Burridge et al. (2014)
Lobster adult	1 h + 95 h	19 (3.9–34)		8.5	Burridge et al. (2014)
Lobster stage III	1 h + 95 h	37 (25–53)		20	Fairchild et al. (2010)
Echinogammarus finmarchicus	1 h + 95 h	70 (63–80)	47 (43–53)	10	This study
Crangon septemspinosa	1 h + 95 h	142 (104–194)		15	Fairchild et al. (2010)
Lobster stage I	24 h + 72 h	0.8 (0.6–1)		12	Burridge et al. (2014)
Lobster stage II	24 h + 72 h	0.6 (0.3–1)		12	Burridge et al. (2014)
Lobster stage IV	24 h + 72 h	1.7 (0–4.8)		12	Burridge et al. (2014)
Mysis sp.	24 h + 72 h	1.4 (0–3.6)		12	Burridge et al. (2014)
Echinogammarus finmarchicus	24 h + 72 h	9.4 (8.1–11)	6.7 (2.6–18)	10	This study
Lobster adult	24 h + 72 h	15 (11–19)		12	Burridge et al. (2014)
Crangon septemspinosa	24 h + 72 h	27 (14–40)		12	Burridge et al. (2014)
Eohaustorius estuarius	48 h + 48 h	16 (13–19)	4.2 (1.8–9.6)	15	EC-ALET unpublished[b]
Excis® (cypermethrin)					
Lobster stage II	5 min + 12 h	660–1690		10–12	Pahl and Opitz (1999)
Praunus flexuous	1 h + 95 h	>142		14	DFO unpublished
Echinogammarus finmarchicus	1 h + 95 h	220 (130–390)	180 (140–220)	10	This study
Lobster stage II	12 h + 12 h	58–365		10–12	Pahl and Opitz (1999)
Lobster adult	24 h	140		10	Burridge et al. (2000b)
Praunus flexuous	24 h + 72 h	33 (25–44)		14	DFO unpublished
Echinogammarus finmarchicus	24 h + 72 h	77 (70–83)	20 (17–24)	10	This study
Lobster stage I	48 h	180 (20–320)		10	Burridge et al. (2000b)
Lobster stage II	48 h	120 (60–180)		10	Burridge et al. (2000b)
Lobster stage III	48 h	60 (30–90)		10	Burridge et al. (2000b)
Lobster stage IV	48 h	120 (80–170)		10	Burridge et al. (2000b)
Lobster adult[c]	48 h	81		15	Burridge et al. (2000a)
Eohaustorius estuarius	48 h	1000–3600	7–40	15	Ernst et al. (2001)
Amphiporeia virginiana	48 h	6900–7400	3.4–30	15	Ernst et al. (2001)
Amphiporeia virginiana	48 h + 48 h	12 (0–20)		15	Ernst et al. (2001)

[a] Immobility.
[b] Personal communication P. Jackman, Environment Canada-Atlantic Laboratory for Environmental Testing (EC-ALET).
[c] Repeated exposure mean test concentration.

observed in other studies of acute, single-pulse exposure of invertebrates to pyrethroids. Following a 1-h pulse exposure to 100 or 300 ng/L esfenvalerate, delayed mortality was observed in 70–100% of juveniles and 15% of adults of the freshwater amphipod *Gammarus pulex*, within15 d post-exposure (Cold and Forbes, 2004). Delayed mortality in 25–75% of organisms occurred between Days 2 and 10 in the freshwater cladoceran *Daphnia magna*, following a 24-h pulse exposure to 600 to 3200 ng/L fenvalerate (Reynaldi and Liess, 2005). The delayed toxicity observed in these and the present study demonstrates the importance of monitoring for effects after a single-pulsed exposure.

The toxicity of these pesticides varies across non-target species and previous studies found the amphipod *E. estuarius* to be more sensitive to AlphaMax® than stage III and IV lobster and *Crangon* shrimp in both 1-h single-pulsed and standard 96-h exposures (Fairchild et al., 2010). More recent studies have examined the acute toxicity of AlphaMax® in single-pulsed 1- and 24-h exposures of shrimp and various life stages of lobster (Burridge et al., 2014) and 48-h exposures of *E. estuarius* (see summary in Table 5). *E. finmarchicus* in the present study was less sensitive to AlphaMax® than larval stage lobsters, *Mysid* shrimp, and *E. estuarius*, and was more sensitive than *Crangon* shrimp. Acute toxicity data for Excis® (or even technical grade cypermethrin) are limited for exposures ≤24 h (Table 5). *E. finmarchicus* was less sensitive to Excis® than the shrimp *Praunus flexuous* in 1- and 24-h single-pulsed exposures. In 48-h exposures to Excis® all lobster life stages were more sensitive than the amphipods *E. estuarius* and *Amphiporeia virginiana* (Burridge et al., 2000a, 2000b; Ernst et al., 2001). Although it is not possible to directly compare all the studies because of the different test conditions, *E. finmarchicus* appears to be at lower risk from aquaculture pesticide use than other non-target marine crustaceans.

Effluent plumes of anti-sea lice pesticides from aquaculture sites have been detected several hours post-release, at concentrations equivalent to 1000- to 2000-fold dilutions of the pre-release concentrations (Ernst et al., 2001). The 1-h thresholds determined for *E. finmarchicus* in the present study represent 23- to 43-fold dilutions of the associated aquaculture treatment concentrations. Work on modeling effluent plumes from aquaculture sites (by Ernst et al., 2001, 2014 and Page et al., DFO unpublished) suggests that dilutions of these magnitudes could occur >1 to 2.5 h post-release of effluent from a cage site. Therefore, amphipods within the vicinity of an effluent plume may be exposed to aqueous concentrations above the 1-h thresholds for a sufficient duration to cause adverse effects, including delayed toxicity. The 24-h thresholds in the present study represent 65- to 300-fold dilutions, which are predicted to occur around aquaculture cages within 3–4 h post-release (Ernst et al., 2001, 2014 and Page et al., DFO unpublished), but effects are not expected from a single anti-sea lice treatment because amphipods are unlikely to be exposed to threshold concentrations for as long as 24 h. However, it is possible that multiple cages are treated at one site within a short time frame (i.e., same day or subsequent days), and this could increase both the pesticide concentrations and the length of time over which non-target organisms are exposed to toxic concentrations.

Few studies have examined the toxicity of these pyrethroids in sediments to marine organisms, despite sediment being a potential sink. Spiked-sediment studies with other amphipod species have reported the following 10-d LC50s (95% C.I.): 0.54 (0.48–0.61) ng/g for *E. estuarius* exposed to AlphaMax® in sand (EC-ALET, 2014, personal comm.), 8 (6.4–9.6) ng/g for *Corophium volutator* exposed to Excis® in sediment (Mayor et al., 2008), and 11 ng/g for *E. estuarius* exposed to technical grade cypermethrin in formulated sediment (Anderson et al., 2008). However, it is important to note that these thresholds are based on nominal concentrations of active ingredient, which are less conservative than measured concentrations. *E. finmarchicus* was much more tolerant than these two other amphipod species to deltamethrin

and cypermethrin in sediment, by approximately 30- and 10-times, respectively (based on measured values versus the above nominal LC50s). These differences in sensitivity may partly be attributed to the much smaller size of amphipods in those studies (~5 mm adults), which may have increased uptake of the chemical into the animals due to their higher surface area to volume ratio (Sprague, 1995). Cold and Forbes (2004) observed that smaller adults (7–8 mm) of the freshwater amphipod *Gammarus pulex* were up to 1.5-times more sensitive to the pyrethroid esfenvalerate than larger adults (10–14 mm) in 24- to 96-h water-only, lethality tests. Juveniles (<3 mm) of both *G. pulex and Gammarus fossarum* were reported to be 14- to 22-times more sensitive to deltamethrin than adults (>7 mm) in 48-h water-only, lethality tests (Adam et al., 2010). The lower sensitivity of *E. finmarchicus* observed in the present study may be attributable to organism size and not solely, species-specific sensitivity. In comparison to sediment tests, *E. finmarchicus* was only 5-times more tolerant to deltamethrin in water-only tests than *E. estuarius* (cf. Fairchild et al., 2010 in Table 5). This suggests that sediment-specific factors may also influence the differences in relative sensitivity observed between studies, but without measured concentrations in the other sediment studies it is difficult to speculate on this further.

There is limited environmental data for pesticide concentrations in sediments related to aquaculture use and chemical monitoring around aquaculture sites is not a requirement in every jurisdiction, Canada included. The Scottish Environmental Protection Agency (SEPA) conducted surveys around marine fish farms and reported concentrations of cypermethrin in sediment ranging from 0.03 to 7.2 ng/g dw (SEPA, 2004, 2005, 2006, 2007), with most sites below the SEPA predicted-no-effect-concentration (PNEC) of 2.2 ng/g. Deltamethrin was not included in those surveys, despite its use in Scottish aquaculture and there being a proposed PNEC of 0.33 ng/g (SEPA, 2008). The 10-d sediment LC50s reported for *E. finmarchicus* in the present study are much higher than reported environmental concentrations in sediment, assuming cypermethrin concentrations would reflect those for deltamethrin. For this amphipod species, this suggests a low potential for acute toxicity from sediment exposures; however, this does not consider chronic exposure. While the SEPA PNECs for deltamethrin and cypermethrin in sediment are well below the thresholds for *E. finmarchicus*, they may not be sufficiently protective for a more sensitive amphipod such as *E. estuarius*. The lack of environmental chemistry data for sediment around cage sites remains a large source of uncertainty when assessing the potential for adverse effects to benthic invertebrates.

5. Conclusions

The toxicity of the sea-lice pesticides AlphaMax® and Excis® have not been thoroughly studied for sensitive non-target crustaceans such as marine amphipods in both aqueous and sediment exposures. Results of 1-h single-pulsed exposures in the present study with the amphipod *E. finmarchicus* suggests that organisms in the vicinity of effluent plumes could potentially be exposed to toxic concentrations of deltamethrin and cypermethrin for sufficient duration to cause lethality or immobility, even delayed effects after exposure. In sediment tests, the 10-d LC50s reported for *E. finmarchicus* for these pesticides are much higher than the existing environmental data, suggesting low potential for risks via this route of exposure. However, other species of amphipods appear to be more sensitive. Regardless, the lack of environmental chemistry data in sediment around cage sites remains a large source of uncertainty when assessing the potential for adverse effects to benthic invertebrates. The risk to benthic invertebrates would be dependent on the magnitude and extent of pesticide accumulation in sediment, which would be influenced by treatment frequency, and physical, chemical, and oceanographic conditions.

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