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A study of dichlorvos (Nuvan; 2,2 dichloroethenyl dimethyl phosphate), a therapeutic agent for the treatment of salmonids infected with sea lice (*Lepeophtheirus salmonis*)

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ABSTRACT

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Larval lobsters (*Homarus americanus*), juvenile lobsters, adult lobsters (*H. americanus*), species of zooplankton, species of phytoplankton, mussels (*Mytilus edulis*) and periwinkles (*Littorina littorina*) were exposed to dichlorvos at various concentrations in the laboratory and mortalities were recorded. Dichlorvos was also released from an ocean field site and at a fish farm while stage IV larval or juvenile lobsters (*H. americanus*) were suspended in the water column in areas adjacent to the release site to determine the effects of dichlorvos on their survival. Results indicate that dichlorvos is not toxic to mussels or periwinkles at 1.0 ppm, for 1 h exposure, but is toxic to larval lobsters, adult lobsters, zooplankton and phytoplankton. In field trials larval and juvenile lobsters housed adjacent to sea-cage operations were not killed following treatment with dichlorvos.

INTRODUCTION

Sea lice (*Lepeophtheirus salmonis*), ectoparasites of salmonids, are creating concern within the aquaculture industry of Canada. The prophylactic agent of choice to treat for these parasites is dichlorvos (trade name Nuvan, Ciba Giegy, Inc.). Despite the success of dichlorvos treatments this chemical is not legally available for use in Canada, partially because of the paucity of information on the effect of the chemical on the flora and fauna at and near treatment sites. Special attention was given to the use of dichlorvos because of reported mortalities of European lobsters exposed to this chemical (Egidius and Moster, 1987). The purpose of the study was to determine the rate of mortality of species of zooplankton, species of phytoplankton, periwinkles

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(*Littorina littorina*), mussels (*Mytilus edulis*), and larval and adult American lobsters (*Homarus americanus*) exposed to various concentrations of dichlorvos.

MATERIALS AND METHODS

Larval lobsters (Homarus americanus)*: laboratory test*

Twenty-day-old, stage IV larval lobsters were obtained from the Department of Fisheries and Oceans in St. Andrews, N.B. These larvae were transferred to the laboratory and acclimated at 30‰ and 20°C in a recirculating saltwater system for 3 days before experimentation.

Ten larval lobsters were exposed in 1 l of saltwater with dichlorvos concentrations of 1.0, 0.1, 0.01, and 0.001 ppm, respectively. A control group of 10 lobsters was handled similarly but not exposed to dichlorvos. After the exposure, larval lobsters were transferred through three rinses of noncontaminated saltwater for a period of 1 h per rinse. After the rinse baths all surviving larvae were transferred to the holding facilities. Larval lobsters were divided into two replicate groups of five and held in 3.5-l polyethylene containers. Recirculating seawater was maintained at 30‰ salinity at 20°C. Larval lobsters were fed twice daily *ad libitum* with *Artemia*. Larvae were observed for mortalities and behavioural changes for 30 days during which time they molted to stage V juvenile lobsters.

Adult lobsters (Homarus americanus)

Adult lobsters (average carapace length 9.2 ± 1.6 cm) were purchased from a local lobster pound. They were acclimated for 15 days in the laboratory at 30‰ salinity and 20°C in a recirculating saltwater system before testing. Lobsters were exposed in groups of 10 in 50 l of aerated saltwater to concentrations of dichlorvos of 1.0, 0.1, 0.01, and 0.001 ppm for 1 h. A control group of 10 lobsters was maintained in conditions similar to the treated groups. After the hour exposure all lobsters that survived the treatment were transferred through three saltwater rinse baths of 1 h each before being placed in the holding facility. Lobsters were held for 30 days post-exposure in individual compartments measuring 33 cm in length, 13 cm in width and 11 cm in depth. Recirculating saltwater temperature was maintained constant at 20°C, with a salinity of 30‰. Lobsters were fed on alternate days with a pelleted lobster feed. The lobsters were monitored daily for mortalities and abnormal behaviour.

Mussels (Mytilus edulis)

Cultured mussels were collected from a commercial longline lease site in Murray River, P.E.I., Canada and acclimated for 10 days in the laboratory at 30‰ salinity and 20°C. Mussels were exposed to dichlorvos using the same

methodology as previously described for larval lobsters. Mussels were suspended in polyethylene mesh in replicate groups of five in 18°C, recirculating seawater for 30 days, post-exposure, and mortalities were monitored daily.

Periwinkles (Littorina littorina)

Periwinkles were collected from a local beach, brought to the laboratory and acclimated for 10 days to 30‰ and 20°C recirculating saltwater. These animals were exposed in groups of 20 as described in the larval lobster experiment. The periwinkles were held in replicate groups of 10 under conditions similar to those described for the larval lobsters except the animals were not fed and were observed daily for mortalities.

Zooplankton

Zooplankton was collected by pumping seawater from a local beach through a 100-μm plankton mesh. Living zooplankters were collected and placed in saltwater in a polyethylene container and transported on ice to the laboratory. The zooplankters were acclimated to 18°C over 24 h. Replicate groups of 10 individuals of these mixed species of zooplankton were then exposed to 1.0, 0.1, 0.01 and 0.001 ppm of dichlorvos for 1 h. After the 1-h exposure the zooplankters were pipetted, using a wide-bore pipette, through three rinses of noncontaminated water at 1 h per rinse. The groups of zooplankton were then placed in Petri dishes with 15 ml of water at 18°C and mortalities observed at 1, 2, 3, 24, and 48 h post-exposure.

Phytoplankton

A concentrated algal culture was obtained from the Department of Fisheries and Oceans in Miminegash, P.E.I., Canada. The algal species included: *Platymonas* sp., *Chaetoceros calcitrans, Chaetoceros gracillis* and *Isochrysis galbana*.

Algal nutrient solution was prepared using the formulation proposed by Guillard (1975). Algae were exposed to dichlorvos concentrations of 1.0, 0.1, 0.01 and 0.001 ppm in 10 ml of the nutrient solution. In this experiment 0.002 g of algal paste was added to each of the dichlorvos treated nutrient solutions plus controls consisting of nutrient solutions without dichlorvos. After the algae were added, the solutions were suspended with the aid of a vortex shaker and allowed to stand for 1 h, and the mixtures were centrifuged at 2600 rpm for 5 min. To rinse the dichlorvos from the algae, the centrifuge tubes were decanted, filled with 10 ml of fresh nutrient solution, vortexed and allowed to stand for 1 h. Each mixture was rinsed three times.

Resuspended algal solutions were placed 5 cm in front of a balanced spectrum fluorescent light (Sylvania Gro-lux) to encourage growth. Algal growth was measured daily with the aid of a spectrophotometer (Spectronic 20). The experiment was terminated on day 9.

Dichlorvos exposure of larval lobster: field test 1

Fourth stage larval lobsters (20 days old) were transported in saltwater in polyethylene bags on ice from St. Andrews, N.B., Canada, to the field site at Owls Head Bay, N.S. (Fig. 1). Larval lobsters were acclimated for 1.5 h to the ambient surface seawater temperature of 12.0°C. The larval lobsters were then transferred to holding tanks consisting of polyethylene containers surrounded by 200-μm mesh. Ten lobsters were placed in each holding tank. Tanks of larval lobsters were then secured by ropes to an anchor line and submerged slowly to four depths: 0 m, 3 m, 5 m, and 10 m. Anchor lines with attached tanks were placed at five locations in Owls Head Bay (Fig. 2). These locations were 0 m, 10 m, 100 m, and 1000 m away from the point of dichlorvos release and a control group was placed in a bay adjacent to Owls Head Bay approximately 1 km from the 1000-m location.

One l of dichlorvos was mixed in 20 l of seawater. The mixture was poured into the Bay along a 30-m line across site 0 at a depth of 1 m. After the mixture

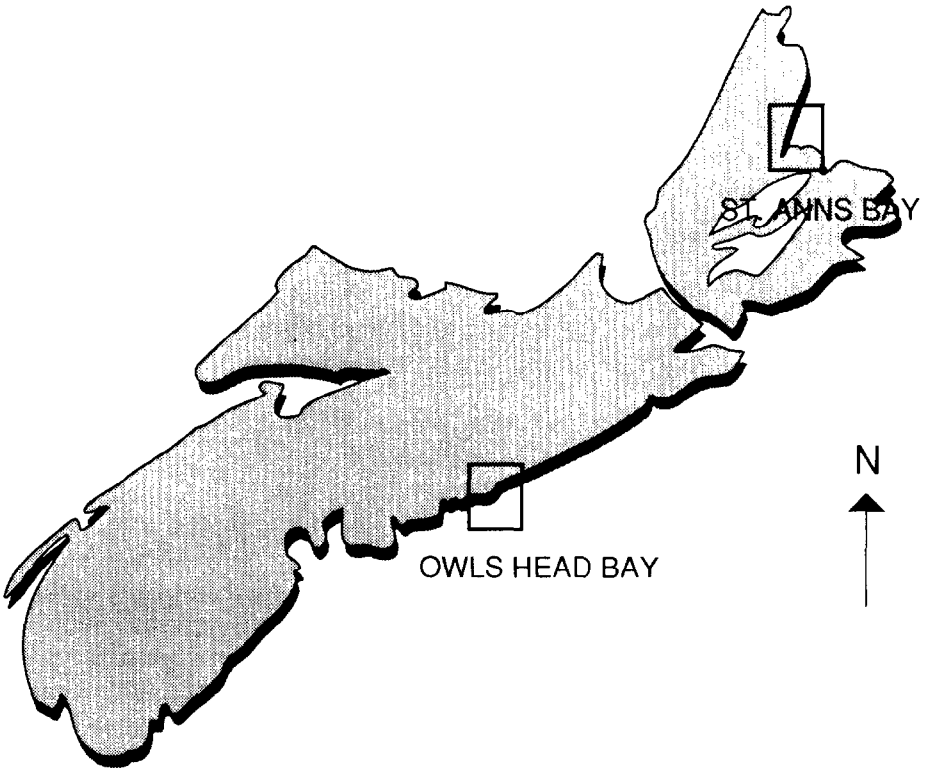

Fig. 1. Map of Nova Scotia showing the location of the larval lobster field test 1, Owls Head Bay, and field test 2, St. Anns Bay.

Fig. 2. Location of suspended larval lobsters in Owls Head Bay during dichlorvos exposure of larval lobsters in field test 1. 1 = Site of dichlorvos release and a site of suspended larval lobsters; 2 = site of suspended larval lobsters 10 m away from the dichlorvos release site; 3 = site of suspended larval lobsters 100 m away from the dichlorvos release site; 4 = site of suspended larval lobsters 1000 m away from the dichlorvos release site; 5 = site of suspended larval lobsters, the control group.

was poured into the water a boat with an outboard motor was revolved around the release site to mix the chemical.

Dichlorvos was added to the water at slack tide followed by the tide moving out of the Bay. At the time of dichlorvos release the surface current was measured at 0.9 km/h in the direction of the suspended larval lobsters, the wind was light from the northeast.

After 6 h the larval lobsters were removed from the holding containers and checked for mortalities. Lobsters were considered dead if no movements could be observed.

Dichlorvos exposure of juvenile lobsters (Homarus americanus)*: field test 2*

Stage V juvenile lobsters were transferred in saltwater on ice from the Atlantic Veterinary College, P.E.I., to St. Anns Bay, N.S. The lobsters were acclimated to ambient seawater temperature of 17 °C for 1 h. The juveniles were then transferred to 500-ml polyethylene holding tanks surrounded by 200-μm mesh. Ten lobsters were placed in each holding tank. Tanks were then secured by ropes to anchor lines and submerged at four locations in the direction of the current (Figs. 1 and 3)..

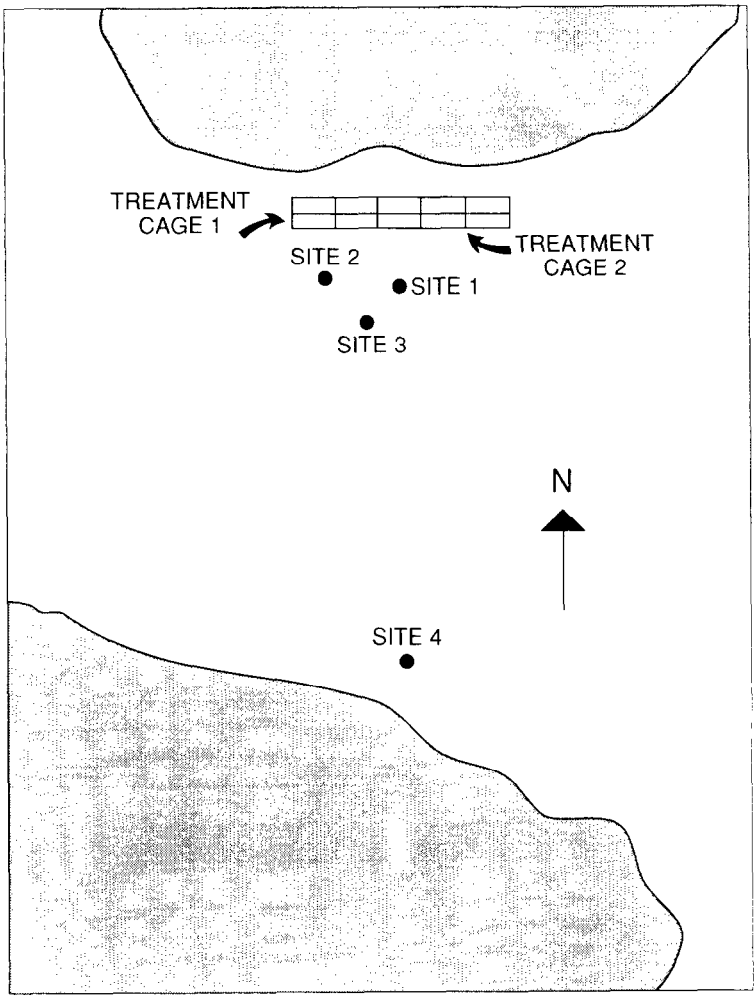

Fig. 3. Locations of suspended larval lobsters in St. Anns Bay during exposure of larval lobsters in field test 2. 1 = site of suspended lobsters 10 m from treatment cage 2; 2 = site of suspended lobsters 10 m from treatment cage 1; 3 = site of suspended lobsters 100 m from treatment cages 1 and 2; 4 = site of suspended lobsters approximately 1000 m from treatment cages 1 and 2.

The tanks containing juvenile lobsters at site 1 were 10 m from treatment cage 1 in the direction of the current. One group of 10 juvenile lobsters was suspended at 2 m depth at site 1. Site 2 was located 10 m from treatment cage 2. Groups of 10 juvenile lobsters were suspended at 40 cm below the surface and another 10 were suspended at 2 m below the surface. Site 3 was located approximately 100 m from the treatment cages 1 and 2 and had 10 juvenile lobsters suspended at 40 cm below the surface and 2 m below the surface. Site

4 was approximately 1000 m from the dichlorvos release sites and contained two groups of juvenile lobsters, one at the surface and another at 2 m in depth.

Thirty-eight ml of dichlorvos were poured into treatment cage 1 containing approximately 20 m^3 of saltwater surrounded by a plastic tarpaulin with 1000 rainbow trout (*Oncorhynchus mykiss*) with attached sea lice. After 1 h the tarpaulin was removed and the dichlorvos/seawater mixture dispersed out of the cage. A second cage was treated in the same fashion except only 25 ml of dichlorvos were used. Five and 24 h post-dichlorvos treatment the juvenile lobsters were observed for mortalities. Mortalities were recorded if all visible movements ceased.

RESULTS

Larval lobsters

After 3 min post-exposure to dichlorvos there was a noticeable increase in the level of activity of larval lobsters. The amount of activity increased progressively from controls to 1.0 ppm. At 20 min post-exposure the same high level of activity was maintained. By 30 min post-exposure larval lobsters in the 1-ppm groups became disoriented and began to convulse. The rapid movements at this stage included rapid turning, dorsoventrally. By 38 min post-exposure to 1 ppm, seven of the 10 specimens were dorsally recumbent and showing signs of convulsions. At the same time those animals in 0.1 ppm were upright but the level of activity was much higher than controls. At 50 min post-exposure all larval lobsters in 1.0 ppm were in dorsal recumbency. Motion was restricted to rapid movements of the swimmerets except for occasional seizures.

By 60 min post-exposure, when the larvae were transferred to noncontaminated seawater, two of 10 larval lobsters in 1.0 ppm had died. There were no mortalities in other groups. At 70 min post-exposure three lobsters had died in the 1.0-ppm group. By 100 min post-exposure all lobsters in 1.0-ppm groups had died. At 100 min post-exposure the larval lobsters in the 0.1-ppm and 0.01-ppm groups still displayed increased levels of activity compared to controls. There was little noticeable difference between the 0.001-ppm group and controls. Although the level of activity in the 0.1-ppm group was increased it was never as high as that seen in the 1.0-ppm groups.

By 140 min post-exposure larval lobsters in the 0.1-ppm group were still more active than the other groups but in general the amount of activity had decreased. By 200 min post-exposure (i.e., 140 min in noncontaminated saltwater) the level of activity in all groups, including the controls, was the same and appeared to be normal. There was no difference in mortality rate among the groups of lobsters held for 30 days post-exposure.

Adult lobsters

Adult lobsters exposed to dichlorvos responded to the treatment in a similar fashion as those in the larval lobster experiment. There was an increase in the level of activity from the 0.01-ppm to 1.0-ppm groups. The 1.0-ppm group was more active than any other group. There were no discernible differences between the 0.001-ppm group and controls. By 30 min post-exposure all the adult lobsters in 1.0 ppm had died. There were no mortalities in any other groups 30 days post-exposure. Increased activity in the 0.1-ppm group was maintained for 2.5 h post-dichlorvos treatment.

Mussels

No mussel mortalities resulted from dichlorvos exposure. There was no noticeable difference in behaviour between control and treatment groups.

Periwinkles

There were obvious behavioural modifications between periwinkle treatment groups. The periwinkles in 1.0 ppm showed very little movement. At no time during the 1-h exposure did the animals adhere to the glass, climb or perform feeding behaviour. This lack of activity was in contrast with those animals in the control group which moved freely up and down in the container and performed normal grazing behaviour. In all other dilution groups (0.1, 0.01, and 0.001 ppm) there was movement. This included adherence to the glass surface and grazing behaviour. There was no discernible difference between 0.01, 0.001 and controls. However, less activity was observed in the groups of periwinkles exposed to 0.1 ppm dichlorvos.

After the periwinkles were transferred to the noncontaminated rinse baths, the level of activity in 1.0- and 0.1-ppm groups increased. After 5 min in the rinse bath no difference could be detected between all groups including the controls. There was no difference in mortalities between groups of periwinkles held for 30 days.

Zooplankton

There were significantly more mortalities in zooplankters exposed to 1.0 ppm dichlorvos. There was no difference in the number of mortalities among any of the other groups (Fig. 4). The level of activity in 0.1-ppm groups was greater than 0.01, 0.001 and controls but there appeared to be no difference in activity between 0.01, 0.001 and control groups.

Algae

One replicate in the 0.01-ppm group was deemed invalid because of a broken centrifuge tube in which the algae were growing and one replicate in the control group was deemed invalid because the algae failed to grow after 3 days in solution. Values for these two samples were discarded for statistical analysis.

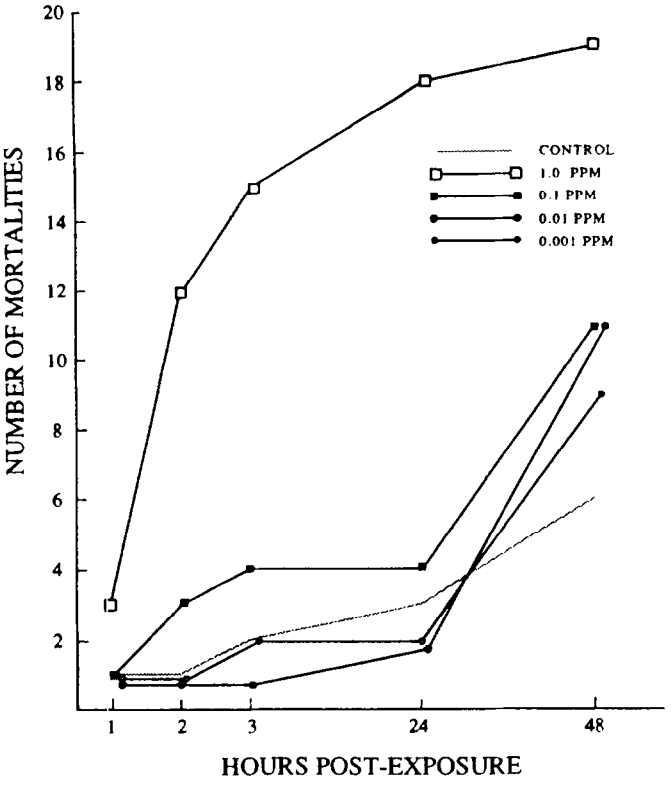

Fig. 4. Number of mortalities of zooplankters exposed to varying concentrations of dichlorvos vs. time in hours post-exposure to dichlorvos.

Algal growth was significantly reduced by dichlorvos ($P > 0.05$). The amount of growth, as measured by the percentage light transmission, was inversely proportional to the logarithm of the dichlorvos concentration (Fig. 5).

Dichlorvos exposure of larval lobsters

The released dichlorvos was visualized as a white plume which flowed in the direction of the suspended larval lobsters. The plume could be seen floating through the tank of suspended lobsters at the surface of sites 1 and 2. By site 3 (100 m from the release site) the dichlorvos had dissipated to such an extent that the white plume was no longer visible. After 6 h post-dichlorvos release only two of the 200 suspended larval lobsters were dead. One mortality occurred at the 10-m depth of site 2 while the other mortality occurred at site 4 at 3 m depth. These two mortalities are considered insignificant because of the fragility of lobsters at this stage of their life cycle.

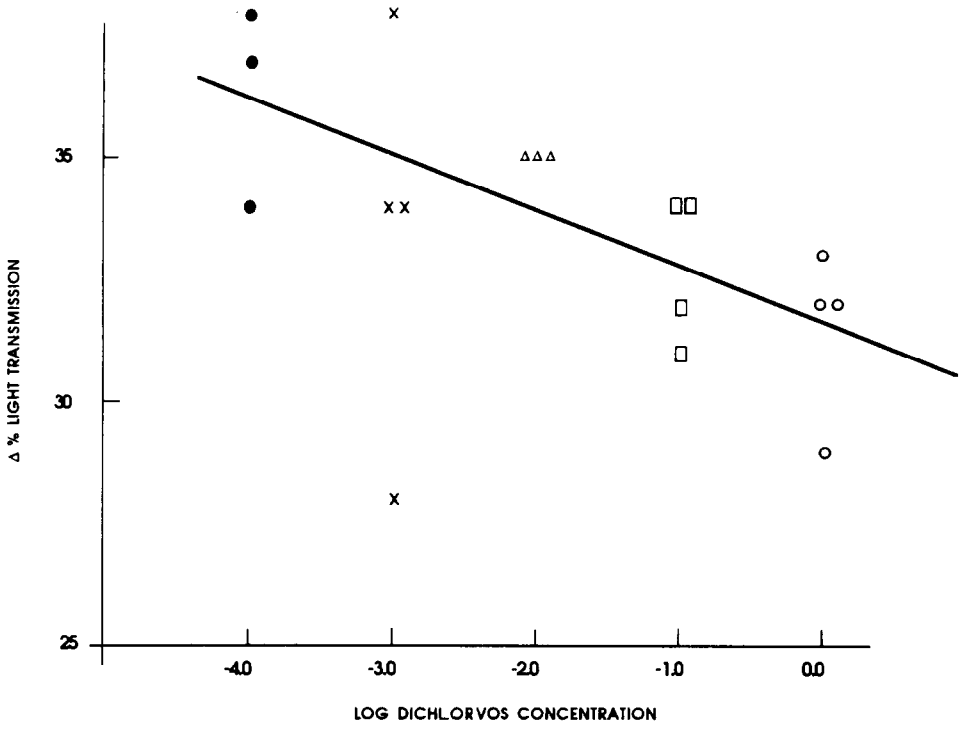

Fig. 5. Regression of the change in light transmission of growing cultures of algae exposed to dichlorvos at varying concentrations represented in logarithmic transformation; indicating decreased growth at higher dichlorvos concentrations ($Y=31.7-1.1X$; $P>0.05$).

Dichlorvos exposure of juvenile lobsters

Fifteen min after the addition of dichlorvos to treatment cages 1 and 2 the swimming activity of the rainbow trout increased for the remainder of the 1-h treatment. Sea lice were seen dropping off the trout 20 min after dichlorvos exposure. The white plume of dichlorvos dissipated within seconds of its introduction to the treatment cages. After 5 h post-dichlorvos release only one of the 70 suspended juvenile lobsters was dead. At 24 h post-dichlorvos treatment four of the 70 suspended juvenile lobsters were dead. These low mortalities were considered insignificant because of the fragility of lobsters at this stage of their life cycle.

DISCUSSION

The present study assessed the effects of dichlorvos on various species, including larval lobsters, adult lobsters, mussels, periwinkles, zooplankton and phytoplankton. This work is, to our knowledge, the first to test the effect of dichlorvos on American lobsters (*Homarus americanus*). Death of adult

lobsters began after only 25 min exposure to 1.0 ppm dichlorvos. These results correspond closely with those of Egidius and Moster (1987) who found the European lobster died within 30 min of exposure to 1.0 ppm of dichlorvos. Egidius and Moster (1987) also reported adult European lobsters surviving for the 24-h exposure to 0.01 ppm dichlorvos.

Our study is the first reported work on the effect of dichlorvos on marine zooplankton and phytoplankton. At 1.0 ppm the mixed zooplankton population declined more rapidly than control groups. Phytoplankton growth was inversely proportional to dichlorvos concentrations. In freshwater systems Krzeczkowska-Woloszyn (1979) reported that trichlorfon, which degrades to dichlorvos, inhibited phytoplankton growth in carp ponds. Lewkowicz et al. (1979) demonstrated that zooplankton populations were reduced by trichlorfon. Pal (1983) reported that a freshwater species of zooplankton *Diaptomus forbesi* was killed at concentrations of 0.123 ppm dichlorvos, but the time of exposure was not recorded. Pal and Konar (1985a) reported that populations of phytoplankton and zooplankton were significantly reduced when exposed to dichlorvos. As in the present work, phytoplankton growth was progressively reduced as the concentration of insecticide increased. Pal and Konar (1985b) reported that zooplankton were reduced progressively with increased dichlorvos concentrations.

No mortalities were recorded from exposures of periwinkles (*L. littorina*) or mussels (*M. edulis*) to concentrations of dichlorvos ranging from 0.001 to 1.0 ppm. Egidius and Moster (1987) also reported that mussels were unaffected by dichlorvos exposures at concentrations of 1.0 ppm for 24 h. Periwinkles did respond behaviourally to the chemical, probably as a result of irritation. Normal grazing behaviour returned 5 min after the organisms were removed from the chemical bath.

There are no previous published reports on results of exposure of larval lobsters to dichlorvos. Larval lobsters, in this experiment, died at 1.0 ppm within 60 min of dichlorvos exposure. No mortalities were recorded in any of the other groups. The results of our larval and juvenile lobster field tests suggest that rapid dilution of dichlorvos in the water column enables larval and juvenile lobsters adjacent to treatment sites to survive. In the first larval lobster test 1 l of dichlorvos was released. This quantity is enough to treat 80 cages at the manufacturer's suggested doses of 10–15 ml of dichlorvos in 10 m^3 of saltwater. Although the white plume of released dichlorvos was seen making contact with tanks of suspended larval lobsters the vast majority survived the observation period. The few mortalities that did occur are considered incidental given the fragile nature of lobsters at this stage of their life cycle.

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