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EFFECT OF FENITROTHION ON THE FORAGING BEHAVIOR OF JUVENILE ATLANTIC SALMON

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Abstract – Juvenile Atlantic salmon (*Salmo salar*) were exposed for 7 d to sublethal concentrations of technical grade fenitrothion and an operational formulation containing 11% fenitrothion Foraging behavior of the salmon was then tested in a stream tank Concentrations of 0 02 and 0 16 μl/L of technical grade fenitrothion and the operational formulation containing 0 08 and 0 16 μl/L of fenitrothion caused a significant decrease in the efficiency of the salmon's attack sequence These concentrations, and a concentration of 0 005 μl/L technical grade fenitrothion and 0 004 μl/L fenitrothion in the operational spray, produced a significant decrease in the salmon's reaction distance to prey All concentrations except for 0 004 μl/L fenitrothion in the operational formulation caused a significant decrease in the number of ingestions of prey made by the fish These results suggest that foraging behavior in salmon is impaired by exposure to very low levels of fenitrothion

Keywords – Fenitrothion *Salmo salar* Foraging Reaction distance

INTRODUCTION

Fenitrothion [*O,O* dimethyl *O*-(3-methyl-4-nitrophenol)phosphorothioate] is an organophosphate insecticide used in Canada to control the spruce budworm (*Choristoneura fumiferana*) and the hemlock looper (*Lambdina fiscellaria*) [1,2] It is regularly sprayed in areas near Atlantic salmon rivers and ponds [2], creating the potential for exposure of salmon to this pesticide Exposures, if they occur, would almost invariably be to sublethal concentrations of the pesticide A maximum of 0 02 μl/L of fenitrothion has been found in waterways following spray operations employing buffer zones [2,3] Sublethal levels of fenitrothion are known to inhibit the activity of brain acetylcholinesterase, thereby disrupting synaptic transmission in the cholinergic part of the nervous system [4] This inhibition can cause severe behavioral changes such as decreasing the ability to forage efficiently Decreased foraging efficiency could lead to decreased growth or even death of the affected individuals [5]

Fenitrothion is known to cause decreased foraging, as well as changes in dominance hierarchy and learning among salmonids [6–10] These effects were found when salmon were exposed to concentrations of fenitrothion near their 96-h LC50 and to a formulation of the pesticide no longer in widespread use The purpose of this study was to examine the effects of fenitrothion, not only on overall feeding level but also on finer details of the foraging behavior of juvenile Atlantic salmon (*Salmo salar*) Foraging was examined following exposure, at concentrations well below the 96-h LC50 level, to technical grade fenitrothion and to an operational formulation of the pesticide that is currently in use in forestry operations in Canada

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MATERIALS AND METHODS

Toxin formulations

Two formulations of fenitrothion were used, technical grade and the operational spray formulation The technical formulation was 95% fenitrothion (Mobay Chemical Corp) The operational formulation was that used to control the hemlock looper in Newfoundland, Canada It consisted of 11% fenitrothion, 40% Cyclosol 63 (a solvent) and 49% insect diluent 585 (stove oil)

96-h LC50 determinations

All LC50 tests were conducted in 10 liters aerated water under static conditions Fish were housed in groups of five in 20-liter polycarbonate test containers Each container held 10 liters fenitrothion treated water, which was changed daily The temperature of the water was ambient For the technical grade tests this was 14 9 ± 0 35°C ($\bar{x}$ ± SD) and 12 7 ± 0 74°C for the operational formulation. For all LC50 determinations, 7-d exposures and foraging trials, the pH of the water was 6.01, the alkalinity was 3.0 mg/L $CaCO_3$, the hardness was 9.6 mg/L $CaCO_3$ and the partial pressure of oxygen was greater than 150 torr. Fish used in the technical grade tests were 5.6 ± 0.92 cm total length and those exposed to the operational spray formulation were 10.1 ± 0.75 cm total length. All fish were fed daily with commercial trout food and any dead individuals were removed.

7-d exposure

Five fish were treated for 7 d in each 20-liter bucket containing 10 liters aerated, fenitrothion-treated water that was changed daily. The water temperature was ambient (technical grade, 14.7 ± 1.8°C; operational formulation, 13.0 ± 1.4°C). Fish used in the technical grade tests were 5.9 ± 0.54 cm while those in the operational tests were 10.0 ± 0.59 cm. Fifteen fish were exposed to each of four concentrations of the toxins, the greatest of which was 10% of the 96-h LC50 level. For the technical grade formulation these concentrations were 0, 0.005, 0.02 and 0.16 μl/L and for the operational formulation 0, 0.04, 0.72 and 1.44 μl/L. For the operational formulation these concentrations corresponded to 0, 0.004, 0.08 and 0.16 μl/L fenitrothion, respectively. Concentrations will be expressed as μl/L of fenitrothion throughout and will be given as nominal concentrations only. There are 1.33 mg fenitrothion in 1 μl of the liquid. There were no deaths during fenitrothion exposure at any concentration tested of either formulation. All fish were fed daily with commercial trout food.

Samples were taken periodically over 24 h from concentrations covering the range of those used in this study to ensure the presence of fenitrothion. Water samples (500 ml for the 96-h LC50 concentrations, 1 liter for the highest and lowest sublethal concentrations used) were suctioned from the exposure buckets through Teflon tubing into precleaned volumetric flasks. The samples were then transferred to separatory funnels and extracted with dichloromethane (3 × 30 ml). After transferring the extract to round-bottom flasks, the dichloromethane was removed in a rotary evaporator (20°C under vacuum). Recovery of fenitrothion was 92%. The residue was then dissolved in 1 ml methanol, filtered and 10 μl injected into the HPLC for analysis. A Perkin-Elmer series 4 HPLC was used in conjunction with a Perkin-Elmer LC85 detector (1.4 μl flowcell) and a Perkin-Elmer CHROM-2 data system. Two Perkin-Elmer 2C18Cr 3 × 3 columns in series were connected to the detector with a minimum length of 0.009-inch i.d. stainless tubing. A solvent flow rate of 2 ml per min was used. The columns were equilibrated for 2.5 min with 35% acetonitrile in deionized, distilled and filtered water. After injection of the sample, the acetonitrile concentration in the mobile phase was increased linearly over 8 min to 70% and then exponentionally (curve 0.5) to 95% over 5 min. The acetonitrile concentration was held at 95% for 5 min and then returned to 35% acetonitrile to equilibrate the column. Standards prepared by diluting technical fenitrothion were used for quantitation, assuming 95% fenitrothion in the technical product.

Foraging experiment

The foraging trials were conducted in 25 × 30 × 70-cm stream tanks. A 4.5-cm/s current was produced by an external pump that removed the water from one end and returned it to the opposite end of the tank. One fish was contained in a 25 × 40-cm section of each tank by fine mesh screens. A clear Plexiglas cover with a small hole was placed over the tank, with the hole positioned just downstream from the mesh at the inflow end of the tank. The tanks were illuminated from above and a 10 × 30-cm sheet of black plastic, placed on the Plexiglas cover, provided shade at the downstream end. A 5 × 5-cm grid was attached to the back of the tank in the section containing the salmon to allow measurement of distances. Following 7 d of exposure to fenitrothion the Atlantic salmon were transferred individually to the stream tanks and allowed to acclimatize for approximately 4 h before trials began. Four hours provided sufficient time to ensure response of the fish to the prey items. During the trials, an observer sat 50 cm from a tank and introduced 10 live adult brine shrimp to the tank as prey. The brine shrimp were introduced one at a time with a disposable pipette through the hole in the Plexiglas cover. The presence of the observer did not seem to affect the foraging behavior of the fish. The salmon held position at the opposite (downstream) end of the tank and moved forward to capture the brine shrimp. A prey item was not introduced while the previous brine shrimp was still available to the salmon. One foraging trial required 10 to 15 min to complete.

For each prey item introduced, the reaction distance of the salmon and the components of its attack sequence were recorded. Reaction distance was defined as the distance between the snout of the salmon and the prey item when the salmon oriented (see below) toward the brine shrimp. The attack sequence was divided into four components: orient, approach, attack and capture. Orient is a movement that aligns the fish's body axis towards the prey. Approach is a directed movement of the salmon toward the prey. Attack is an attempt to grasp the prey with the mouth. Capture is the closing of the salmon's mouth around the brine shrimp, ending in ingestion of the prey. The attack sequence can be broken off at any point by the salmon, which then must begin the next sequence with another orient. When the attack sequence was ended, the salmon returned to its station downstream or stopped its forward movement toward the prey. This, combined with the obvious movements of orientation and attack, made the components of the attack sequence easily distinguishable. The frequency of each component of the attack sequence was recorded for each brine shrimp presented. The ratios of the frequency of each act in the salmon's predation sequence to the frequency of the preceding act were calculated as a measure of foraging efficiency. This assumes that an "ideal" predator would move from orient through to capture, without interruption, for each attack sequence initiated. The resulting ratios of the components of the attack sequence would then be equal to one. The number of ingestions was also used as a measure of efficiency.

All proportional data contained a large number of high and low values and were, therefore, transformed using a modified arcsine transformation [11]. The transformed data were analyzed using analysis of variance (ANOVA) and Tukey's multiple comparison tests. The ingestion data were analyzed using χ^2, and the reaction distance data (which could not be satisfactorily transformed) were analyzed with Kruskal-Wallis and Dunn's multiple comparison tests. The significance level was set at 0.05.

RESULTS

The 96-h LC50 for the technical grade fenitrothion was 1.6 μl/L (95% fiducial limits 1.28–1.87 μl/L) while for the operational spray formulation it was 14.4 μl/L (95% fiducial limits 12.5–17.6 μl/L). This concentration of the operational spray formulation contains 1.6 μl/L fenitrothion.

The HPLC analysis indicated that fenitrothion was present in the water during the entire 24-h period between the addition of fenitrothion-treated water and its replacement the next day (concentrations are given in Table 1). The levels were generally higher than the nominal concentrations for the lowest concentrations used and declined over the 24-h period.

Exposure to technical grade fenitrothion produced a significant decline in the ratios of each of the attack-sequence components to the previous component of the sequence with increasing concentration (approach per orient: $F = 13.66$, $df = 3$, $p < 0.001$; attacks per approach: $F = 10.54$, $df = 3$, $p < 0.001$; captures per attack: $F = 19.38$, $df = 3$, $p < 0.001$; Fig. 1). The decline in the attack sequence ratios was only significant at concentrations of 0.02 and 0.16 μl/L (Tukey's multiple comparisons). The 0.005 μl/L level had no effect on the value of these ratios. There was a significant decrease in the number of ingestions as concentration increased ($\chi^2 = 161.1$, $df = 3$, $p < 0.001$). The frequency of ingestions at the 0.005 level were significantly less than that of the controls and the frequencies at the 0.02 and 0.16 μl/L concentra-

Table 1. Concentrations (μl/L) of fenitrothion in exposure containers as measured by HPLC

	Measured concentration			
Nominal concentration	0 h	4 h	8 h	24 h
Technical grade				
0.005	0.009	0.01	0.008	0.004
0.16	0.152	–	0.086	0.012
1.6	0.51	0.649	0.366	0.285
Operational spray				
0.004	0.009	0.012	0.017	0.014
0.08	0.036	0.028	0.04	0.023
1.6	0.609	0.422	0.282	0.193

One μl/L equals 1.33 mg/L fenitrothion.

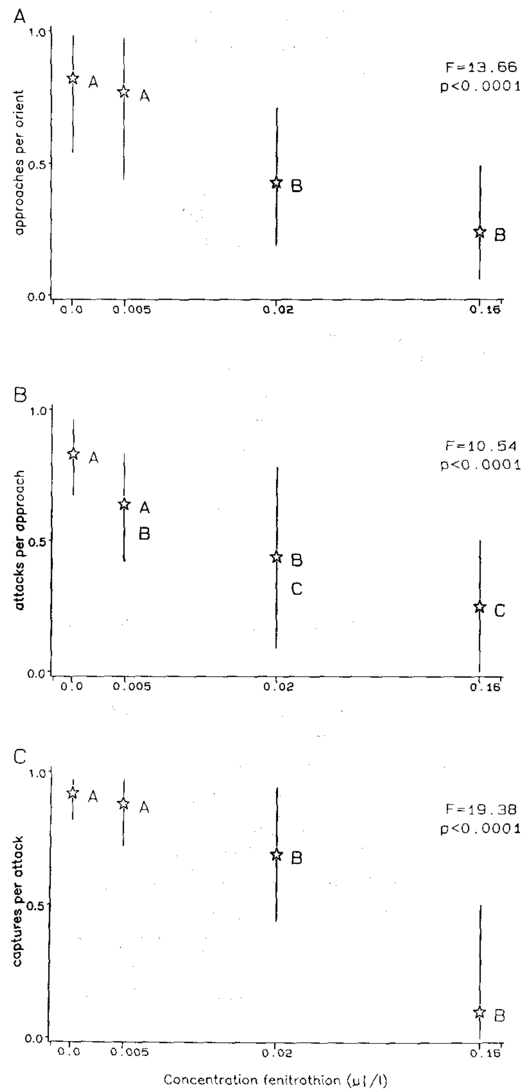

Fig. 1. Mean (+SD, n = 15) ratios of the frequency of each component in the attack sequence to each previous component for salmon exposed to technical grade fenitrothion. For each ratio, concentrations with the same letter are not significantly different (Tukey's multiple comparison test). Also shown are the results of ANOVA on data transformed by a modified arcsine transformation, comparing data for each ratio across fenitrothion concentration.

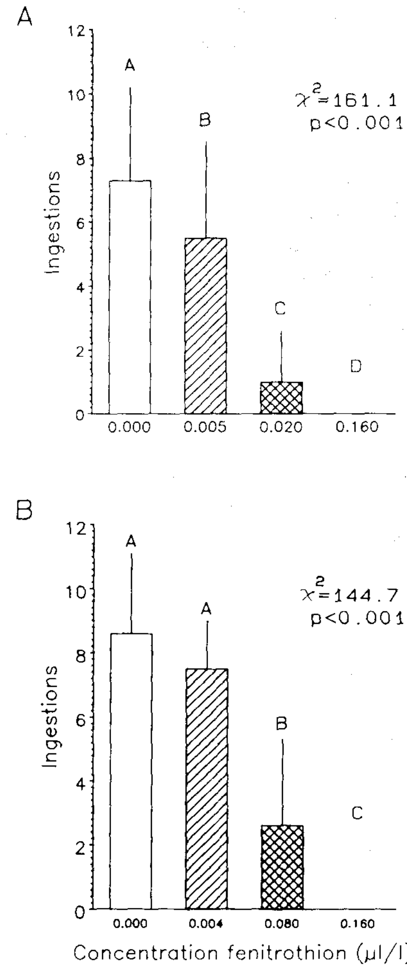

Fig. 2. Mean (+SD, n = 15) number of ingestions of prey by salmon exposed to (**A**) technical grade fenitrothion and (**B**) the operational formulation of fenitrothion. The results of χ^2 tests comparing the number of ingestions across concentration are shown. For each formulation, concentrations with the same letter are not significantly different (χ^2).

tions were less than that at the 0.005 μl/L dose (Fig. 2A, χ^2 tests). The reaction distance of the salmon to the prey was significantly less at the 0.005 μl/L concentration than for the controls and the distances at the 0.02 and 0.16 μl/L exposures were significantly less than that at the 0.005 μl/L level (Fig. 3A, Dunn's multiple comparisons).

The ratios of each of the attack-sequence components to the previous component of the sequence were also significantly affected by the operational spray formulation (approaches per orient: $F = 31.67$, df = 3, $p < 0.001$; attacks per approach: F = 15.80, df = 3, $p < 0.001$; captures per attack: F = 18.08, df = 3, $p < 0.001$; Fig. 4). There was a significant decrease in each of the ratios at 0.08 μl/L fenitrothion and 0.16 μl/L but no significant

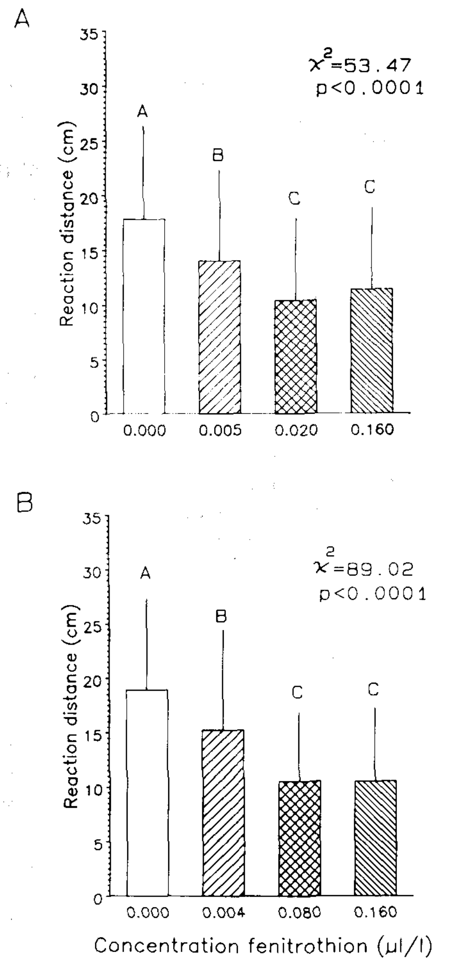

Fig. 3. Mean (+SD, $n = 15$) reaction distance to prey of salmon exposed to (**A**) technical grade fenitrothion and (**B**) the operational formulation of fenitrothion. The results of Kruskal-Wallis tests comparing reaction distance across concentration are shown. For each formulation, concentrations with the same letter are not significantly different (Dunn's multiple comparison tests).

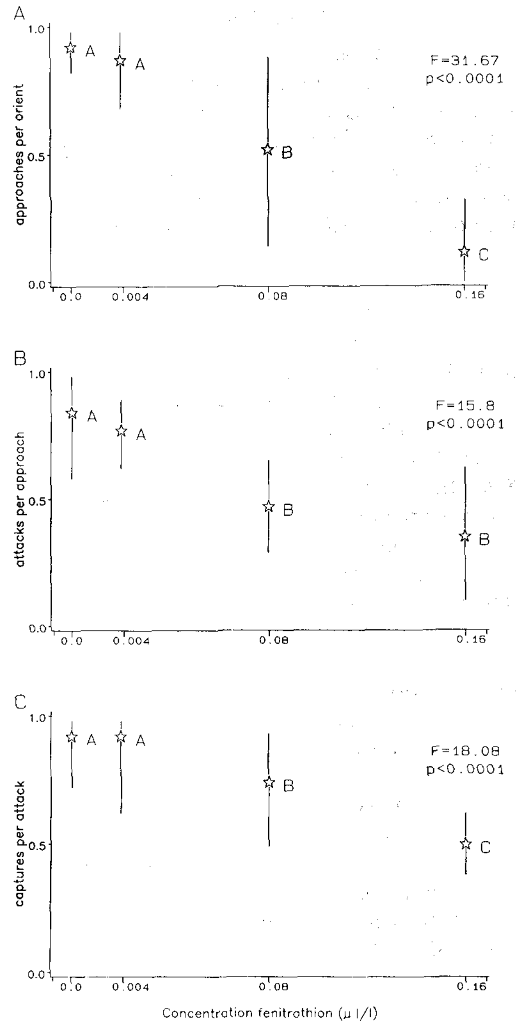

Fig. 4. Mean (+SD, $n = 15$) ratios of the frequency of each component in the attack sequence to each previous act for salmon exposed to the operational formulation of fenitrothion. For each ratio, concentrations with the same letter are not significantly different (Tukey's multiple comparison test). Also shown are the results of ANOVA on data transformed by a modified arcsine transformation, comparing data for each ratio across fenitrothion concentration.

decline at the 0.004 µl/L level (Tukey's multiple comparisons). There was also a significant decline in the number of ingestions at the 0.08 and 0.16 µl/L concentrations but no effect of an exposure to 0.004 µl/L (Fig. 2B, χ^2 tests). The reaction distance of the salmon was significantly less than that of the controls at the 0.004 µl/L level with the distance at the 0.08 and 0.16 µl/L concentrations being less than that at the 0.004 µl/L level (Fig. 3B, Dunn's multiple comparisons).

For both formulations of fenitrothion, those Atlantic salmon that were exposed to the pesticide tended to allow the brine shrimp to move closer before beginning an attack sequence. Once the attack sequence was begun, exposed salmon were more likely to end the sequence before capturing the prey, with those exposed to higher concentrations ending the attack earlier in the sequence.

DISCUSSION

Both formulations of fenitrothion produced a significant negative effect on the foraging behavior of Atlantic salmon. At the higher exposure concentrations all components of the attack sequence were depressed. As a result, Atlantic salmon were less efficient at foraging on the prey items once they had responded to them. This decreased efficiency contributed to a decline in the number of prey consumed.

All concentrations of fenitrothion produced a decreased reaction distance of Atlantic salmon to prey. Assuming a semicircular scanning area around the salmon [12,13], the observed decline in reaction distance could produce a reduction in the total reaction field of the salmon of as much as 50 to 80%. In a natural situation, this could significantly decrease the probability of responding to prey items and, therefore, lead to a decrease in the number of prey ingested. The possible decrease in consumption of prey could produce a decrease in growth if exposure were to continue or if the effects are long lasting. The time required for Atlantic salmon to recover normal behavior following exposure to fenitrothion is not known. The effect of a decrease in reaction distance may be compounded for those fish exposed to higher concentrations of fenitrothion, which would also experience a decrease in foraging efficiency.

A quantitative comparison of the effects of the two formulations was not possible due to a difference in the behavior of the controls. The 6-cm fish used in the technical grade experiment had a significantly higher number of nonresponses to the prey than did the 10-cm fish used in the operational formulation experiment ($\chi^2 = 13.8$, $df = 1$, $p < 0.001$). However, a qualitative comparison of the two formulations is possible. Similar concentrations of fenitrothion in the two experiments appeared to have the same effect on the foraging behavior of Atlantic salmon. Also, the 96-h LC50, expressed as concentration of fenitrothion only, is identical for the two formulations. It would appear, therefore, that the toxicity of the operational spray formulation is due mainly to the presence of the fenitrothion and not to the other components of the formulation. A study by Thellan [14] also concluded that the effects of the spray formulation were due to the presence of the active ingredient.

Other studies have shown effects of fenitrothion on the behavior of salmonids. Fenitrothion has been shown to produce a significant decrease in predator avoidance [7], in learning [8], in the numbers of fish maintaining territories [9] and in feeding [6]. The concentrations used in these other studies were higher than all but the highest concentration used here, although the duration of the exposures were shorter. Also, the operational formulation used in these previous studies was different than that used here. However, these findings, taken together with the results of the present study, indicate that fenitrothion has the potential to produce a significant impact on the overall behavior of fish.

The biological impact of fenitrothion will depend on the magnitude and duration of exposure. A maximum of 0.02 μl/L fenitrothion has been found in streams and ponds following commercial spray programs [2,3]. This may pose less of a threat to fish in streams, where fenitrothion disappears within a few days [1], than in ponds, where it can persist at low levels for 5 to 35 d [3,15]. The results of this study indicate that Atlantic salmon exposed to fenitrothion at concentrations as low as 0.005 to 0.01 μl/L for this period of time would experience reduced foraging efficiency and feeding rates. The impact of such a decline in foraging ability could be confounded since prey items may also be affected by the toxin and ease of capture may compensate for lowered foraging capability. Also, if Atlantic salmon quickly recover following exposure, then a small decrease in foraging ability may not translate into a significant impact either at the individual or population level. Nevertheless, the probability of exposure should continue to be minimized by the use of adequate buffer zones around waterways.

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