EFFECT OF THE MOSQUITO LARVICIDE, ABATE (TEMEPHOS) ON FINGERLINGS OF *Oreochromis mossambicus* (PETERS)

ASOKA PATHIRATNE AND JANAKIE M. RANASINGHE

*Department of Zoology, University of Kelaniya, Kelaniya.**(Date of receipt : 18 December 1991)**(Date of acceptance : 22 April 1992)*

Abstract : The acute toxicity of Abate, an organophosphate used in the control of malaria mosquitoes in Sri Lanka, to the fingerlings of *Oreochromis mossambicus* was investigated under static laboratory conditions. The LC₅₀ values of Abate for 24, 48 and 96 hours were 27.6 (20.1 - 35.4), 15.2 (9.9 - 20.8) and 12.9 (8.7 - 17.2) ppm respectively. Exposure to high concentrations of Abate (4-16 ppm) for 96 hours, significantly depressed the oxygen consumption of the fingerlings. Sublethal concentrations (< 0.5 ppm) had no significant effects. Histopathological studies of the gill tissue revealed that the exposure to high concentrations of Abate (> 0.5 ppm) was associated with gill lesions namely epithelial separation, epithelial hyperplasia and telangiectasis of the gill lamellae. It is concluded that Abate could be used in controlling mosquito larvae at levels of less than 0.5 ppm without apparent short term effects to the fingerlings of *O. mossambicus*.

Introduction

Abate an organophosphate which is a proprietary preparation of 'Temephos' (o,o,o',o'-tetramethyl o,o'-thiodi-p-phenylene phosphorothioate) is now widely used in some countries to control the larvae of mosquitoes and black fly.^{1,2} Recently, the use of Abate as a larvicide has been adopted for the control of anopheline mosquitoes in aquatic environments in Sri Lanka. This larvicide has been found to be relatively safe to non-target organisms, moderately biodegradable and less persistent in the experimental environments.^{2,3}

Abate has a low solubility and in suspension, it behaves like DDT, and rapidly accumulates on solid surfaces.¹ After spraying, the insecticide acts as a mat on the water surface and reduces the natural aeration. This property can also affect other aquatic organisms including fish.

While many workers have investigated the effects of insecticides on temperate fish species,⁴ the effects of Abate on tropical fish, particularly cichlids, have not been so well studied. Since inland fish are increasingly being used as a source of protein by the rural poor, it is necessary to monitor the effects of Abate used for controlling mosquito larvae, on fish and particularly the fingerlings in inland water bodies. *Oreochromis mossambicus* is an important food source in the inland water bodies of Sri Lanka. The present study is an attempt to evaluate the effects of different concentrations of Abate on fingerlings of *O. mossambicus* with particular reference to behaviour, survival, oxygen consumption and gill structure.

Materials and Methods

Fish : Fingerlings of *O. mossambicus* (2.9 ± 0.8 cm in total length) were collected from a freshwater pond in Kelaniya. Abate (50% E.C.), used in this study was obtained from Anti-Malaria Campaign, Narahenpita. The fish were adapted to laboratory conditions, in glass aquaria with aged tap water, under natural photoperiod for 2-3 weeks. The aquaria were aerated continuously. During the acclimatization, the fish were provided with commercially prepared food pellets daily. They were starved 24 hours prior to use in experiments. No mortality was observed during the acclimatization period.

Toxicity tests : The static, acute toxicity tests were conducted in rectangular glass tanks (30x30x60 cm) containing 24 l of aerated aged tap water following Ward and Parrish.⁵ The fingerlings used were 1.2 ± 0.3 g in weight and 3.4 ± 0.9 cm in length ($n = 360$, stocking density = 20 fish/24 l/tank). The stock solution of Abate (1g l^{-1}) was prepared separately and the desired degree of concentrations (0.25-36 ppm) were prepared by dilution. In all experiments, three replicates were used and a control set was maintained simultaneously in the glass tanks, with only the test water. The mortality rate was observed for each concentration at 24, 48 and 96 h. The physical characteristics of the test water were as follows : temperature, $28-30^{\circ}\text{C}$; pH, 6.5-7.0; and dissolved oxygen 5.6-6.8 mg l^{-1} . At the end of the experiments, oxygen consumption of control and exposed fish was determined according to the procedure described earlier.⁶

After the measurement of oxygen consumption, the fish in control and exposed experiments were sacrificed by pithing for histopathological studies. The gills (second gill arch of left or right side) were removed and were preserved in 10% formalin. The preserved gills were embedded in paraffin wax following the standard histological procedures. Sections were cut in $5\text{ }\mu\text{m}$ thickness and stained with haematoxylin and eosin.

The concentrations of Abate-mortality data were statistically analyzed by Probit analysis for daily LC_{50} values and their corresponding 95% confidence limits.⁷ Results of the oxygen consumption of control and Abate exposed fishes were analysed by one way analysis of variance (ANOVA). Scheffe's test⁸ was used to compare the means of exposed groups with that of controls at $\alpha = 0.05$.

Results

Behavioural and morphological changes in the fish exposed to Abate

Behavioural changes: The fish exposed to Abate (>4 ppm) were lethargic and showed impaired swimming. They could not change their swimming directions actively as the fish in control experiments. They swam fairly well at bottom levels of the tank and rarely came up near the surface.

Morphological changes: The skin covering the entire body, specially the cranium, became very transparent after the treatment with Abate (>4 ppm). Black pigments of skin on each side of the body began to fade gradually. The fish exposed to higher concentrations of Abate (8-36 ppm) showed intense gill haemorrhage. These changes developed with the increase in duration of the exposure to Abate, as well as with the increase in the concentration of Abate.

Effect of Abate exposure on survival of fish

No mortality of fish was observed in the control tanks or in the tanks containing 0.25-1 ppm Abate within 96 hours. Four ppm of Abate caused 10% fish mortality after 96 hours. The most lethal concentration observed was 36 ppm. Eighty percent of the fish were dead within 24 hours of the exposure. The estimated LC₅₀ values of Abate are presented in Table 1: The 24, 48 and 96 hour LC₅₀ values were found to be 27.6, 15.2 and 12.9 ppm respectively.

Table 1 : LC₅₀ values obtained for *Oreochromis mossambicus* fingerlings exposed to Abate

Exposure time (hr)	LC ₅₀	
	mean	95% confidence limits
24	27.6	20.1 - 35.4
48	15.2	9.9 - 20.8
96	12.9	8.7 - 17.2

Fish used were 1.2 ± 0.3 g in weight and 3.4 ± 0.9 cm in length.

Effect of Abate on oxygen consumption of fish

The data on rates of oxygen consumption of the fish exposed to different concentrations of Abate for 96 hours are presented in Table 2. The exposure of fish to 1 ppm of Abate had no significant effect on the rate of oxygen consumption. However, the exposure of fish to the levels of 4 ppm of Abate or greater, significantly decreased the rates of oxygen consumption upto 84% of the fish in the control experiment (p < 0.05).

Effects of Abate on gill histology

The gills of the fish in the control experiment (Figure 1) and the fish exposed to 0.25 and 0.50 ppm of Abate were devoid of microscopic lesions. However, lesions were observed in the gills of fish exposed to the levels of Abate greater than 0.50 ppm. The observed gill lesions (Figure 2) were; (a) separation of the epithelial tissue layer of the gill lamellae from the underlying connective tissue (b) epithelial hyperplasia of cells

Table 2: Effect of exposure to sublethal and lethal levels of Abate on oxygen consumption of *Oreochromis mossambicus* fingerlings

Concentration of Abate (ppm)	Oxygen consumption after 96 hrs. (mg/g/hr)
0 (control)	2.42 ± 0.43 ^a
1	2.20 ± 0.20 ^a
4	1.35 ± 0.15 ^b
8	1.10 ± 0.10 ^b
10	0.90 ± 0.04 ^b
14	0.58 ± 0.03 ^b
16	0.41 ± 0.02 ^b

Results are expressed as mean ± standard deviation, n = 3.

^{a-b} Means not followed by the same letter are significantly different from controls (p < 0.05).

basally located in the gill filament, leading to obliteration of the space between adjacent gill lamellae and fusion of the latter and (c) lamellar telangiectasis where blood capillaries become swollen at the edges of the lamellae and seen as globules. The lesions varied in severity and frequency among fish exposed to different levels of Abate (Table 3). The gills of fish exposed to 1 ppm of Abate showed lamellar telangiectasis slightly and 12 ppm of Abate showed lamellar telangiectasis and epithelial separation. There was a greater development of epithelial separation, hyperplasia and lamellar telangiectasis in the gills of the fish treated with 18, 24 and 36 ppm of Abate. The gills of the fish exposed to 36 ppm Abate showed severe epithelial separation, hyperplasia and lamellar telangiectasis.

Table 3 : Occurrence of gill lesions seen in *Oreochromis mossambicus* fingerlings exposed to sublethal and lethal levels of Abate

Concentration of Abate (ppm)	Epithelial separation	Hyperplasia	Lamellar telangiectasis
0 (control)	-	-	-
0.5	-	-	-
1.0	-	-	+
12	+	-	++
18	+	+	++
24	++	+	++
36	+++	++	+++

n = 3, (-) : absent, (+) : present.

Figure 1: The gill of control *Oreochromis mossambicus* (X 200), e - epithelium, L - lamellae.

Figure 2 : The gill of *Oreochromis mossambicus* exposed to high concentrations of Abate showing gill lesions (X 200), es - epithelial separation, h - hyperplasia, LT - lamellar telangiectasis.

Discussion

Many morphological and behavioural changes have been reported earlier following exposure of fish to various insecticides, either to high concentrations for brief periods or to sublethal concentrations for extended periods.^{4,9} Exposure of *O. niloticus* to high concentrations of two organophosphates, Monocrotophos and Fenthion for 48 hours has been reported to cause discolouration of the body and erratic swimming patterns prior to their death.¹⁰ In the present study, marked morphological and behavioural changes in *O. mossambicus* were also observed following exposure to Abate. These changes can prevent the fish from functioning effectively and efficiently in the environment.

In this study, 48 hour LC₅₀ in *O. mossambicus* fingerlings exposed to Abate was found to be 15.2 ppm. The LC₅₀ values of three other organophosphates, Endosulfan, Endrex and Supersumithion for the fingerlings of the same species of fish for 48 hours have been reported to be 0.0055, 0.375, and 10.05 ppm respectively.¹¹ By comparing the LC₅₀ values, it is clear that Abate is less toxic to this fish than the other three insecticides currently used for agricultural purposes in Sri Lanka. Using the pesticide safety level for organophosphates suggested by the FAO,⁴ the safety range for Abate from this study corresponds to 1.29-0.13 ppm (1/10 LC₅₀ - 1/100 LC₅₀, 96 hour). The dosage of Abate, currently used in controlling mosquito larvae in Sri Lanka is 0.1 kg/hectare (Dr M. Wickramasinghe, Anti-Malaria Campaign, Personal Communication) which corresponds to a concentration of 0.1 ppm in water bodies with 10 cm depth of water. This value is within the safety range of Abate obtained from this study. No fish mortality occurred at the concentrations less than 4 ppm. It is therefore evident that Abate could be used in controlling mosquito larvae, at a concentration of 0.1 ppm without any apparent harm to *O. mossambicus* fingerlings.

Respiratory distress is one of the early symptoms of insecticide poisoning. Organochlorines such as DDT, dimethoate and Methoxychloro have been reported to stimulate oxygen consumption of fish at sublethal levels and inhibit the oxygen consumption at lethal concentrations. Fenitrothion, an organophosphate has been reported to depress the oxygen consumption of *Labeo rohita*.⁴ In the present study, exposure of *O. mossambicus* to high concentrations of Abate (> 4 ppm) for 96 hours decreased the oxygen consumption rates of this fish significantly. Sublethal concentrations (< 1 ppm) had no significant effect on the oxygen consumption. The present study also showed that exposure to high concentrations of Abate for 96 hours induce various histopathological changes in the gills such as epithelial separation, epithelial hyperplasia and lamellar telangiectasis. Sublethal concentrations elicited minor changes in gill histology. The observed decrease in oxygen consumption of these fish exposed to high concentrations of Abate can be attributed to the gill lesions induced by Abate. Such gill lesions are particularly deleterious to the fish which inhabit tropical water bodies where fish are subjected to lower dissolved oxygen

concentrations. These lesions impair the normal respiratory function in the gills and may eventually contribute to the death of these fish.

The sublethal concentrations of Abate tested in this study (0.25 - 0.50 ppm) are two and a half to five times in excess of those used in the current malaria mosquito larvicide programme in Sri Lanka and have not led to any serious short-term effects in *O. mossambicus* fingerlings. It has been found that Abate does not accumulate indefinitely in fish,¹³ therefore it seems unlikely that serious effects might occur after longer exposure times although this remains to be definitively established. Hence, the use of 0.1 ppm of Abate for controlling larvae of malaria mosquitoes is unlikely to harm the wild *O. mossambicus* populations, especially the fingerlings.

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