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Behavioral Changes in an Air-Breathing Fish, *Anabas testudineus*, Exposed to Malathion

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Malathion is a widely used organophosphorus pesticide. A large amount of this pesticide is being used in India. It is used in agriculture, in horticulture, in various public health and domestic applications, in poultry and in eradicating ectoparasites from cattle and pig. It reaches the aquatic environment by direct application, spray drift, aerial spraying, washing from the atmosphere by precipitation, erosion and run-off from agricultural land, in factory effluents and in sewage (Edwards 1973).

Anabas testudineus is an edible freshwater fish. It is of great economic importance. Therefore this fish was selected for studying the behavioral changes due to malathion exposure. The concern of neurotoxicity in human populations has motivated a large number of psychophysiological studies on the effect of toxic substances on animals. It is believed that behavioral changes are the most sensitive measures of neurotoxicity (Doving 1991). Warner et al. (1966) declared that "The behavior (or activities) of an organism represents the final integrated result of a diversity of biochemical and physiological processes. Thus a single behavioral parameter is more comprehensive than a physiological or biochemical parameter." Fisher et al. (1980) by their experiments on the effects of sublethal doses of hydrazine on the behavior of bluegills supported the concept that toxicant-caused stresses upon organisms can be quantified by methods other than mortality. Changes in behavior have been suggested for use as a sensitive indicator of chronic sublethal toxicant exposure (Orsatti and Colgan 1987). Some fish behaviors (e.g., locomotor activity and avoidance) are extremely sensitive to pollutant chemicals, whereas others (e.g. aggression) seem to be rather refractory (Doving 1991; Richmonds and Dutta 1992; Dutta et al. 1992a).

Scherer and Harrison (1979) described a technique to elicit the optomotor response in fish and invertebrates, by rotating circular screens marked with black and white vertical stripes around a cylindrical test chamber. This response is considered to be an essential one for maintaining position within the habitat, and for schooling in fish. This optomotor response test can detect the impairment of visual orientational

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functions. Macmillan (1987) investigated the effects of herbicides alachlor and atrazine on the optomotor response of fathead minnows. Since the optomotor response can be quantified, it was selected for this study.

MATERIALS AND METHODS

The fish A. testudineus (21-26 g, 15-20 cm) were collected from unpolluted fresh water habitats in and around Bhagalpur, India. Procedures for holding, acclimation and exposure were according to the guidelines of the American Public Health Association et al. (1981). The fish were maintained in large aquaria in the laboratory. These tanks had a layer of gravel on the bottom. The tanks were fitted with aerators and filters filled with nylon wool and charcoal. The fish were acclimated for at least 4 wk prior to experimental manipulations. They were fed 3 times daily, until satiation with groundnut cake and commercial fish food sticks. Water quality characteristics were determined periodically. The mean values for the water quality characteristics are as follows: temperature 27 $\pm$ 1 degree C, dissolved oxygen 7.1 $\pm$ 0.5 mg/L, pH 7.1 $\pm$ 0.2, total hardness 118 $\pm$ i mg/L as $CaCo_3$, alkalinity 123 $\pm$ 4 mg/L as $CaCo_3$. Since commercial grade malathion is used in all spraying operations against pests, we obtained commercial grade malathion containing 50% of active ingredients from North Minerals Ltd. Haryana, India. The 24-hr LC50 for technical grade malathion is reported as 28 mg/L (Dutta et al 1992b). The sublethal exposure concentrations selected for this study were 1 mg/L, 2 mg/L, 3 mg/L and 4 mg/L.

Exposure to malathion was done in large glass aquaria. The water in the exposure tank was aerated for 24 hr before adding the pesticide. The water was not aerated after the addition of pesticide. The reason for this is that maloxon, the oxygen analog of malathion is highly toxic compared to malathion. A test solution with the required concentration was prepared from a previously prepared stock solution of the pesticide. The fish were exposed for 24 hr. Feeding was stopped 24 hr before the exposure and the fish were not fed during the exposure.

Ten fish were exposed for control and each exposure concentration. The optomotor responses of the control and experimental fish were measured using the optomotor response test apparatus (Macmillan 1987, Dutta et al. 1992a and Richmonds and Dutta 1992). This apparatus consisted of a plastic bucket, a turn table and a one gallon glass mason jar. The inner wall was pasted with 1.2 cm wide strips of black electrical tape placed at 7 cm apart at an angle of 55°. The jar was suspended inside the bucket. The glass jar was filled with water containing the same concentration of pesticide to which the fish were exposed. The bucket was made to rotate at a speed of 15 RPM with the direction of the movement, being in the downward slope of the stripes.

An illumination of 20 lux was used in the experiment. Fish that were previously exposed to a particular malathion concentration were placed in the glass jar containing the same concentration of the pesticide to which the fish were exposed. Control fish were treated in the same way except that no malathion was added to the water in the exposure tanks.

The bucket was made to rotate and the fish were allowed to settle down with the bucket rotating. The rotation of the bucket was stopped for 6 min. This was done to acclimate the fish to the optomotor response test apparatus. This was followed by an observation and recording period of 3 min. After this, the optomotor response was recorded for another 3 min with the bucket stopped.

Every 90° turn or movement, referred to as a "quarter turn" was recorded as one movement. The quarter turns in the direction of the drum movement were recorded as "followings" and those in the opposite direction of the rotation of the drum were recorded as "reversals." Their means and SDs were calculated (Table 1). T-value with level of significance was calculated between the control (0) and different concentrations of malathion during the "following" and "reversal" turns (1, 2, 3, and 4 of Table 1).

RESULTS AND DISCUSSION

Table 1 shows the mean scores of the optomotor response at different exposure concentrations of malathion and the statistical analysis of these scores. The mean scores for "following," while the bucket was rotating was significantly ($p \leq 0.05$) lower at 2, 3, and 4 mg/L exposure concentrations compared with the scores of control fish and fish exposed to a concentration of 1 mg/L (Table 1). The mean scores for "following" during the bucket stopped condition was significantly lower at 3 mg/L ($p \leq 0.05$) and 4 mg/L ($p \leq 0.0001$) exposure concentrations compared with control fish and fish exposed to all other concentrations.

The scores for "reversal" while the bucket was rotating showed a significant ($p \leq 0.001$) decrease only at the highest exposure concentration namely 4 mg/L compared to control fish and fish exposed to all other lower concentrations. During the bucket stopped condition, the scores for "reversal" were significantly ($p \leq 0.01$) lower at 2, 3, and 4 mg/L exposure concentrations compared to control fish and fish exposed to a concentration of 1 mg/L. All these scores showed a general trend of decrease with increasing exposure concentrations (Figures 1 and 2). Fish entered into a state of hypoactivity around an exposure concentration of 2 mg/L of malathion. This is evident from the low scores of optomotor response from the different categories except the scores for "reversal" while the bucket was rotating. At the highest exposure concentration, 4 mg/L, fish were highly lethargic.

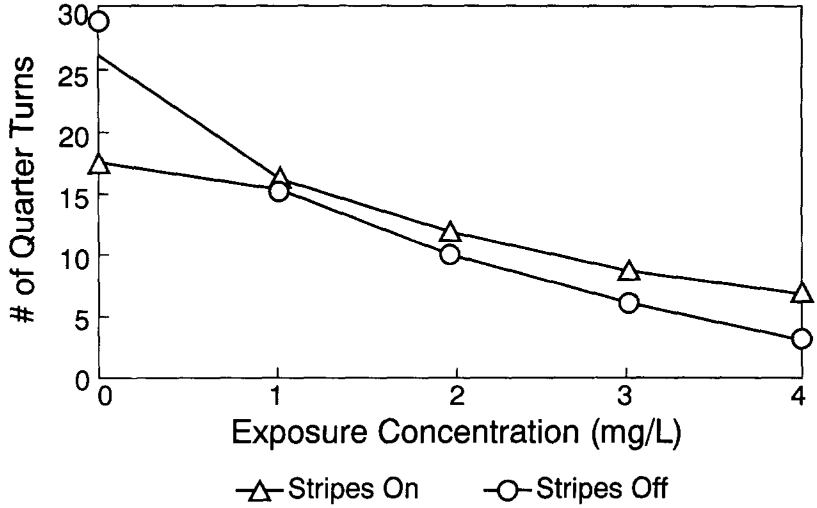

Figure 1. Scores of "following" response in control and malathion exposed fish

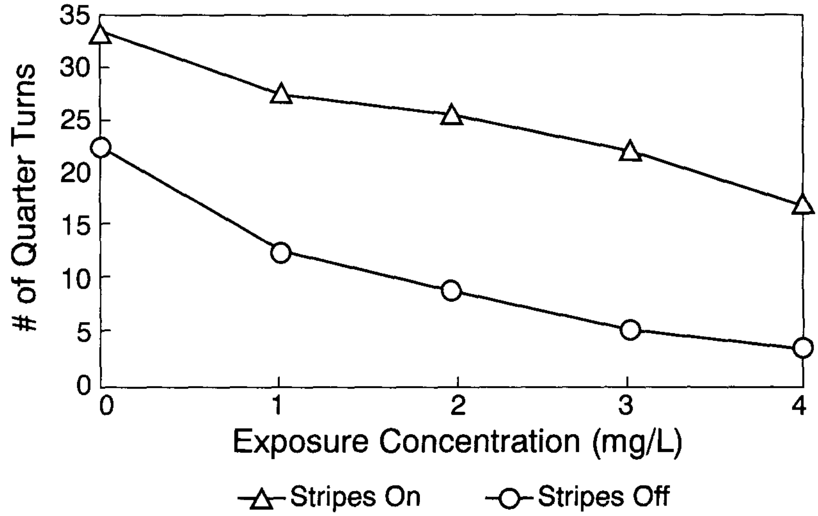

Figure 2. Scores of "reversal" response in control and malathion exposed fish

Table 1. Statistical Analysis of Optomotor Response Test Score (n=10 for each condition)

Exposure Concentra- tion prob mg/L	Bucket Rotating Mean	S.E.	t value	2-tail prob	Bucket Stopped	Mean	t S.E.	2-tail value
				"Following"				
0	26.15	5.32			17.45	3.36		
1	16.30	3.54	1.47	0.176	15.10	4.42	0.54	0.605
2	11.50	2.52	2.57	0.030*	9.75	2.99	1.60	0.144
3	8.45	2.11	2.72	0.023*	6.05	1.77	2.89	0.018*
4	6.55	2.19	3.13	0.012*	2.55	0.93	4.74	0.001***
				"Reversals"				
0	33.75	5.41			22.65	5.57		
1	27.70	4.41	0.86	0.415	12.75	3.48	1.39	0.197
2	25.95	3.77	1.45	0.181	8.95	3.54	2.48	0.035**
3	22.70	2.35	2.08	0.067	5.40	1.13	3.00	0.015**
4	17.30	1.30	3.31	0.009**	3.45	0.91	3.62	0.006**

$^{*}p \le 0.05$, $^{**}p \le 0.01$, $^{***}p \le 0.001$

Fish showed some rapid and erratic swimming movements immediately after introduction into the water in the exposure tanks in all concentrations of toxicant. The fish were excited at this time. At times, during the exposure, the fish came to lie on their sides.

Behavioral changes are sensitive indicators of pollutants such as malathion and other pesticides and toxicants. The optomotor response is essential for behaviors such as searching for food, orientation toward food odor, location of a mate, escaping from a predator and avoidance of an adverse situation. Two major behavior changes such as "hypoactivity" and "lethargy" were noticed in fish that were exposed to different concentrations of malathion.

Hypoactivity was observed at an exposure concentration of 2 mg/L. Sharma et al. (1983) reported erratic swimming movements followed by lethargy in Clarias batrachus exposed to 0.25 to 2.00 mg/L malathion. In A. testudineus, in addition to hypoactivity, lethargy was seen at the higher exposure concentrations namely 3 mg/L and 4 mg/L.

Dutta et al (1992a) have recorded that the fish Labeo rohita (Ham) exhibited hyperactivity at an exposure concentration of 0.2 to 0.4 mg/L of malathion. But in this study A. testudineus entered into a state of hypoactivity at an exposure concentration of 2 mg/L of malathion. L. rohita became lethargic around an exposure concentration of 0.6 mg/L, whereas A. testudineus became lethargic around an exposure concentration of 4 mg/L. There seems to be a six- to ten-fold difference in the concentration of the pesticide that can bring about behavioral changes in these two different genera of fish. This difference can be attributed to the mode of respiration of these two different genera of fish. L. rohita is a completely water-breathing fish in contrast to A. testudineus which is an air-breathing fish. Dutta et al (1992a) have shown that the LC 50 for air-breathing fish is higher than that of the water-breathing fish.

The lethargic condition that results due to pesticide exposure would affect the fish in several ways. The fish that became lethargic would fall easy prey to predators. Feeding and food-capture will be hampered by lethargy and loss of orientation caused by the action of the pollutants.

Fish living in streams may not be able to maintain their position and may be swept downstream. Bull and McInerney (1974) reported that many juvenile coho salmon were unable to maintain position and were swept downstream after being exposed to sumithion, an organophosphorus insecticide, in a stream aquarium.

The results of this study show that exposure of fish to pesticides in the aquatic environment may not cause immediate death, but it certainly can bring about some undesirable behavioral changes. These undesirable behavioral changes may lead to a reduction in the number of fish in the population which will result in a disturbance in the aquatic ecosystem in which these fish live.

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