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Effect of Malathion on the Brain Acetylcholinesterase Activity of Bluegill Sunfish *Lepomis macrochirus*

Chelliah R. Richmonds and Hiran M. Dutta

Department of Biological Sciences, Kent State University, Kent, Ohio 44242, USA

The harmful effects of pesticides on aquatic organisms are due to the indiscriminate use, careless handling, accidental spillage, or discharge of untreated effluents into natural waterways. There was a shift in the types of insecticides used in the mid-1960s from the organochlorine to the less stable organophosphate and carbamate classes (Henry 1984). Malathion belongs to the widely used organophosphorus group of pesticides. Malathion has a low toxicity to mammals and a relatively high toxicity to fish (Mount and Stephan 1967). 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). Frank et al. (1990) surveyed the farm wells during 1986 and 1987 for pesticides in Ontario, Canada. Their survey showed contamination of farm wells by various pesticides including malathion.

The toxic effects of the organophosphorus compounds result from their ability to inhibit the enzyme acetylcholinesterase (AChE), which in turn disrupts the transmission of nerve impulses. Malaoxon, an oxygen analog of malathion appears to be the active part that will bind to AChE (O'Brien et al. 1974). Abiola et al. (1991) studied the blood cholinesterase activity of field pesticide applicators. Their results showed an inhibition of cholinesterase activity ranging from 5% to 28% Several studies, including the one by Cook et al. (1976) have indicated that AChE measurement is the most sensitive indicator of organophosphorus poisoning in fish. Bluegills are more sensitive to malathion when compared to fathead minnows and goldfish (Pickering et al. 1962). Therefore we decided to study the effect of malathion on the brain AChE activity of bluegill sunfish, Lepomis macrochirus.

MATERIALS AND METHODS

Bluegills, body length 12-13.5 cm, weight 32-45 g, were obtained from a local fish hatchery. Because there is no agricultural land nearby where pesticides are applied, this source for the supply of

Send reprint requests to H. Dutta at the above address

fish was selected. Fish were transported to the laboratory in containers with battery-operated aerators and placed in large glass aquaria. Tap water was collected in large fiberglass tanks and allowed to stand for at least a week before it was used for any experimental purpose. The mean values for the water quality characteristics are as follows: dissolved oxygen 6.91 ± 0.38 mg/L; pH 7.27 ± 0.16; total hardness (mg/L as $CaCO_3$) 125.55 ± 3.35; alkalinity (mg/L as $CaCO_3$) 34.57 ± 1.63. Water temperature was 21 ± 1^0 C and a 14/10 hr light-dark cycle was maintained by using auto-control fluorescent lighting throughout the holding and exposure periods. Fish were fed three times daily with live guppies and commercial fish food sticks, Tetra Doro Min manufactured by Tetra Werke, West Germany.

Cook et al. (1976) reported concentrations of up to 0.05 mg/L of malathion in some surface waters. Therefore it was decided to select exposure doses that fall within this limit. Malathion concentrations used in the present study were 0.002, 0.004, 0.008, 0.016, 0.032 and 0.048 mg/L and the duration of exposure was 24 hr.

A test solution with the desired concentration of the pesticide was prepared from a previously prepared stock solution of the pesticide. Commercial grade malathion is more toxic than technical grade malathion to the fish Tilapia mossambica. The relatively high toxicity of commercial grade malathion may be due to other ingredients present as emulsifiers which act as synergists (Sailatha et al. 1981). Therefore commercial grade malathion was used in this study.

Exposure to malathion was done in large fiberglass tanks. Replicates of five fish were exposed at a time. Ten fish were used for control and each exposure concentration to estimate AChE activity. The mean values for the water quality characteristics are as follows: dissolved oxygen 6.41 ± 0.18 mg/L; pH 7.32 ± 0.34; total hardness (mg/L as $CaCO_3$) 93.78 ± 2.78; alkalinity (mg/L as $CaCO_3$) 24.60 ± 0.86. Feeding was stopped 24 hr before the start of the exposure. There was about 2 L of water per gram weight of fish. Controls were treated under the same conditions without malathion.

Fish were pithed and the brain was carefully removed intact with sharp-pointed scissors and forceps, after making a median incision in the dorsal side of the skull and excising the brain free by cutting the optic nerves and spinal cord. Care was taken to avoid any blood clots, bones, skin or portions of olfactory and optic nerves. The removed brains were kept frozen and analyzed for brain AChE activity within a week.

The estimation of AChE activity of the brain tissue was performed according to the methods of Ellman et al. (1961). Homogenization was performed using a Tri-R® Stir-R model #S 63C variable speed motor attached to a homogenizer. The enzyme activity was calculated by measuring the increase in yellow color produced by thiocholine when it reacts with dithiobisnitrobenzonate ions. A Bausch and Lomb Spectronic 20 colorimeter was used for estimating AChE activity.

The analyses were performed at 25 ± 1° C at a wavelength of 412 nm. Duplicate measurements were done on each sample and the mean value was taken. Data were analyzed using one-way ANOVA to test for the overall effect of malathion dose on AChE activity. Student-Neuman-Keuls test procedure was applied to detect differences between specific pairs of mean values.

RESULTS AND DISCUSSION

The results are shown in Table 1, which includes the exposure concentration, mean AChE activity as µM of substrate hydrolyzed/min/g brain tissue, and percent of inhibition. Statistical analysis of data revealed no significant difference in AChE activity between control fish and fish exposed to 0.002, 0.004 and 0.008 mg/L of malathion. A significantly ($p < 0.0001$) decreased mean AChE activity was seen above an exposure concentration of 0.016 mg/L malathion, compared to controls and all other lower levels of exposure concentrations. At 0.032 and 0.048 mg/L exposure concentrations, there was a significant ($p < 0.0001$) reduction in the mean AChE activity compared to the mean activity estimated from the controls and all other lower levels of exposure concentrations.

Table 1. Brain Acetylcholinesterase Activity in Control and Malathion Exposed (24 hr) Fish (n=10)

Conc mg/L	Activity mean‡‡	S.E.M. +/-	Neuman-Keuls test grouping‡	% Inhibition†
0.000	11.36	0.41	A	0
0.002	11.26	0.26	A	0.88
0.004	11.06	0.31	A	2.65
0.008	10.60	0.36	A	6.91
0.016	8.51‡	0.23	B	25.09
0.032	5.84‡	0.28	C	51.45
0.048	4.57‡	0.21	D	59.77

‡ Means with the different letters are significantly different ($p < 0.0001$)

‡‡ Activity mean = µM substrate hydrolyzed/min/g brain tissue

† Compared with control

Organophosphorus pesticides inactivate the enzyme AChE and are specific in action (Heath 1961). Considerable decrease in brain AChE activity occurred at higher exposure concentrations. At the highest exposure concentration of 0.048 mg/L, about 60% reduction of AChE activity was noticed. There was no death among the fish exposed during this part of the study. The fish withstood 60% reduction of total AChE activity. There were conflicting reports in the literature about the amount of AChE reduction required to cause death. Most of the estimates fall within a range of 70-85% reduction in total AChE activity to result in the death of the fish. Coppage and Matthews (1974), after their 24-hr exposure studies on estuarine

fishes, claimed that mean reductions in AChE activity of about 80% are critical in short-term poisoning of the fish tested, and this may apply to fish in general.

Depression of brain AChE may cause numerous physiological and behavioral modifications that reduce the survival ability of animals (Richmonds and Dutta 1989). Significant decrease in AChE activity was reported from all the regions of rabbit brains following malathion administration (Vijayakumar and Selvarajan 1990). At sublethal pesticide levels there is impairment in mechanisms that are critical to survival (Heath 1961). This impairment will reduce the ability of the fish to perform normal functions such as obtaining food, finding a mate, and escaping from predators.

Galgani and Boequene (1990) studied the inhibition of AChE from four marine species by organophosphates and carbamates. For all organophosphates studied, these authors found the sensitivity to be the highest for fish, compared with shrimp and mussel. Malathion induced changes in the AChE activity of bluegills may be used as an important and valuable index for assessing the environmental pollution by malathion.

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