Toxicity of malathion and its effect on the activity of acetylcholinesterase in various tissues of the grass carp, *CtenOPharyngodon idella* Val.

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ABSTRACT

Malathion, a widely used insecticide, is known to cause serious metabolic disturbance in non- target organisms, like fish. Bioassay tests were conducted on the grass carp fish to evaluate the acute toxicity of malathion by determining their LC50 after 24, 48, 72 and 96 h exposure. The safe concentration of this biocide was calculated on the basis of LC50 for 96h. The fish showed characteristic changes in behavior when exposed to various concentrations of malathion with difficulty in respiration, convulsions and short erratic jerky body actions. The sublethal toxic potency of malathion was indicated by inhibiting acetyl cholinesterase activity in the brain, muscle, gill and liver tissues of the grass carp. The changes in acetyl cholinesterase activity in the above tissues were decreased significantly up to 96h. Maximal inhibition of the enzyme was observed at 24 and 48 h intervals. So, by acquiring knowledge of LC50 values of a toxicant, its discharge into nearby water resources may be regulated to protect aquatic life.

Key words: Malathion, toxicity, Grass carp, acetyl cholinesterase.

INTRODUCTION

OrganOPhosphorus (OP) compounds are widely used in agriculture, medicine and industry. OP pesticides, in addition to their intended effects like the control of insects or other pests, are sometimes found to affect non- target organisms including humans (Chantelli- Forti *et al*., 1993; Chaudhuri *et al*.,1999). Exposure to OPs is also cause of longer- term damage to the nervous system, with reports of poor mental health and deficits in memory and concentration (Davis, 1991; Mason, 2000; Nigg and Knaak, 2000). Because of the serious environmental problems resulting from the use of pesticides in the agricultural sector, several countries are seeking to employ biological and other nonpolluting methods for combating pests.

Malathion is one of the most widely used organOPhosphate insecticides throughout the world. It is used to control pests affecting agricultural crOPs, ornamentals, green houses, livestock, stored grain, forests, buildings, households and gardens. Contributing to its pOPularity is its relatively low acute

mammalian toxicity (Brenner, 1992; Hazarika *et al*., 2003). However, like other pesticides that have been found to cause irreparable damage to humans and the environment (Brenner,1992). They also cause serious metabolic disturbances in non- target species, like fish and freshwater mussels (U.S.E. prot. Agen. 1972).

Malathion contains potent neurotoxic molecules that exert their toxicity by blocking the breakdown of acetylcholine by the enzyme acetylcholinesterase (AchE). Acetylcholine is the primary neurotransmitter in the sensory and neuromuscular systems in most species. The activity of this system is vital to normal behavior and muscular function and represents a prime target on which some toxicants can exert a detrimental effect. Inhibition of the Ach E enzyme results in a build up of acetylcholine, causing a continuous and excessive stimulation of the nerve/ muscle fibres, which leads to tetany, paralysis and eventual death.

Measurement of AchE inhibition in aquatic organisms has already been used as a biomarker of neurotoxic contaminants (Habig and Di Giulo, 1988 ; Galgani *et al*., 1992 ; Payne *et al*., 1996 ; Mc Henery *et al*., 1997 ; Kirby *et al*., 2000 ; Sole` *et al*., 2000; Abdel- Halim *et al*.,2006).

The present investigation was undertaken to determine the followings:

1. The lethal level of malathion to the grass carp (*CtenOPharyngodon idella* Val.) and its safe levels that recommended for employment near water resources.
2. The effect of malathion on the activity of acetylcholinesterase in the grass carp.

MATERIALS AND METHODS

Specimens of the grass carp (wt. 30 ± 5gm) were obtained from Delta Breeding Station (DBS), Cairo. The fish were acclimatized in the laboratory aquaria for 2 weeks before being subjected to bioassay tests.

Technical grade malathion of 95% purity was obtained from pesticides market. The fish were exposed to different concentrations of malathion. Ten specimens of grass carp species were exposed to each concentration. Mortality was observed at 24h intervals for 96h. Fish were considered dead when they did not respond to probing with a glass rod. Dead specimens were removed immediately since dead organic material in static bioassays may deplete the DO, affecting tolerance limits of other specimens in bioassay tests (Schreck and Brouda, 1975), while safe concentrations were computed on the basis of LC50 for 96h, using application factors of 0.024 for organOPhosphate as mentioned by Bansal *et al*. (1980).

LC50 values of malathion for 24, 48, 72 and 69 h exposures were computed on the basis of probit analysis (Finney, 1964) as shown in Table (1).

1.0 mg/l from malathion was chosen to represent sublethal concentration, because the concentrations of malathion detected in the main stream of the River Nile in Cairo and at the beginning of each of Damietta, Rosetta branch as well

as Ismailia canal lied in the ranges 0.1-2.0, 1.0, 1.0, 0.21-3.6 mg/l respectively (Nile Basin Initiative, 2005). Acetylcholinesterase level was estimated beginning from 12 h to 96h, since there was no significant change in the values of controls at different periods,

Ten fish from aquaria and at the specified exposure time were anesthetized in 120 mg/l tricaine methanesulfonate (MS $_{222}$), solution. Immobilization was completed with in 20 seconds. Brain, muscle, gill and liver were removed for homogenization in cold 0.25M sucrose. Acetylcholinesterase activity was determined by the method of Metcalf (1951).

Statistical analysis

Studentst-test was employed to calculate the significance of the differences between control and experimental means. P values of 0.05 or less were considered statistically significant (Fisher, 1950).

RESULTS AND DISCUSSION

The data on LC50 values (Table 1) and the acute toxicity ranges at different time intervals reveal that acute toxicity of malathion ranged between 3.728 mg/l and 2.138 mg/l for grass carp. The safe concentrations of malathion was 0.0513 mg/l as shown in Table (1).

Table (1): Acute toxicity, confidence limit and safe concentration of malathion to the grass carp fingerlings.

Fish species	Biocide used	Acute toxicity (LC50 values) and confidence limit (mg/l)			
		24 h	48 h	72 h	96 h
Grass carp	Malathion	3.728 (2.383-5.831)	2.838 (1.709-4.711)	2.444 (1.684-3.548)	2.138 (1.459-3.134)
		Safe concentration (mg/l) of malathion on the basis of 96hLC50			
		0.0513			

The fish showed characteristic changes in behavior when exposed to various concentrations of malathion. Difficulty in respiration, convulsions and short erratic jerky body actions were observed. The skin of exposed specimens became light in color and secreted an excessive amount of mucus. Some specimens frequently dashed against the wall of the experimental aquaria. Subsequently, the fish became progressively lethargic. They lost their sense of equilibrium and had fast jerky body movements. The fish settled at the bottom before death. In low concentration of malathion, the responses were of a lesser degree. Control fish behaved normally. Similar observations have been made in different fish species following exposure to various biocides (Verma *et al*., 1978; Verma *et al*., 1980; Singh *et al*., 1981; Singh *et al*., 1984; Sanjay *et al*., 2005).

The intense OPercular movements in the grass carp following exposure to malathion may be due to hypoxic stress accompanied by a sub sequential inhibitory influence of this compound on the respiratory mechanism. Reddy and Gomathy (1977) and Verma *et al*. (1979) have demonstrated that toxicity of organic biocides to fish involved a modification of gas exchange process at the gills with subsequent hypoxia at tissue level.

Mount and Stephan (1967) have suggested that the safe level of malathion for fishes lies somewhere between 1/15 and 1/45 of the 96h LC50 concentration. Their studies suggest that levels of malathion at or below 1/45 of the 96h LC50 will not harm the growth or reproduction of the fathead minow (*Pimephales promelas*), and they suggested the same application of this level for use on other fish species. For the grass carp, this would mean that at a level of malathion greater than 0.0513 mg/l, damage to the fish's well-being could possibly occur.

The changes in acetylcholinesterase activity in the brain, muscle, gill and liver tissues of malathion exposed fishes decreased significantly up to 72h. Maximal inhibition of the enzyme was observed at 24 and 48 h intervals (Table 2).

Table (2): Acetylcholinesterase (AchE) activity (µmol/ mg protein /h) in various tissues in normal and malathion exposed fish at different hours of exposure.

Activity of AchE (µmol/ mg protein /h)							
Tissue	control	Hours of malathion exposure					
			12	24	48	72	96
Brain	26.35	M	24.083	20.55	15.033	18.6	24.717
	±0.812	S.D.	±1.078	±0.873	±0.864	±0.616	±0.685
		P	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Muscle	17.483	M	15.233	11.233	8.899	13.666	16.883
	±0.614	S.D.	±0.606	±0.450	±0.289	±0.653	±0.402
		P	P<0.01	P<0.01	P<0.01	P<0.01	N.S.
Gill	11.483	M	9.567	8.217	6.25	7.183	9.433
	±0.531	S.D.	±0.476	±0.366	±0.327	±0.232	±0.484
		P	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Liver	5.55	M	3.55	2.083	1.233	2.633	4.083
	±0.451	S.D.	±0.414	±0.293	±0.294	±0.301	±0.147
		P	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

Each value is the mean ± S.D. , P= t' test , N.S. = Not significant.

Since the inhibition of acetylcholinesterase activity gradually increased from 12 to 24 and 48 h and decreased from 48 h to 96 h (Table 2), it can be suggested that inhibition of the esterase by malathion is dependent on the

duration of exposure. After 96h of exposure, the enzyme activity in all the tested tissues as compared to that in normal tissue, thus indicating the reversal of inhibition in treated fishes. It is likely that the effect of malathion decreases after 48h probably due to its degradation.

The differential inhibition (descending order) of acetyl cholinesterase activity in the four tested tissues (brain> muscle> gill>liver) may be due to the presence of isozymes with different affinities for the substrate and the inhibitor. Further, the pesticide itself may be present in different amounts in the different tissues, producing differential inhibition or the inhibitor may be metabolized at different rates. This assumption has been described by a number of authors as Wilson (1967) and Kabeer Ahammad *et al*. (1980).

In conclusion, by acquiring knowledge of LC50 values of a toxicant, its discharge into nearby water resources should be regulated to protect aquatic life. The safe concentration determined in the present investigation is actually fractions of LC50 values and therefore, would be useful in regulating their discharges.

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