

## **Impact of malathion on acetylcholinesterase in the tissues of the fish *Tilapia mossambica* (Peters)—A time course study**

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**Abstract.** The sublethal toxic potency of malathion in inhibiting acetylcholinesterase activity of brain, muscle, gill and liver tissues of the fish, *Tilapia mossambica* was studied at 12 h intervals. Maximum inhibition at 36 and 48 h, and complete revival of acetylcholinesterase activity after 72 h was noticed, suggestive of the loss of inhibition of the enzyme activity was probably by suitable (acetylcholine) accumulation,

**Keywords.** Malathion; acetylcholinesterase; acetylcholine; *Tilapia mossambica*.

### **Introduction**

Malathion, a widely used insecticide is known to cause serious metabolic disturbances in non-target species, like fish and fresh-water mussels (U.S.E. Prot. Agen. 1972). Malathion is known to affect the nervous system by inhibiting acetylcholinesterase (AChE), the enzyme that modulates the amount of the neurotransmitter acetylcholine (Fukuto, 1971). There are several metabolic routes by which an organism can detoxify organophosphorus insecticides. In addition, The physiological condition of the organism during toxic impact must be considered to understand the influence of pesticide. In the present study malathion was chosen to evaluate its influence on the acetylcholinesterase activity and acetylcholine content in the tissues of the fish *Tilapia mossambica* at different times after exposure to malathion.

### **Materials and methods**

The fish, *Tilapia mossambica* was collected from streams around Tirupati and acclimated to laboratory conditions for about a week. They were fed daily with groundnut-cake (solid material obtained after extracting oil from the seeds) and frog muscle twice a week. Technical grade malathion of 95% purity was used. The lethal concentration for 50% killing ( $LC_{50}$ ) values was computed on the basis of probit analysis (Finney, 1964) and was found to be 5.6 ppm. Two ppm malathion was chosen to represent sublethal concentration. Malathion was added only once and the fish tanks were aerated at 12 h intervals.

Fishes weighing  $8 \pm 2$  g were separated in to 7 batches of 10 each. One batch of fishes were exposed to 80 litres of tap water in two 50 litres glass-tanks. The remaining 6 batches of fishes were exposed to 480 litres of 2 ppm malathion in tap-water in 12 glass tanks. The period of exposure was adjusted to obtain fishes at two time-inter-

vals exposure per day. Acetylcholinesterase and acetylcholine levels were estimated every 12 h. Since there was no significant change in the value of controls at different periods, only average values are represented in the results choosing the value for one fish at each interval.

After exposure of the fish to malathion, brain, muscle, gill and liver were removed for homogenisation in cold 0.25M sucrose. Acetylcholine and the esterase activity were determined by the method of Metcalf (1951). Protein was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

## Results and discussion

The changes in acetylcholinesterase activity in the brain, muscle, gill and liver tissues of malathion-exposed fishes decreased significantly upto 60 h. Maximal inhibition of the enzyme was observed at 36 and 48 h intervals (table 1). Concomitant with the decreased AChE activity, the ACh content correspondingly increased after exposure for 36 and 48 h (table 2).

Since the inhibition of acetylcholinesterase activity gradually increased from 12 to 36 and 48 h and decreased from 48 to 72 h (table 1), it can be suggested that inhibition of the esterase by malathion is dependent on the duration of exposure. After 72 h of exposure, the enzyme activity in all the tissues as comparable to that of normal tissues, thus indicating the reversal of inhibition in treated fishes. It is likely that the effect of malathion decreases after 48 h probably due to its degradation.

The differential inhibition of acetylcholinesterase activity in the four 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 metabolised at different rates.

Corresponding to maximal inhibition of acetylcholinesterase activity at 36 and 48 h of exposure, acetylcholine content also showed a corresponding increase (data not a substrate of the esterase, the changes observed in its content in the four tissues at acetylcholine. During 72 h when the esterase activity was restored to the normal level the acetylcholine content also returned to normal levels. Since acetylcholine is a substrate for the esterase, the changes observed in its content in the four tissues at different times of exposure were compatible with alterations in the enzyme activity.

It is well known that the inhibitory effects of malathion result from the relatively longer half life of the phosphorylated enzyme, as compared with the acetylated enzyme of the physiological reaction (Wilson, 1967; Albridge, 1971). Since there was only a single addition of malathion to the water without any change of water, it is likely that the phosphorylation of the enzyme may have occurred only upto 48 h and later the amount of the phosphorylated enzyme could have decreased due to lowered levels of the pesticide.

It is evident from our earlier findings, that malathion inhibits acetylcholinesterase in four tissues of *T. mossambica*, competitively (Kabeer and Ramana Rao, 1980). In the presence of high amounts of acetylcholine, the inhibitory effect produced by malathion is known to be reduced (Augustinsonn and Nachmansohn, 1949). Thus the accumulation of high concentrations of acetylcholine during 36 and 48 h may contribute towards reversal of the inhibition of enzyme activity. However, the possibility is rather doubtful since acetylcholine can compete for the free enzyme and not for the already phosphorylated enzyme. The most possible explanation is that the accumulated acetylcholine may induce the formation of increased amounts of acetylcholinesterase leading to the revival of affected fishes.

**Table 1.** Acetylcholinesterase (AChE) activity of brain, muscle, gill and liver tissues of normal and malathion exposed fishes at different hours of exposure.

Tissue	Control	Specific activity of AChE ( $\mu$ mol mg protein. h)						
		Hours of malathion exposure						
		12	24	36	48	60	72	
Brain	4.8 $\pm$ 0.2	M	3.9	2.6	2.3	2.4	4.1	4.7
		S.D.	$\pm$ 0.2	$\pm$ 0.4	$\pm$ 0.3	$\pm$ 0.2	$\pm$ 0.3	$\pm$ 0.3
		PD	-19.1	-46.2	-50.7	-49.3	-15.3	-1.9
			$P < 0.001$	N.S				
Muscle	3.6 $\pm$ 0.2	M	3.0	2.5	1.9	1.8	3.0	3.8
		S.D.	$\pm$ 0.3	$\pm$ 0.3	$\pm$ 0.1	$\pm$ 0.2	$\pm$ 0.4	$\pm$ 0.3
		PD	-17.2	-31.5	-47.4	-48.3	-15.5	-5.4
			$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.025$	N.S
Gill	2.4 $\pm$ 0.1	M	2.0	1.8	1.4	1.4	2.0	2.4
		S.D.	$\pm$ 0.1	$\pm$ 0.2	$\pm$ 0.2	$\pm$ 0.2	$\pm$ 0.2	$\pm$ 0.1
		PD	-15.4	-28.1	-40.6	-41.9	-17.4	-1.0
			$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.05$	N.S
Liver	1.4 $\pm$ 0.1	M	1.2	1.1	0.9	0.0	1.2	1.4
		S.D.	$\pm$ 0.1	$\pm$ 0.1	$\pm$ 0.8	$\pm$ 0.2	$\pm$ 0.1	$\pm$ 0.0
		PD	-14.0	-22.1	-34.3	-37.5	-16.6	-2.7
			$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.025$	N.S

Each value is the mean  $\pm$  S.D. of 6 individual observations. PD=Percent deviation from normal. P='t' test, NS=Not significant.

**Table 2.** Acetylcholine content of brain, muscle, gill and liver tissues of control and melathion exposed (ME) fishes at different hours of exposure.

Tissue	Control	ACh content ( $\mu$ mol/g wet wt)					
		Hours of malathion exposure					
		12	24	36	48	60	72
Brain	43.4 $\pm$ 4.9	M 56.3	62.7	73.7	73.8	58.0	45.1
		S.D. $\pm$ 3.7	$\pm$ 2.4	$\pm$ 3.7	$\pm$ 4.6	$\pm$ 4.5	$\pm$ 4.1
		PD +29.6	+44.2	69.6	+69.7	+33.4	+3.7
		$P < 0.001$	$P < 0.001$	$P < 0.001$	0.001	$P < 0.025$	N.S
Muscle	33.9 $\pm$ 3.0	M 44.5	53.5	60.0	57.6	41.8	37.8
		S.D. $\pm$ 3.9	$\pm$ 3.8	$\pm$ 2.7	$\pm$ 2.8	$\pm$ 2.7	$\pm$ 2.8
		PD +31.1	+57.6	+76.9	+69.6	+23.1	+11.5
		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.025$
Gill	27.6 $\pm$ 3.1	M 33.2	45.9	55.2	54.2	39.1	31.5
		S.D. $\pm$ 2.3	$\pm$ 2.7	$\pm$ 3.2	$\pm$ 3.3	$\pm$ 2.5	$\pm$ 2.3
		PD +20.3	+66.3	+99.9	+97.7	+41.6	+14.1
		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.025$
Liver	24.4 $\pm$ 2.1	M 33.2	46.1	50.0	41.5	32.1	26.6
		S.D. $\pm$ 2.6	$\pm$ 2.7	$\pm$ 2.2	$\pm$ 3.2	$\pm$ 2.5	$\pm$ 1.6
		PD +35.7	+88.3	+104.3	+69.7	+31.2	+4.4
		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.025$

Each value is the mean  $\pm$ S.D. of 6 individual observations, P.D. -Percent deviation from normal,  $P = t'$  test. N.S. = Not significant.

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