EFFECTS OF AN ORGANOPHOSPHATE PESTICIDE (PARATHION) ON OXYGEN CONSUMPTION OF TILAPIA MOSSABICA

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Abstract: Parathion is an organophosphate insecticide and extensively used to control harmful insects of agriculture. The present study is aimed to study the toxicological impacts of parathion (Methyl Parathion) on oxygen consumption. The toxicity tests were conducted by static renewal bioassay method on the juveniles of fish Tilapia mossambica (Peters). The teleost fish *Tilapia mossambicus* of 15±1g was exposed to three different sub lethal concentrations of methyl parathion for 24,48,72,96 hours. The probit analysis showed that the LC_{50} (lethal concentration) for 24, 48, 72 and 96 h were 4.5, 2.8, 1.8 and 1.02 mg/L. Hepatic metabolic parameters like protein and glycogen were also determined as oxygen consumption is directly reflects their content.
One third (330 µg/1) one tenth (100 µg/1) and one fifth (200 µg /1) of the LC50 values were selected for sub lethal studies. An initial increase in the oxygen consumption was observed in low sub lethal concentrations ($1/5^{th}$ and $1/10^{th}$ exposure) but a sharp decrease in oxygen consumption was observed in all sub lethal concentrations. Results also clearly reveal that protein, glucose and glycogen content of liver were significantly reduced in all experimental groups treated with sub lethal concentrations of parathion. The reduction in glycogen content of liver in all the experimental fishes might be due to the utilization of carbohydrates for energy production as a result of toxicant induced hypoxia which has also been reflected in oxygen consumption. Fish under sub lethal concentration were found to be under stress and duration of the exposure is found to be an important factor for inducing the toxic effects. Hence, respiratory dysfunction and hepatic metabolic markers like glucose and glycogen can be used as an index of Parathion toxicity.

Keywords: Hepatic markers, LC_{50} concentration, oxygen consumption, parathion

Introduction: In an agricultural country like India, insecticides represent the major components of aquatic chemicals as pollutants. Environmental contaminants such as metals, pesticides, and other chemicals cause severe risks to many aquatic organisms. For that reason, an immense deal of previous research has described physiological mechanisms of toxicity in animals exposed to pollutants. On the contrary, effects of pollutants on fish behavior are not regularly studied. Since behavior links physiological function with ecological processes, behavioral markers of toxicity appear ideal for evaluating the effects of aquatic pollutants on fish populations. Toxicant exposure often completely reduces the performance of behaviors that are necessary for fitness and survival in natural ecosystems, frequently after exposures of lesser magnitude than those causing significant mortality. Unfortunately, the behavioral toxicity of many contaminants is still unknown, necessitate their future study.
Physiological effects of toxicants in the literature include disruption of sensory, hormonal, neurological, and metabolic systems, which are likely to have profound implications for many fish behaviors. On the other hand, little toxicological research has required integrating the behavioral effects of toxicants with physiological processes. The most commonly observed links with behavioral disruption include cholinesterase (ChE) inhibition, altered brain neurotransmitter levels, sensory deprivation, and impaired gonadal or thyroid hormone levels[1]. Oxygen uptake is widely used in physiology as a biological indicator that integrates the overall metabolic activity of an animal in response to specific environmental factors [2] because it reflects energy expenditure and, ultimately, the food requirements. The changes in the oxygen consumption of fish as an index of toxicity to various pesticides have been studied by several investigators. [3], [4], [5], [6], [7].
Parathion is also known as parathion-ethyl or diethyl parathion and locally known as Folidol. It is an organophosphate compound and is a potent insecticide. It is lipid soluble and highly toxic to non-target organisms, including humans. Exposure may occur from the use of parathion as an insecticide on agricultural crops. EPA has classified parathion as a Group C, possible human carcinogen [8]. Most of methyl parathion is discharged with urinary and some of it is discharged with stools [9]. The methyl parathion which is entered into the body is completely and rapidly absorbed by gastrointestinal system. It was observed that the plasma cholinesterase enzyme (ChE) activity in the fish was inhibited in the rate of 90% right after 4 h following the application [10].
Liver is the first organ where methyl parathion is metabolized and detoxified. [11] In one more study which was carried out with chickens exposed to 14% LD50 dosage of methyl parathion, showed that the parathion was rapidly absorbed by gastrointestinal

way then it was spread to plasma, liver, kidney, brain and gastrointestinal tissues after 8 hours of application. Studies showed that four different mechanisms have a role in metabolysis of methyl parathion. Fishes are particularly very sensitive to the water pollution. Hence, contaminants such as insecticides, herbicides may significantly influence some physiological and biochemical processes when they enter into the organs of fishes. [12], [13], [14] More over the insecticides mainly crash on liver [15] and affects the carbohydrate metabolism by declining the glycogen content [16]. Therefore, the present study is aimed to investigate the affects of parathion (Methyl parathion) on oxygen consumption and carbohydrate metabolism in a locally available fish, Tilapia mossambica.

Materials and Methods:

Sample Collection and Maintenance: Healthy and active *Tilapia mossambica (Peters)* juveniles of same size, length and weight approximately (22gm-25gm) (irrespective of age and sex) were procured from the local fishermen at Jhabua, Madhya Pradesh, India. Fish were brought to the laboratory in large aerated containers and were acclimatized for 20 days in 100 L glass aquaria containing dechlorinated tap water. They were fed with commercial dry feed pellets (Nova, Aquatic P. Feed). Physico- chemical characteristics of water were analyzed following the methods mentioned in APHA [17]. Water was changed every day and a 12-12 h photoperiod was maintained during both acclimatization and test periods. The fish were fed on a regular basis with commercial fish food pellets during acclimatization and test periods, but feeding was stopped two days prior to exposure to the pesticide.

Acute toxicity test: Parathion **(Folidon 50 Ec - Methyl Parathion 50% Ec)** was procured from the local market (Chemet chemicals Pvt. Ltd, Ahmedabad, Gujarat, India). A stock solution was prepared in acetone and mixed in water to obtain required dilutions. Required quantity of Parathion was drawn directly from this stock solution using micropipette. Required dilutions of the acetone formulation were made with tap water. The LC50 value for 96 hours of Parathion was determined by procedure of Finney [17].

A group of 10 healthy fishes were accommodated in 45 liters of test solution in the aquarium. Each group were exposed to different concentrations of pesticide to calculate the medium lethal concentration LC_{50} value using probit analysis method and was found to be 1.02 mg/L.. The experiment was conducted in triplicate. Dead fishes were removed immediately from the test medium to avoid deterioration.

The fish, in bathes of 10, were exposed to varying concentrations of parathion with 20 liters of water using three replicates for each concentration. One third (330 µg l-1) one tenth (100 µg l-1) and one fifth (100µg l-1) of the LC50 values were selected for sub lethal studies. Behavioral patterns and oxygen consumption were observed in all experimental and control groups. During the experimental period the control and experimental fish were kept under constant observation to study swimming behavior and whole animal oxygen consumption.

Whole body animal oxygen consumption: The whole body animal oxygen consumption was measured for all sub lethal concentrations along with the control by following the methods Welsh and Smith [18] and modified by Saroja [19]. Difference in the dissolved oxygen content of the water before and after experiment yielded the amount of oxygen consumed by the fish during the period of experimentation. The oxygen consumed by normal and treated fish was determined. After experimentation, the fishes were individually weighed and their unit metabolism was calculated and expressed as milliliters of oxygen consumed per gram wet weight of fish per hour. The data were analyzed by T test for the study of significance.

Total protein estimation: The total Protein content of was estimated according to modified standard method of Lowery et al. [20]. An amount of 5% homogenate of liver was isolated and precipitated with 5% trichloro acetic acid (TCA) and centrifuged at 3000 rpm for 15 minutes. The precipitate was dissolved in 1 ml of 1 N NaOH solution and 0.2 ml of extract taken into test tube and mixed with 5 ml of alkaline copper solution was added. To this 0.5% ml of 50 % folin phenol reagent was added. In the time following 30 minutes, the optical density was measured at 540 nm against a blank. The standard graph was plotted by using Lowry method with bovine serum albumin. The values were expressed as mg/g wet weight of the tissue.

Glycogen estimation: The glycogen was estimated by the standard method of Kemp et al. [21]. A 2% homogenate of liver tissue was prepared in 80% methanol and centrifuged at 3000 rpm for 10 minutes. The tissue residue was suspended in 5 ml of trichloro acetic acid (TCA), boiled for 15 minutes at 1000C, and then cooled in running water. The solution was made up to 5 ml with TCA to compensate the evaporation and then centrifuged. From this, 2 ml of supernatant was taken into the test tube and 6 ml of concentrated H_2SO_4 was added and the mixture was boiled for 10 minutes. The mixture was cooled and the optical density was measured at 520 nm. The standard graph was plotted with D-glucose by using the above mentioned method. The glucose was converted to glycogen by the multiplication factor of 0.98 [22] and is expressed as mg of glycogen/g wet weight of the tissue.

Statistical analysis: The data was subjected to one-way analysis of variance (ANOVA) using Microsoft Excel-2007 and the significance difference was set up at $p < 0.05$. These values were expressed as Mean ± SD for all parameters in the experiment.

Results and Discussion:

Acute toxicity study of Parathion: The findings of research related to acute toxicity study of Methyl parathion in *Tilapia mossambica* (Peters) in terms of treatment (concentration) and cumulative response (mortality) at 24, 48, 72 and 96 h and corresponding LC50 values with 95% confidence limits are presented in Table 1.

Fishes exposed to sub lethal concentrations of an organophosphorus pesticide, parathion for a short-term exposure were studied in terms of oxygen consumption and carbohydrate metabolism. The LC50 value of parathion for Oreochromis mossambicus was 1002 ug/L at 96 hr (Table.1). Fish mortality increased with increase in concentration of the pesticide. Gulping air, irregular swimming, loss of equilibrium, rapid opercular movements and over secretion of mucus on the skin was observed during experimental period. Besides, body surface acquired dark color in all fishes treated with sub lethal concentrations.

Gulping of experimental fish at water surface perhaps a protective behavior which helps to keep the animal away from contact of toxic medium. One more reason might be more demand of higher oxygen level during the exposure period [23].

Oxygen consumption: The oxygen consumption or respiratory potential is a reflection of metabolism of an individual. Hence it can be used as an important physiological tool to assess the toxic stress also useful to correlate the behaviour of the animal. The results of the present study clearly shown that methyl parathion influenced the oxygen consumption rate of fish in all experimental groups.(Table 2). Fish exposed to various sub lethal concentration shown increased oxygen consumption on first day but started decreasing along with dose and duration of exposure. In one tenth and one third sub lethal concentration exposure, rate of oxygen consumption increased in first few hours but the end of the first day onwards it shown decreasing trend with duration. Even it was found that the rate of oxygen consumption was slowly decreased in control group which may be due to the starved conditions and the reduced metabolic rates of the starved fish [24]. Initially the fish were in more stress during first hour and later they shown signs of recovery. From beginning to end of the experimental period the fish showed painstaking respiratory distress with rapid opercular movements leading to the higher amount of toxicant uptake.

It is well known that pesticides can cause respiratory distress and even failure by affecting respiratory centers of the brain (or) tissue involved in breathing[25],[26]. Fish exposed to sub lethal concentration showed increased oxygen consumption in Group II but decreased in other treated groups along with increased concentration of pesticide. The fluctuated response in respiration may be endorsed to respiratory distress as a result of the demolition of oxidative metabolism as in *Tilapia mossambica* [27] due to cypermethrin toxicity. The analysis of data from the present investigation evidenced that Methyl parathion is highly toxic and had intense impact on both behavior and oxygen consumption in *Tilapia mossambica* in all sub lethal concentrations. Consequently it has led to the distorted fish respiratory physiology. Differences in the oxygen consumption rate in experimental fish treated with parathion are perhaps due to damaged oxidative metabolism and pesticide induced respiratory stress. Hence, dysfunction of behavior and respiration can serve as an index of pesticide toxicity. The outcome of our research suggests that the altered rates of respiration of Tilapia may also serve as a quick biological monitor to evaluate the impacts of parathion on other biotic communities.

Biochemical analysis of total protein and glycogen: Results are shown table 3**.**

The change in the biochemical parameters (increase or decrease) is dependent on the health status and metabolism of the individual. Most of the toxicants including pesticides act as metabolic depressor and influence the metabolism of biologically active molecules such as proteins, glycogen, carbohydrates and lipids [27]. In the present study total protein content significantly and gradually decreased in experimental fish gradually along with increase in the pesticide concentration. The depletion in protein content in liver might be due to the damaged or low protein synthesis under the toxic stress condition as reported the earlier workers [28], [29], [30].Besides, special catabolic reactions like proteolysis, formation of lipoproteins to repair the cell and to fulfill energy requirement in cells are also responsible for reduction of protein content [31]. [32].

Glycogen is a polysaccharide and major reserve food and first source of energy for many animals. It plays a significant role in the glucose cycle that can be quickly transferred to meet an unexpected need for glucose [33]. In the present experiment, results clearly indicated that the glycogen content of liver was decreased with increased Methyl parathion concentration. The reduction of liver glycogen content in all the experimental fishes might be due to the utilization of carbohydrates for energy production as a result of toxicant induced hypoxia which has also been reflected in oxygen consumption. Similar reports were observed in common carp *Cyprinus carpio* exposed to sub lethal

concentrations of endosulfon showing a decrease in levels of plasma glucose and slight variation in the serum protein [34]. The depletion in glycogen content may also due to active glycogenolysis and glycolytic pathway to provide excess energy in stress condition [35].

Similar findings were also found in the glycogen content of the liver of *Colisa fasciatus* and *Sarotherodon mossambicus* exposure to various toxicants including Thiodan and Arsenate [36], [37],[38],[39],and [40]. In the present investigation the reduction in liver glycogen might be due to the hypoxic condition created by pesticides during the period of experimentation.

Conclusions: In the present study, we have observed the abnormal behavioral and metabolic consequences in Tilapia in all experimental groups treated with sub lethal concentrations of Parathion. We also found that the sub lethal exposure of the parathion, an organophosphorus pesticide proved to be highly toxic to *Oreochromis mossambicus* and which affected the rate of oxygen consumption, depletion in total proteins and glycogen levels liver. The reduction of liver glycogen content in all the experimental fishes might be due to the utilization of carbohydrates for energy production as a result of toxicant induced hypoxia which has also been reflected in oxygen consumption. The depletion in protein content in liver might be due to the damaged or low protein synthesis under the toxic stress condition or due to special catabolic reactions like proteolysis, formation of lipoproteins to repair the cell and to fulfill energy requirement in cells.

Table.1: Determination of Lethal Concentration $(LC)_{50}$ of Methyl parathion for Tilapia mossambica. (Normal laboratory conditions)

Sr. No.	No. of Animal	Methyl parathion con.ug/L	Mortality				% Mortality
			24h	48h	72h	96h	
1	10	Control	–	–	–	–	–
2	10	500	–	–	–	2	20
3	10	600	–	–	–	3	30
4	10	700	–	–	1	3	40
5	10	800	–	1	1	4	50
6	10	900	–	1	2	4	60
7	10	1000	1	2	2	5	60
8	10	1100	1	1	2	7	70
9	10	1200	1	1	3	9	90
10	10	1500	1	2	3	9	90

Table2. Oxygen consumption (ml of oxygen consumed/gm/hr wet wt. of fish/) of the fish, *O.mossambica* following exposure to different sub lethal concentrations of Methyl parathion.

S.No	Hours of exposure	Control	Sub-Lethal concentrations		
			1/3	1/5	1/10
A	24	1.132+/- 0.1281	1.198+/-0.110	1.298+/-0.110	0.98+/-0.010
B	48	1.914+/-0.1718	0.902+/-0.0558	0.812+/-0.018	0.812+/-0.058
C	72	1.056+/-0.1137	0.850+/-0.0407	0.780+/-0.0407	0.761+/-0.007
D	96	1.158+/-01146	0.751+/-0.072	0.718+/-0.0328	0.708+/-0.028

Table 3: Effect of sub lethal concentration of Methyl parathion on total proteins and total glycogen content in liver of *Tilapia mossambicus*

Hours of exposure	Control(**X ± SD**)		Treated with Sub-Lethal concentrations(**X ± SD**)					
			1/3		1/5		1/10	
	Protein(mg/g)	Glycogen mg/g)	Protein(mg/g)	Glycogen mg/g)	Protein(mg/g)	Glycogen mg/g)	Protein(mg/g)	Glycogen mg/g)
24	151.11	39.18	131.11	38.18	130.2	41.18	141.11	48.08
48	149.57	40.21	130.17	33.14	129.11	31.10	126.14	27.11
72	159.2	40.31	119.12	28.11	115.01	24.2	114.12	20.11
96	148.44	41.21	118.14	22.03	98.14	18.41	89.53	17.81

Results are mean (X ± SD) of 5 observations indicates the standard deviation values and are significant at $P < 0.05$

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