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# ADAPTIVE CHANGES IN RESPIRATORY MOVEMENTS OF AN AIR BREATHING FISH *TILAPIA MOSSAMBICUS* EXPOSED TO ENDOSULFAN

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## ABSTRACT

**The acute toxicity of endosulfan as the freshwater fish *Tilapia mossambicus* has been studied here, The sublethal ($LC_0$) Median ($LC_{50}$) and Lethal ($LC_{100}$) concentration of endosulfan for 120hrs were assessed as 190, 240 and 260 ppm respectively.From the 120 hrs $LC_{50}$ value, the 1/3rd and 1/6th sublethal concentration were calculated as 80ppm and 40ppm respectively, to which the fishes were exposed. The pattern of changes in the respiratory movements of endosulfan exposed *Tilapia mossambicus* were taken to suggest that the fish showed adaptive increase in its frequency of surfacing with a tremendous drop in its opercular movements during first hour of exposure to higher concentration of endosulfan.The reduced opercular movement is also considered adaptive for the fish to prevent itself.**

## INTRODUCTION

Pesticide pollution is known to affect a number of physiological systems of fish. Most organic environmental pollution taken up by organism undergoes extensive biotransformation before being excreted. The rate and extent of biotransformation as well as the chemical nature of the products can vary widely between species and even among individuals within the same species. Most information concerning xenobiotic metabolism has been derived from studies on liver. However, the ability to metabolize xenobiotics is not restricted to the liver. Endosulfan (thiodon - 6-7-8-9-10 -10 - Hexachloro -1, 5, 5a, 6, 9, 9a - hexahydro 6-9 metano 2, 4, 3 benzodioxathiepin -3-oxide) a brown crystalline solid, is soluble in organic solvents and sparingly (20ppm) soluble in water. It is used in agriculture against a wide variety of insects including Lepidopteran larvae, beetles and aphids. It has been especially effective against certain wood and stem bores such as peach tree borer, bronz birch borer and locust borer.Toxicology is the branch of biology dealing with the damages caused to the organism by any toxicant. These toxicants are generally pesticides industrial chemicals heavy metals and even drugs. Among these toxicants, pesticides are undesired highly dangerous pesticides are applied in the agriculture to kill various pests of which insects are the most important. Pesticides directed against the insects are called "Insecticides". Insecticides have been developed to affect specific target mechanisms in the insects. There are several major kinds of insecticides like Organochlorines, Organophosphates, Carbamates, Pyrethrioids, etc., (Satake *et al*. 1997). Like phosphamidan, Dichlorocos, Malathion etc., are acetylcholine esterse (AchE) in-

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hibitors (Sarin and Gill 1998). AchE is an enzyme in the post synaptic of neurons. It inactivates the excess quantum of actylchloline released. During the transmission of nerve impulses across the synapses. If AchE is not present to inactivate the excess actyl choline, the post synaptic will continue to remain in the stimulated state. The consequent disturbance in the neural mechanism is responsible for the death of the insect (O' Brien, 1976).

The technical endosulfan contains isomer $\alpha$ and $\beta$ in a 4:1 ratio, which differ markedly in terms of their persistence and toxicity, the former being at least 20times more toxic than the latter (Devi *et al*. 1981). Although $\alpha$- endosulfan disappear < 100days residues of the $\beta$ isomer persist in the environment for as long as 2 years (Me Ewen and Stephanson, 1979).

Respiration is one of the important parameters on which depend many of the vital functions like growth and reproduction of fish. one of the early symptoms of acute pesticide poisoning is alteration of failure of respiratory metabolism (Holden, 1972). A survey of literature on fish and pesticide pollution clearly indicates that same pesticides increase respiratory metabolism (Davis 1973; Brafield and Mathiessen 1976; Lunn *et al*. 1976, Bakthavathachalam and Srinivasa Reddy, 1983). And some pesticides decrease the o2 uptake fish (Ferguson *et al*. 1966; Lee 1969, Gopalakrishna Reddy and Gomathy 1977; Gopalakrishna Reddy *et al*. 1977).

To supplement the information available on the increase and decrease of oxygen uptake of fish exposed to different pesticides an attempt is made in the present study to examine the changes in the respiratory movements of air breathing fish *Tilapia mossambicus* upon exposure to different concentration of Endosulfan.

## MATERIALS AND METHODS

Fresh water fish Tilapia mossambicus were collected from freshwater pond around Musiri ( Near River Cauvery). They were stocked and acclimated to laboratory conditions by keeping them in rectangular cement tank (36"x36"x18") which was already washed and sterilized with artificial feed and excess of the food and excreta were siphoned out after feeding to prevent fungal growth and contamination of water medium, the tank water was renewed daily. About five days before the commencement of experiments, a suitable number of healthy fishes were transferred and maintained in a small plastic trough. Feeding was stopped two days prior to experimentation to reduce additive effects of the animal's excreta. In the test medium as suggested by Arrora *et al*. (1984). The fishes ranging from 5 - 8cm in length and 7-10grams in weight were used for the experiments.

### Water critieria of the medium

The chlorine free water was used as test water in all ther experinments. The conditions of test water mainted at contast characterisation as recommended by committee on water quality criteria, (Ramakrishnan and Sivakumar,1993) throughout the test were depicted in the following Table -

| Parameter | Values |
|---|---|
| Temperature | 28$^o$C+2$^o$C |
| Salinity | 2.7+1ppt |
| pH | 7.0+1 |
| Dissolved Oxygen | 7.7+Ml/L |
| Free $CO_2$ | 1.5+0.01Mg/L |

### System of treatment

In the present investigation, the commercial grade of endosulfan 35EC were selected and used by diluting it in required volume or any other organic solvents. This is in accordance with the method employed by the farmers who simply dilute the grade with water and apply in paddy fields.

### Acute toxicity studies

Acute toxicity tests were conducted to determine the impact of toxicant on aquatic animals with in a short period of 2 hours, simultaneously the toxicity of pesticides on the test fish *Tilapia mossambicus* , 6, 12, 24 , 48, 72, 96 and 120hours were determined. The $LC_{50}$ is statistical estimate of the concentration of the toxic materials in water that kills 50 percent of the test animals under experimental conditions at specific time intervals (Sprangue, 1973). The value is ideally suited for toxicity studies as it gives a more accetable and reproducible concentration required to affect 50percent of the organisms that any other value (Pickering and Henderson, 1966).

### Range finding test

In the toxicity evaluation studies to avoid delay, expense and to save time and effort, the preliminary exploratory tests were conducted. These tests determine the appropriate range of pesticide concentration which should be covered in the full scale tests solution (Endosulfan) were first prepared over acute range of concentration from 100ppm to 400ppm with an interval of 25ppm (Endosulfan) small plastic trough having 5 liters capacity were used in which 3liters of the endosulfan solution of the different concentrations were taken separately.

**Full scale test for toxicity evaluation**

For assessing the toxicity the concentration of the pesticide in the test solution was selected on the basis of previous tests (preliminary test). About 5 fishes were exposed in 3 liters of solution taken in a plastic trough. To maintain the pesticide concentration and DO level of water and to avoid the accumulation of excreatory wastes during the experimental period, the test solution in the trough was renewed after every 24 hours with least distrubance to the fishes the control experiments were also carried out simultaneously. The rate of mortality was observed at an interval of 1,6,12,24,48,72,96 and 120 hours. The fishes showing no resporatory movement. The responses to tactile stimuli were considered dead and were removed immediately to avoid concentration of the medium. The concentration at which 100 percent mortality was observed with in 120hours was considered as lethal concentration (120hrs, $LC_{100}$) and the concentration having 100percent survival till 120hours was considered as sublethal concentration (120hours, $LC_0$).

**Enumeration of opercular movement**

Control and endosulfan exposed fish were kept in glass jars (containing either pesticide - free tap water for control fish or a particular concentration of endosulfan exposed fish) and acclimated for 10minutes. After 10minutes the number of operculum beat was enumerate for 10minutes using a stopwatch. Enumeration was repeated for 10times and mean value was calculated to get the opercular movement of particular fish. Similarly enumeration of opercular movement was made in control and endosulfan exposed *T.mossambicus* after different periods of exposure opercular movements of the fish was expressed as number of beats per minute. Enumeration of frequency of surfacing After a period of acclimatization (as described above) frequency of surfacing per hour it was also carefully observed and enumerated in control and endosulfan exposed fish for different periods of exposure. Frequencies of surfacing in control endosulfan exposed fish were represented as frequency of surfacing per hour changes in opercular movements and surfacing frequency (either increase or decrease) of endosulfan exposed T.mossambicus from those of control levels were calculated as percentage and tested for their significance.

## RESULTS

Toxic impact of the pesticide endosulfan of *T.mossambicus* was recorded from the mortality value of the experimental animals in relation to different concentration level of the pesticides. The impact of the toxicity of endosulfan to *T.mossambicus* was assessed and presented in the form as sublethal median lethal and lethal concentration. The $LC_{50}$ value of endosulfan to T.mossambicus for 120hrs was found to be 240 ppm as 50% of mortality, But the concentrations of 190 ppm of endosulfan, no mortality was recorded till the end of 120hrs. At the same, at the concentration of 280 ppm of pesticide , all the exposed experiments fishes died showing 100% mortality with in 120hrs, indicating it as the lethal / most toxic to the animal. Thus, it is evident from the present study that the sublethal 0%mortality, 50% median lethal mortality and 100% lethal mortality in T.mossambicus occurred at 190, 240 and 280ppm of endosulfan respectively as show in Table 1.

**Opercular movement**

The opercular movement in terms of number of beats per minute of control and Endosulfan exposed *T. mossambicus* and given in (Table 2) together with percent change in opercular movements of Endosulfan exposed fish from control fish.

*Tilapia mossambicus* a control level of opercular movement of about 36 ± 2 beats per minute, Table 2 clearly indicates that endosulfan caused unequivocal changes in opercular movement of the fish. When exposed to (190ppm). Endosulfan for first 1hr the fish showed a spurt in its opercular movements. However, following prolonged exposure for sublethal concentration 1/3rd and 1/6th of $LC_{50}$ value of endosulfan (80ppm and 40ppm). The fish showed a tremendous reduction in its opercular movement. A progressive inhibition of opercular movement up to 120 hours followed by slight recovery after 96th of endosulfan exposure could be observed in fish exposed to sublethal concentraction, where as under higher concentration the fish showed progressive inhibition even up to 120hrs of exposure (Table 2).

**Frequencey of surfacing**

Table 3 provides surfacing frequency of control and endosulfan exposed Tilapia mossambicus along with the percent changes in frequency of surfacing of endosulfan exposed fish from that of control fish. The patterns of changes in frequency of surfacing of endosulfan exposed *Tilapia mossambicus* were found to be different from those of opercular movements with a frequency of surfacing of about 46 per/10minutes in control fish. Unlike opercular movement, surfacing frequency is formed to be increased first 24hr of exposure in all the concentration of endosulfan studied, when exposed to sublethal,1/3rd and 1/6th concentration of $LC_{50}$ value of endosulfan the fish showed progressive reduction in the frequency of surfacing up to 96hrs of exposure. A progressive inhibition was observed in the frequency of surfacing of fish exposed to sub lethal concentration upto 120

**Table 2.** Opercular movement of control and Endosulfan exposed *Tilapia mossambicus* expressed as number of beat/ minutes values are means of five observations ± SD.

| Concentration of Endosulfan | Exposed for Hours | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 24 | 48 | 72 | 96 | 120 | 144 |
| Sub lethal (190ppm) | 36 ± 0. 2 | 39 ± 0. 1 | 32 ± 0.2 | 28 ± 0. 2 | 23 ± 0. 2 | 30 ± 0. 2 | 31 ± 0. 2 |
| 1/3rd Concentration of $LC_{50}$ (80ppm) | 36 ± 0. 2 | 40 ± 0. | 136 ± 0. 1 | 31 ± 0.1 | 29 ± 0.2 | 30 ± 0.3 | 30 ± 0.5 |
| 1/6th Concentration of $LC_{50}$ (40ppm) | 36 ± 0.2 | 29 ± 0. 1 | 24 ± 0. 1 | 21 ± 0. 1 | 30 ± 0.1 | 29 ± 0.2 | 30 ± 0.2 |

**Table 3.** Frequency surfacing of control and Endosulfan exposed *Tilapia mossambicus* expressed frequency of surfacing per hour values are mean of 5 obsevatins ± standard deviation

| Concentration of Endosulfan | Exposed for Hour | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 24 | 48 | 72 | 96 | 120 | 144 |
| Sub lethal (190ppm) | 46 ± 0.5 | 64 ± 0. 6 | 51 ± 0. 5 | 33 ± 0. 2 | 29 ± 6. 2 | 35 ± 0.2 | 38 ± 0. 1 |
| 1/3rd Concentration of $LC_{50}$ (80ppm) | 46 ± 0.5 | 63 ± 0. 6 | 45 ± 0. 4 | 35 ± 0. 3 | 32 ± 0.2 | 33 ± 0. 2 | 38 ± 0.2 |
| 1/6th Concentration of $LC_{50}$ (40ppm) | 46 ± 0.5 | 64 ± 0.2 | 45 ±0. 4 | 35 ± 0.3 | 32 ± 0. 2 | 33 ± 0.2 | 36 ± 0. 2 |

**Table 1.** Mortality of *Tilapia mossambicus* exposed to different concentrations of Endosulfan at different hours exposure

| Concentration ppm | No. of Fish exposed | 6 hrs | 12 hrs | 24 hrs | 48 hrs | 72 hrs | 96 hrs | 120 hrs |
|---|---|---|---|---|---|---|---|---|
| 170 | 30 | - | - | - | - | - | - | |
| 180 | 30 | - | - | - | - | - | - | - |
| 190 * | 30 | - | - | - | - | - | - | |
| 200 | 30 | | | 2 | 4 | 4 | 6 | 6 |
| 210 | 30 | | 2 | 5 | 5 | 5 | 6 | 6 |
| 220 | 30 | 5 | 5 | 6 | 6 | 8 | 10 | 10 |
| 230 | 30 | 6 | 6 | 8 | 8 | 10 | 13 | 13 |
| 240 ** | 30 | 6 | 8 | 11 | 14 | 14 | 15 | 15 |
| 250 | 30 | 10 | 10 | 16 | 16 | 18 | 18 | 19 |
| 260 | 30 | 10 | 15 | 20 | 22 | 22 | 24 | 24 |
| 270 | 30 | 12 | 15 | 22 | 24 | 26 | 26 | 28 |
| 280 *** | 30 | 15 | 22 | 22 | 24 | 26 | 26 | 30 |

* Sublethal concentration ; ** Median lethal or $LC_{50}$ concentration ; *** Lethal concentration

hrs exposure. A progressive reduction up to 96hrs followed by a slight recovery after 120 hours of exposure could be seen in endosulfan-exposed fis

## DICUSSION

In the present study, the 190, 240 and 280ppm of endosulfan was found as sublethal, median and lethal concentrations for 120hrs. However the fishes were exposed to 80ppm, which is 1/3rd of $LC_{50}$ value of Endosulfan is sublethal to fishes. The same view that the time and concentration dependent mortality has been reported of Agarwal (1971) in Channa punctatus exposed to mercuric chloride, Similarly Maruthanayagam and Sharmila (2004) have reported that the $LC_{50}$ value of monocrotophas to Channa punctatus and also Singh (1984) have reported the $LC_{50}$ value of dimethoate to *Barbus stigma*.

Pesticides are known to after the oxygen uptake of fish. A depression in oxygen consumption to about 50% has been reported in Colisa lalia following exposure to sublethal concentration of a-BHC (Uthaman 1977). Mystus vittatus, when exposed to thiodon showed a 50% decrease in its oxygen consumption (Gopalakrishna Reddy and Gomathy 1977). Gopalakrishna Reddy *et al*. 1977 also reported a similar decrease in oxygen uptake of Colisa lalia exposed to disyston. Sarotuerodan mossambicus, when exposed to thiodon concentration, showed drop in oxygen uptake to about 36-40% (Vasanthi and Ramaswamy, 1987) Natarajan (1981) also studied reduced oxygen uptake. A reduction is haemoglobin content and erythrocyte population resubling in hypochromatic microcytic anaemia have also been suggested as reasons for drop in oxygen uptake in fish exposed to pesticides ( Natarajan 1981; Srivastava *et al*. 1977).

In the present study *Tilapia mossambicus* show unequivoral changes in its opercular movements and surfacing frequency following exposure to Endosulfan concentrations for different periods. The initial increase in opercular movements upto 1hr of exposure to sublethal concentration of 1/3rd and 1/6th concentration indicates that the fish adaptively increased its opercular movement to increase its o2 uptake to meet the pesticide stress. Perhaps the low concentration of endosulofan brings about a triggering mechanism for an increased oxygen uptake. On the other hand, higher concentration of Endosulfan caused inhibition effect on opercular movement even from the first 24hours of exposure. In particular, that drop in opercular movement of fish exposed to higher concentrations of endosulfan and corresponding increase in frequency of surfacing of the fish clearly indicate that the fish adaptively shifts more towards aerial respiration (by obtaining atmospheric oxygen by surfacing) to meet its energy demand. By reducing its opercular movements. The fish perhaps, tries to avoid intimate contact (through the gill opening) with the pesticide.

An increase oxygen uptake, to meet the energy demand during early period of pesticide exposure, inspite of reduced opercular movement, could be possible onl;y air breathing fish, where truly could adaptively employ their extra route of respiratory mechanism (air breathing organs) to increase their oxygen uptake more effectively under pesticide stress, as seen in *Tilapia mossambicus* in the present study.

Progressive decline in opercular movement of fish exposed to endosulfan concentration upto 120hrs and in frequency of surfacing indicates the severe inhibitory effect of the endosulfan on the respiratory movements of the fish. The decreased respiratory movement may be the result of inhibition of enzymes associated with the metabolic activity respiratory movements under endosulfan toxicity.

However, recover of different magnitudes observed in opercular movement following 120hours of exposure to different concentration of endosulfan and in frequency of surfacing following 96hr and 144hr. of exposure indicates the reversible, inhibitory nature of the pesticide endosulfan on the respiratory movements of *T. mossambicus*.

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