ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(2): 545-548
www.allresearchjournal.com
Received: 06-12-2015
Accepted: 08-01-2016

Suvarna Rawal T
Department of Zoology, BNN College (University of Mumbai), Bhiwandi Dist-Thane 421302, Maharashtra, India

Momin Shakir
Department of Zoology, BNN College (University of Mumbai), Bhiwandi Dist-Thane 421302, Maharashtra, India

Correspondence
Momin Shakir
Department of Zoology, BNN College (University of Mumbai), Bhiwandi Dist-Thane 421302, Maharashtra, India

In vivo sublethal toxicity of organochlorine insecticide on glycogen content in the Hepatopancreas and muscle tissues of fresh water fish *Rasbora daniconius* (HAM.)

Suvarna Rawal T, Momin Shakir

Abstract
Changes in the glycogen (polysaccharide) content in Hepatopancreas and muscles of the fresh water fish, *Rasbora daniconius* were studied. When subjected to increasing concentration of endosulfan (organochlorine insecticide) and the time period of exposure to different sublethal concentrations, a decrease in glycogen content was observed in both the tissues which indicates the possible arrival of pesticidal toxicity.

Keywords: Glycogen, liver (Hepatopancreas), muscle, Endosulfan, *Rasbora daniconius*

Introduction
Pesticides are the substances used to control pest including insect, weeds and plant diseases. Pesticides are defined under the Federal insecticide, fungicide and Rodenticide Act as, any substance or mixture of substances intended for preventing, repelling or mitigating any pest, (insect, rodent, nematode, fungus, weed, other forms of terrestrial or aquatic plant or animal life or viruses, bacteria or other micro-organism or in living man or other animals, which the administrator declares to be a pest) and any substances or mixture of substances intended for use as a plant regulator, defoliator or desiccant (Hayes and Laws,1991) [4]

Pesticides enter into the hydrosphere via many pathways including direct application for pest, and disease vector control, urban and industrial waste-water discharge, surface run-off from non-point sources including agricultural soil, aerosol and particulate deposition, rainfall and adsorption from the vapour phase at the air-water interface etc. (Sharma, 1991) [12]. The different aquatic environment like rivers, lakes, river streams, estuaries and even ocean may thus be affected by this deliberate or accidental application of pesticides either by dilution, concentration and degradation. The excess use of pesticides have been found to cause toxic effects to aquatic animal like fishes, crabs, etc. which are economically important as food for human consumption and these pesticides creates ecological and physiological imbalance. These pesticides produce toxicity for a wide range of animals. The effects of pesticide on animals like fresh water fishes is an important aspect of pollution and the information available on the physiological effects of exposure to different pollutant is scanty.

An insecticide, Endosulfan have been commonly used to control pest of fruits, vegetables and ornamental plants in Bhiwandi, has been used for toxicity studies. Endosulfan was developed by Hoechst pharmaceutical and introduced in 1956.It is an organochlorine insecticide. It is readily absorbed by insect cuticle and affects the Ganglia of CNS leading to hypersensitivity, hyperactivity with violent burst of convulsion and finally complete prostration with convulsive movement.

India is currently the largest manufacturer of pesticides in Asia, next only to Japan. More than 60 technical grades of pesticides are manufactured indigenously. In agrochemical, we manufacture significant quantities of synthetic pyrethroid such as fenvalerate and cypermethrin, endosulfan an organophosphate range of organochemical, including monocrotophos. India is also a dominant producer of isoproton, a weedicide accounting for nearly 25% of the worldwide production.

The purpose of study is to investigate the changes in liver and muscle biochemistry of *Rasbora daniconius* by the influence of endosulfan. Extensive and indiscriminate use of pesticide may have toxic effects on a wide range of non-target organism including desirable plants and animals. Fish and other wildlife species has been the victim of pesticide poisoning.

Materials and methods

Fresh water fish, *Rasbora daniconius* have been selected as a model species for the present investigation. The active and healthy specimen of fishes were collected from Diwanshah Lake, Bhiwandi (Maharashtra) with the help of local fisherman's and brought to laboratory. They were examined for any pathological symptoms and washed with 0.1% $KMnO_4$ solution to avoid any fungal infection. The fishes were acclimatized to the laboratory condition for about a month before being used for experiment. Fishes were regularly fed with dried tubifex worms and chironomus larvae. Feeding was stopped two days before being used for experiments. Chlorine free tap-water was used throughout the course of the experiment. Various physicochemical characteristics of test water such as Temperature, pH, total acidity, total alkalinity and total hardness were regularly reported and listed in table no.1

Table 1: Physiochemical parameters of test water

Temperature	27 ^{0}C
P^H	7.3
Do	5.9mg/litre
Free chlorine	Nil
Total acidity	3.5mg/litre
Total alkalinity	44mg/litre
Total hardness {as $CaCO_3$}	31mg/litre

After a general selection for healthy and same group of fishes (4.2±0.5) cm. and (0.850±0.5) in weight, they were transferred to glass aquaria containing 1:4 mixture of lake water and dechlorinated tape-water. After acclimatization, the healthy fishes were selected for experimental purpose without sex-discrimination. 10 such fishes were transferred to glass aquaria of 50 litres capacity, each aquarium containing 20 litres of dechlorinated tape-water.

For selecting sub-lethal concentration of the toxicant to which fishes could be exposed, earlier information of acute toxicity data was made use of. This reveals that the toxicity of pesticide i.e. endosulfan does not increase with the time of exposure. Therefore the toxicant doses selected were close to their 96hrs. LC_{50} values. LC_{50} values of endosulfan to *Rasbora daniconius* was found to be 0.070 mg/litre. 1/3rd, 1/5th and 1/10th of 96hrs. LC_{50} values were selected for sub-lethal concentration of endosulfan listed in table no.2

Table 2: Selected sub-lethal concentration of endosulfan for toxicological studies (mg/litre)

Toxicant	Concentrations		
Endosulfan	0.007(1/10th)	0.014(1/5th)	0.023(1/3rd)

To maintain these concentrations constant throughout the experimental period, and to avoid the accumulation of metabolic waste, the entire water was changed every alternate day.

The *Rasbora daniconius* were sacrificed carefully and the liver and muscles was taken out for biochemical estimation of glycogen. Weighed tissues (liver and muscles 100mg taken in pool from both the control and treated fish) were digested to 5 ml of 30% KOH solution. This was diluted to 20 ml with distilled water. The diluted solution was used for the assay of glycogen content. The estimation was carried out according to the Anthron method as describe by Siefer *et al.* (1950) [11].

Result and discussion

Endosulfan exposed tissues of *Rasbora daniconius* show glycogen content of mg/gm wet weight for muscle and liver as cited in table no: 3 and 4.

Table 3: Glycogen content in the liver of fish *Rasbora daniconius* when exposed to sublethal doses of endosulfan for a period of 4 weeks.

Parameter	Exposure in days	Control	Concentration of endosulfan in mg/litre		
Glycogen mg/gm/wet/weight of tissue			0.007	0.014	0.023
	7 Days	60.8725	60.2075	59.1915*	59.9795*
SD		± 1.0115	±1.00052	±1.0145	±1.0105
PV			(1.092%)	(2.762%)	(1.467%)
	14 days	60.9271	58.5259**	57.0809***	56.9595***
SD		±1.012	±1.0115	±0.0105	±1.0115
PV			(3.941%)	(6.313%)	(6.512%)
	21 DAYS	59.7259	56.9295**	55.9892***	55.2109***
SD		±1.011	±1.012	±1.017	±1.05631
PV			(4.682%)	(6.256%)	(7.56%)
	28 days	58.8271	54.0809***	53.1090***	51.7257***
SD		±1.011	±1.0115	±1.0145	±1.0105
PV			(8.068%)	(9.27%)	(12.072%)

The values in bracket= Percentage variation (PV)
The values in ± = Standard deviation (SD)
>0.05 not significant
0.01 to 0.05 significant*
0.001 to 0.01 very significant**
< 0.001 extremely significant***

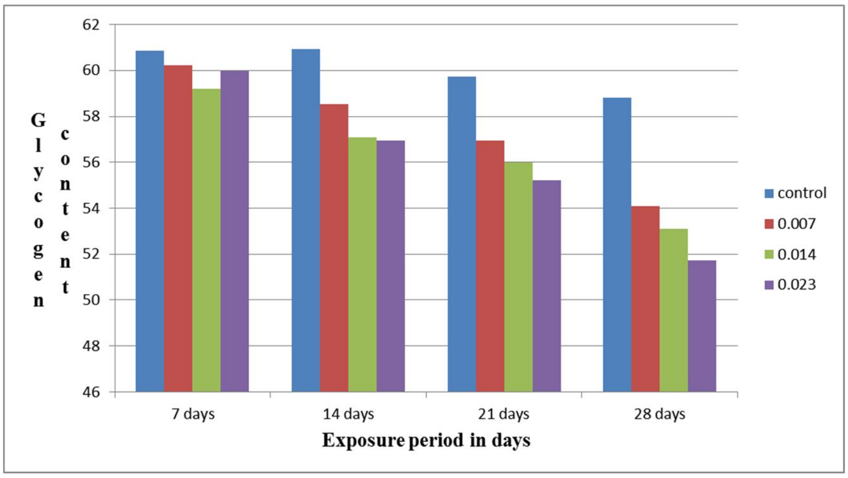

Fig 1: Glycogen content in the liver of fish *Rasbora daniconius* when expose to sub-lethal doses of endosulfan for 4 weeks.

Table 4: Glycogen content in the muscle of fish *Rasbora daniconius* when exposed to sub-lethal doses of endosulfan for a period of 4 weeks.

Parameter	Exposure in days	Control	Concentration of endosulfan in mg/litre		
Glycogen mg/gm/wet/weight of tissue.			0.007	0.014	0.023
	7 days	80.2875	71.0285***	70.7280***	68.7965***
SD		±1.011	±1.012	±1.0125	±1.014
PV			(11.532%)	(11.905%)	(14.312%)
	14 days	80.7920	71.9720***	69.2760***	67.0859***
SD		±1.016	±1.0105	±1.0105	±1.01
PV			(10.917%)	(14.254%)	(16.965%)
	21 days	81.2015	70.2701***	68.7929***	66.1509***
SD		±1.0125	±1.0105	±1.0155	±1.01
PV			(13.462%)	(15.281%)	(18.535%)
	28 days	81.9725	69.7202***	69.5081***	68.2979***
SD		±1.011	±1.0105	±1.014	±1.012
PV			(14.947%)	(15.206%)	(16.682%)

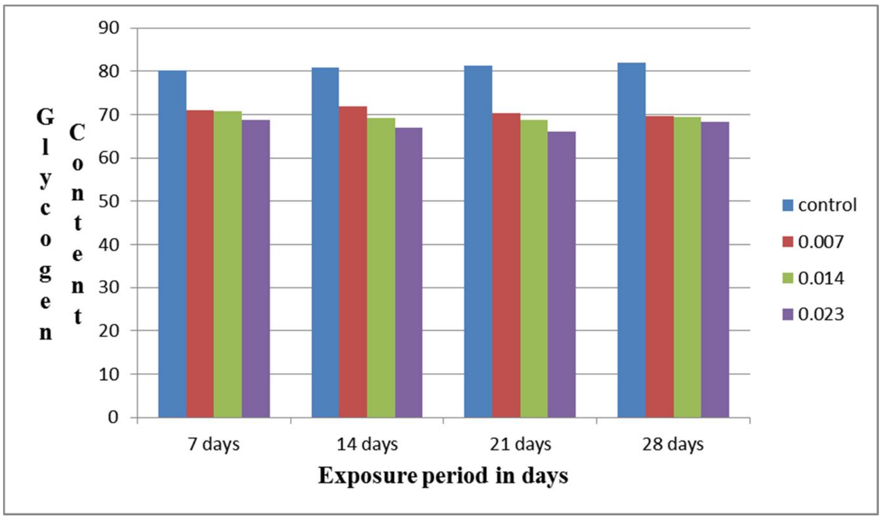

Fig 2: Glycogen content in the muscle of fish *Rasbora daniconius* when exposed to sub-lethal doses of endosulfan for a period of 4 weeks.

Table no. 3 & 4 shows that level of glycogen decreased in the muscle and liver when exposed to endosulfan.

Glycogen is the principle and immediate source of energy. Dange *et.al.* (1984) [1] suggested that the reduction in the glycogen content may be due to rapid breakdown to release glucose in to the circulatory system to meet the energy requirement. Gaikwad (1981) [2] reported that decrease in the glycogen content observed in *Tilapia mosambica* chronically exposed to Thiodon, may be due to tremendous increase in energy demand. Soman (1987) [13] & Gopi (1992) [3] observed decline in the glycogen content of the liver & muscle of the fish *Colisa fasciata* & *Cyprinus carpio* respectively. Ramalingam (1986) [7] studied the effect of DDT and Malathion on the carbohydrate metabolism of the *Sarotherodon sp.* He has suggested that a possible shift from aerobic to anaerobic metabolism, in which sugar were converted in to lactate via Pyruvate. Swaminathan *et.al.* (1990) [14] observed decline in tissue glycogen content of the liver and muscle and related it to the hypoxia condition under which stored glycogen might have been utilized by the fish *Tilapia mossambica* exposed to Thiodon. Mukhopadhyaya and Dehadrai (1980) [6] suggested that the increased glycogenolysis decreases glycogen content in the liver of *Clarius batrachus* exposed to Malathion. This view has been suggested by otherworkers. Reddy & Yellumma (1991) [9] monitored perturbation in the carbohydrate metabolism during Cypermethrin toxicity in *Tilapia mossambica*. They observed decline in the glycogen content of the tissues which had been related to the enhanced oxidation through HMP-pathway.

Ravindra Kumar (2000) [8] suggested the reduction in the carbohydrate content in all tissues may be due to rapid depletion of stored glycogen to provide energy for *Mystus gulio* under stress. Decline in the body muscle glycogen caused severe anaerobic stress resulting in the breakdown of tissue glycogen (McLeay & Brown, 1979) [5].

Tripathi *et.al*. (2003) [15] pointed out depletion of glycogen may be due to direct utilization of energy generation, a demand caused by pyretheroid induced hypoxia. Sastry *et.al.* (1984) [10] suggested in stress condition carbohydrate reserve depleted to meet energy demand. The decrease in the glycogen and glucose suggested the possibility of active glycogenolysis and operation of glycolytic pathway as reported.

Conclusion

The decrease in the glycogen content in the liver & muscle in the present studies observed in *Rasbora daniconius* treated with endosulfan, may be due to glycogenolysis in order to meet energy demand under the pesticide stress as per the view expressed by other workers.

References

1. Dange AD, Masurekar VB. Effect of Naphthalene exposure on activity of some enzyme in Chichlid fish Tilapia mossambica (Peters). Indian journal of exp. Biology. 1984; 2:16-24.
2. Gaikwad SA. Toxicity studies with Thiodon 35 EC & Phenylmercuric Acetate on Tilapia mossambica. Ph.d. thesis university of Mumbai, 1981.
3. Gopi. Chronic toxic effects of Fenthion– the organophosphate insecticide to common fresh water Carp, Cyprinus carpio (Linn.). Ph.D. Thesis, University of Bombay, 1992.
4. Hayes WJ, Laws ER. A handbook of pesticide toxicology Academic press, 1991, I, II, III.
5. Mcleay DJ, Brown DA. Growth stimulation & biochemical changes in juvenile Coho salman exposed to kraft pulpmill effluent for 200 days. J fish res Bd. Can. 1979; 31:1043-1049.
6. Mukhopadhyaya PK, Dehadrai PV. Studies in air-breathing cat-fish Clarius batrachus under sublethal Malathion exposure. Indian journal of experimental biology. 1980; 18:348-382.
7. Ramalingam Effect of DDT & Malathion on tissue SDH & LDH isoenzyme in Sarotherodon mossambica. Proc. Indian Acad. Sci. 1986; 19(4):303-309.
8. Ravindra Kumar. Effect of DDT exposure on physical parameter of the body & tissue cation of the fish Mystus gulio. Environ. biol. 2000; 5:651-654.
9. Reddy, Yellumma S. Toxicity of Cypermethrin in *Tilapia mossambica.* J Environ. Biol. 1991; 5(2):25-30.
10. Sastry KV, Siddique AA. Some haematological, biochemical & enzymological parameters of a fresh water teleost fish Channa punctatus exposed to sub-lethal concentration of Quinalphos pesticide. Biochem. Physiol. 1984; 22:8-13.
11. Seifers S, Dayto Naic B. The estimation of glycogen with Anthrone reagent. Arch. Biochem. Biophys 1950; 25:191.
12. Sharma RK, Shashi Sharma, Smita Shandilya. Effect of Sumithion on mortality rate of Clarius batrachus. Geobios 1991; 8:237-239.
13. Soman. Toxicity of Fenthion, Thiodon, DDT, malathion & Nuvan to the fish colisa fasciata. Enviromental biology &Toxicology, Rastogi & company, meerut, India, 1987, 09-15.
14. Swaminathan MS. Indian agriculture next phase, pesticide. 1990; xxiv(1):17-21.
15. Tripathi G, Harsh. Fenvelerate induced macromolecular changes in cat-fish Clarius batrachus. J Environ biol. 2003; 23:143-146.