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Effect of Methoxychlor on biochemical status of tissues in Channa punctatus (Bloch, 1793)

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Abstract
Effect of the organochlorine pesticide Methoxychlor (MXC) on biochemical composition of tissues of the snake head fish Channa punctatus adults was analyzed. Parameters like total protein, glycogen, total lipids in muscle & liver were determined. Additionally, oxygen consumption of the selected fish was recorded. The fish were exposed to different sub-lethal concentrations of Methoxychlor for a period of 72 hrs & the said biochemical parameters estimated at the end of the period. The 96 hr LC50 of Methoxychlor in Channa punctatus is 71 µg/L. At the selected concentrations of the pesticide, the fish suffered alteration in locomotary behaviour. There was a slight loss of coordination. Opercular ventilation increased as also the frequency of surfacing for gulping air. Muscle & liver protein showed significant decrease. Liver glycogen declined markedly. Muscle glycogen also decreased but marginally. There was a significant increase in consumption of dissolved oxygen.
Keywords: Methoxychlor, Channa punctatus, Toxicity, Pesticide, liver glycogen, muscle glycogen.

Introduction
In India, different pesticides have been used since about 1850 but before 1940s, they were used in relatively small amounts. In the recent past, a great deal of research has been done to evaluate the hazardous effects of organochlorine pesticides on animals. Pesticides are being used on large scale the world. Pesticide concentrations may sometimes reach lethal levels causing fish mortality, which has harmful effect on humans when consumed. Methoxychlor is used as a insecticide to protect food crops and control disease vectors, locusts, and termites (Duffard et al., 1996). Methoxychlor [1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane] is a chlorinated hydrocarbon pesticide structurally similar to dichlorodiphenyltrichloroethane (DDT). MXC is lipophilic, only slightly soluble in water and is poorly volatile. It binds rather tightly to soil. It has a short environmental half-life.

Material and Methods
Experimental fish
Live adult Channa punctatus specimens were procured from local sources. The fishes were acclimatized to the laboratory conditions for one week. Regular & proper feeding was given and change of fresh water was done regularly. Beef liver and poultry waste was fed to the fish during acclimatization. Water was changed every alternate day to remove wastes. The experimental fish were 15 cm long and weighed 50 gm. The test pesticide used in the present study was Methoxychlor, obtained from Sigma-Aldrich.

Exposure to pesticide
The effect of Methoxychlor at three different sub-lethal doses, 17.75 µg/L, 35.5 µg/L & 53.25 µg/L (i.e. 25%, 50% and 75% of 96 Hr. LC50), were studied on liver and muscle glycogen in full grown specimens of *Channa*

punctatus. The fish were starved for 48 hrs prior to exposure. Two sets of fish containing 8 fish each were exposed to Methoxychlor for a period of 96 hours. After completion of exposure period, the experimental fish were sacrificed and liver and muscle tissue samples taken out for glycogen estimation. A control set was also maintained using the same number of fish and water from the same source. The fish were not fed during the exposure period. The aquarium water was aerated continuously.

Determination of biochemical parameters

The experiment was conducted in glass aquaria (75 × 30 × 30 cm) containing 35L of dechlorinated water. The water with the pesticide was changed after every 24 hrs to maintain concentration of pesticide. Assessment of water quality parameters was done as per the Standard Methods (APHA, AWWA, & WEF, 2005). The glycogen content from liver and muscle is biochemically estimated by Anthrone method.

Result & Discussion

At the selected concentrations of Methoxychlor, the fish showed altered state of equilibrium and balance. There was a slight loss of coordination. Opercular ventilation increased as also the frequency of surfacing for gulping air.

Table 2: Tissue glycogen under after the influence of Methoxychlor (mg/gm wet wt).

Parameter	Control	25% LC50	50% LC50	75% LC50
Liver glycogen	2.73±0.14	2.3±0.21	2.01±0.13	1.80±0.14
Muscle glycogen	0.95±0.037	0.83±0.020	0.72±0.019	0.66±0.016

(All values are *mean*±*SD*, n=16)

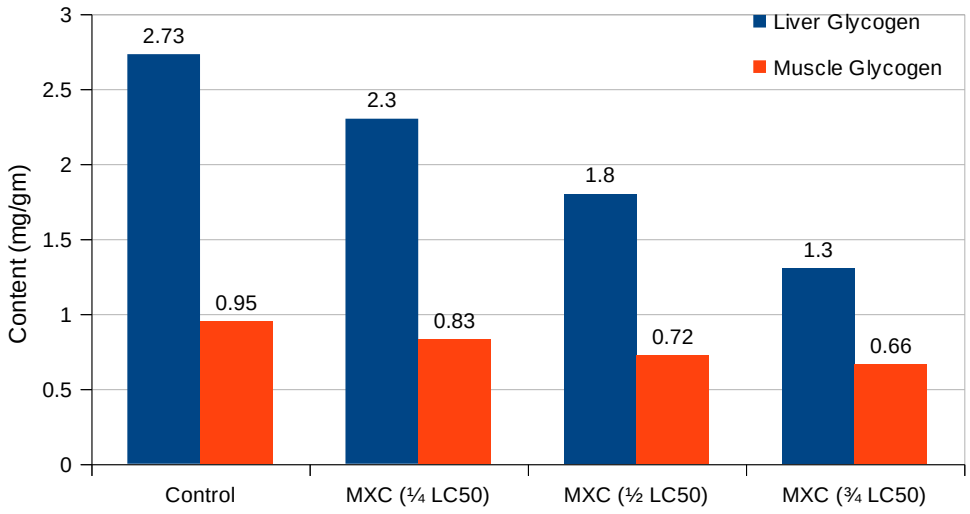

Fig. 1- Effect of Methoxychlor exposure on tissue glycogen content in *Channa punctatus.*

Homeostasis of metabolites in an organism is tightly controlled by hormones and keeps fluctuating with different degrees of activity. Toxicant stress is reported to influence serum levels of cortisol in the rainbow trout, *Onchorhynchus mykiss* (Aldegunde et al., 1999). There is a reduction of glycogen in *Channa punctatus* probably due to repression of glucokinase activity under the effect of parathion. The histological damage to hepatocytes and disappearance of glucose-6-phosphate by pesticide (Dursban) may be a possible cause for decrease in glycogen content of *Channa punctatus* (Jaroli et al., 2005). The present study saw observations similar to work of other investigators. Glycogen content in the experimental fish liver also declined under exposure to Methoxychlor from 2.73±0.075 mg/g in the normal to 2.3±0.058, 1.8±0.025 and 1.3±0.043 mg/g in fish exposed to 25%LC50, 50%LC50 and 75% LC50 concentrations respectively. Similarly, muscle glycogen was affected under experimental conditions; it changed from 0.95±0.08 mg/g in normal to 0.83±0.020, 0.72±0.019 and 0.66±0.016 mg/g when exposed to above concentrations of Methoxychlor. A similar observation was reported in

Channa punctatus under influence of zinc intoxication; liver glycogen content declined after an exposure period of 10 days and onward (Srivastava et al., 2009). Liver glycogen in *Channa punctatus* decreases under influence of sub-lethal and lethal concentrations of Alachlor; glycogen content reported at 35.06 mg/g and 30.26 mg/g after exposure to Alachlor (Tilak et al., 2009). Liver and muscle glycogen decreased steadily with the period of exposure to Endosulfan, probably indicating at impaired glycogen synthesis and utilization of their reserve glycogen for energy needs (Sastry et al., 1983). Insecticides can cause serious impairment to physiological and health status of fish. Therefore, biochemical tests are routine laboratory tests useful in recognizing acute or chronic toxicity of insecticides (Banaee, 2012).

Physico-chemical characteristics of the test water

The physico-chemical characteristics of the test water are presented in Table 1. The dissolved oxygen content was between 5.80 to 7.95 mg/L. The temperature was noted to be from 22.60 to 27.50ºC, *p*H 7.2 to 8.1; salinity ranged from 0.13 to 0.17 mg/L, while total hardness varied from 195 to 226 mg/L $CaCO_3$ during the experimental period.

Table 1: *Physico-chemical parameters of water.*

Parameter	**Range**
Temperature (ºC)	22.60 - 27.50
Dissolved oxygen (mg/L)	5.80 to 7.95
Total hardness (mg/L)	195 to 226
*p*H	7.2 - 8.1

Conclusion

Glycogen content in fish exposed to different concentrations of Methoxychlor show significant decrease compared to control set, this change may be due to its breakdown to glucose under the pesticide toxicity. A similar result was obtained in Channa punctatus under the influence of MalathionEffect of aldrin on peripheral blood of fish Channa punctatus (Bloch).

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