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Chronic toxicity studies on proximate composition of *Cyprinus carpio* exposed to fenthion

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ABSTRACT
The present study indicates proximate composition, including protein, carbohydrates, moisture, LSI and lipids contents of fresh water fish Cyprinus carpio chronically exposed to fenthion. In the present study the significant decrease in glycogen, protein, and increase in lipid and moisture content could be observed. Significant drop in LSI observed in Fenthion treated *C. carpio* clearly indicates fall in nutritional value or quality of food.

Keywords: Fenthion, Cyprinus carpio, glycogen, protein, lipid

1. Introduction
All living organisms need a regular supply of energy for their survival, which is obtained from surrounding sources. Food is an important source of energy used for building up the body tissue which further signifies that complete and balanced diet is necessary for the proper functioning of the body. The estimation of the rate of metabolism in a vertebrate animal has been considered as the most sensitive parameter of pollution stress since it affects many other factors such as enzyme activities, biochemical activities and other physiological processes. Keeping this view in mind it was thought to be very necessary to carry out the proximate composition study i.e. determining the proportions of carbohydrates, proteins and fats present in test tissues of the organism using standard procedure. This method helps to find out whether the insecticide, Fenthion reduces the nutritional quality of the test fish *C. carpio* which is a very good source of food. It has been shown that pesticides alter the physiological and biochemical processes in many aquatic forms [1-11].

The information on biochemical alteration caused by Fenthion toxicity in fishes in general and on *Cyprinus carpio* in particular is not known. With this view in mind present investigation has been conducted to study the effect of sublethal concentrations of Fenthion on proximate composition which includes glycogen, protein and lipid content in the tissues of test fish. Liver somatic index changes of *C. carpio* exposed to Fenthion was also studied.

2. Materials and Methods
The fishes were brought from the Arey fish farm in Mumbai to the laboratory. At the end of successful acclimatization healthy looking fishes of approximately the same size (13 cm.) and 18-20 gm weight were selected as test animals [2]. The fish selected for the test were exposed to three different sublethal concentrations of Fenthion. In order to maintain the concentration of toxicants throughout the period of experiment and to avoid the accumulation of metabolic wastes, entire water was replaced by fresh aged tap water every alternate day. The water analysis for determining pH, acidity, alkalinity, hardness, dissolved oxygen was carried out regularly twice a week following the standard methods described in [2]. The average values of all these parameters are presented in table. Healthy looking fish selected were divided into groups of eight each and were exposed to 0.38, 0.193 and 0.096 mg/l Fenthion for a period of 60 days. A control group was also maintained in duplicate for comparison. For estimation of different metabolites the control and exposed fishes were sacrificed at the end of the 60 days period. The muscle and liver tissues were immediately removed for estimations of glycogen,

protein, total lipids, moisture contents and Liver somatic index.

The glycogen content of liver and muscle tissues was estimated by Anthrone method as described by [29]. The optical density was read at 625 mμ on spectronic-20 (Baush & Lomb model No. 33-31-72). The method suggested by [15] was opted for the determination of soluble protein contents in liver and muscle tissues. The optical density was read at 500 mμ on spectronic-20. The total lipid content of dry liver and muscle tissue samples was estimated gravimetrically following the method of [6]. Moisture was determined according to the method given on [1]. A known weighed quantity of muscle and liver at tissues was dried in an oven at 90 °C to 95 °C for 24 to 36 hours. The dried tissue was weighed again. The moisture content was calculated from the loss in weigh.

Liver somatic index was calculated from the following formula:

3. Results and Discussion

The results of proximate composition of different experimental groups of fish *C. Carpio* exposed to three different sub lethal concentrations of Fenthion for period upto 60 days are given in table 1and 2. From comparison of the results it is evident that *C. carpio* exposed to 0.38, 0.193 and 0.096 mg/l Fenthion showed significant reduction in glycogen and protein contents of muscle and liver tissues. The LSI values decreased in the liver tissue with respect to the concentration of Fenthion exposed. Lipid & moisture contents increased with all concentrations. It can also be seen that the increasing value of lipid & moisture was proportional to the concentration of Fenthion and exposure period.

Table 1: Changes in Glycogen, Protein, Lipid and Moisture (Mg/Gm Wet Weight) Of Muscle in *Cyprinus Carpio* Chronically Exposed To Three Different Sublethal Concentration of Fenthion

Content	Control	0.096 mg/l	0.193 mg/l	0.38 mg/l
Glycogen	60.63 ±2.23	59.123 ±1.20 -2.48%	58.02 ±1.0 - 4.30%	53.84* ±1.3 -11.19%
Protein	118.3 ±1.2	110.49 ±3.7 -6.60%	109.93 ±1.6 -7.0	104.5 ±1.23 -11.66%
Lipid	29.8 ±1.9	31.6 ±1.0 6.04%	36.4 ±2.0 22.14%	39.1** ±2.2 31.2%
Moisture	62.93 ±1.4	64.32 ±1.5 2.20%	67.47 ±1.28 7.21%	71.44 ±1.1 13.52%

± = Standard deviation for 5 determinations in %

* = P<0.05

% = percent change from control after 60 days exposure to Fenthion

Fresh water characteristics (Average values)

Dissolved oxygen: 6.9 ±0.2, pH = 7.8 ±0.5

Carbondioxide = 0.3 ±0.2, Temperature = 29 ± 1°

Acidity in ppm = 5.6, A1ka1inity in ppm = 45.9

Total hardness (CaCO3) in ppm = 31.0

Glycogen represents a principal and immediate source of energy. From table 1 and 2, it can be observed that chronic exposure of Fenthion significantly reduced the glycogen content in both the muscle and liver tissues of *C. carpio.* [11] Reported reduced glycogen content in pelecypod *L. marginalis* exposed to Malathion. [13] Also reported that muscle and liver glycogen contents reduced in *H. fossils* when exposed to Malathion. According to [5] reduction in glycogen content is due to its rapid break down to release glucose into circulatory system to meet the energy requirement. This report can be supported with the increase in blood glucose level noted Table 4, and perhaps this could also be one of the reasons for depression in glycogen content observed in *C. carpio* in the present investigation. [6] Reported that decrease in glycogen content observed in chronically exposed *T. mossambica* to Thiodan is due to tremendous increase in demand of energy. [4] suggested that increased glycogenolysis decreased glycogen content in liver of *Channa punctatus* exposed to Malathion. [21] Noted that the exposure of fresh water fish *H. fossils* to concentration 0.247 mg/1 of Chlordane induced muscle and hepatic glycogenolysis and glycogenesis occurred at 2 and 12 hours exposure period. According to [31] glycogen content decreased with increasing glycogenolysis in *C. fasciatum* when exposed to Lebaycid 1000. [17] reported that g1ycogen content of liver tissue of *Barbus Stigma* was reduced from 50 to 45 mg/g in 0.001 mg/l, 43.5 in 0.002 mg/1 and 40 in 0.003 mg/l of Endosulfan. [28] Observed similar reduction in glycogen contents of the liver and muscle tissues of fish

Channna punctatus chronically exposed to Endosulfan. It is zpossible to say that in the present study, observed reduction in size of the liver of *C. carpio*, might have affected its capacity to store glycogen. Further the depletion in glycogen level noted in the present investigation could also be attributed to increased glycogenolysis to compensate the energy demand table 1, 2, & 4.

Table 2: Changes In Glycogen, Prutein, Lipid And Moisture (Mg/Gm Wet Weight) Of Liver In *Cyprinus Carpio* When Chronically Exposed To Three Different Sublethal Concentrations Of Fenthion.

Content	Control	0.096 mg/l	0.193 mg/l	0.38 mg/l
Glycogen	12.25 ±1.0	11.315 ±1.62 -7.63%	9.638 ±2.2 -21.3%	7.64* ±1.25 -37.6%
Protein	148.3 ±2.1	140.8 ±3.5 -5.0%	134.8 ±2.2 -9.10%	129.7* ±7.4 -12.3%
Lipid	18.4 ±1.2	20.3 ±1.7 +10.3%	22.5* ±1.9 +22.2%	26.2 ±2.0 +42.3%
Moisture	90.110 ±1.23	98.34 ±1.4 +9.13%	103.92 ±1.50 +15.3%	117.13 ±1.12 +29.9%

± = standard deviation for 5 determinations in %

* = P < 0.05.

% = Percentage change from control after 60 days exposure to Fenthion

Fresh water characteristics (Average values)

Dissolved oxygen: 6.9 ±0.2, pH = 7.8 ±0.5

Carbondioxide = 0.3 ±0.2, Temperature = 29 ± 1°

Acidity in ppm = 5.6, A1ka1inity in ppm = 45.9
Total hardness (CaCO3) in ppm = 31.0

Table 3: Changes in Liver somatic Index Value in *Cyprinus Carpio* When Chronically Exposed to Fenthion

Content	Control	0.096 mg/l	0.193 mg/l	0.38 mg/l
Liver Somatic index (LSI)	1.93 ±0.05	1.70 ±0.03	1.15 ±0.03	0.89 ±0.01

From table 1 and 2 it can be revealed that protein contents in both muscle and liver tissues in chronically exposed *C. carpio* (0.38, 0.193 and 0.096 mg/l of Fenthion) decreased significantly during 60 days exposure period. The percentage of inhibition was more in liver as compared to muscle. Similar reduction in protein content was reported in *T. mossambica* exposed to Endosulfan chronically by [32]. According to [7, 31] protein contents in muscle and liver tissues depleted in fresh water fishes exposed chronically to different insecticides. [26] Reported that liver exhibited maximum inhibition than muscle of *T. mossambica* when exposed to Dichlorvos [20, 27, 30] noted depletion in tissue protein in different species of fish exposed to various pesticides. According to [34] protein level decreased to 10% in liver of catfish exposed to Aldrin. Tissue total protein is an energy source for fishes during stress, spawning and muscular exercise as reported by [14]. [17] Observed drop in protein level from 250 mg/g to 233, 221 and 180 mg/g in 0.001, 0.002 and 0.003 mg/l respectively in Endosulfan treated *Barbus Stigma*. According to [25] decline in muscle and liver proteins in *Sarotherodon mossambica* chronically exposed to DDT. Malathion, and Mercury was due to intensive tissue proteolysis. [31] reported that depletion in protein content was due to histopathological changes in tissues of fresh water fish *C. fasciata* when exposed to Lebaycid chronically. Studies conducted by [3, 19, 12, 27] reveal that marked variation in activity of enzymes involved in transamination in fishes may be the cause of protein depletion. Similar changes in transaminase enzymes are observed in the present investigation Thus, in the present study decline in protein levels could be related to energy demand leading to intensive proteolysis and also due to histopathological changes fig 1, 2 and 3.

Table 4: Changes in chemical constituents of blood in fish *Cyprinus carpio* when exposed chronically to fenthion for 60 days.

Chemical	Control (Acetone+water)	0.096 mg/l	0.193 mg/l	0.38 mg/l
Glucose mg/100 ml	62.6 ±0.8	78.8 ±0.9 25.8%	86.5 ±0.4 38.1%	98.5 ±0.2 57.3%
Protein mg/ml	45.9 ±0.6	44.4 ±0.2 -3.26%	40.4 ±0.24 -12.80%	33.0 ±0.35 -28.10%
Lactic acid mg/100 ml	26.4 ±0.5	27.8 ±0.3 5.30%	30.6 ±0.2 15.90%	35.6 ±0.9 34.84%
Haemoglobin gm/100 ml	10.7 ±0.1	9.95 ±0.8 -7.0%	8.6* ±0.2 -19.62%	7.7 ±0.8 -28.0%
Clotting time Sec.	120.0 ±0.3	111.6 ±0.0 -7.0	108.7 ±0.6 -9.41%	80.6** ±0.3 -32.8%

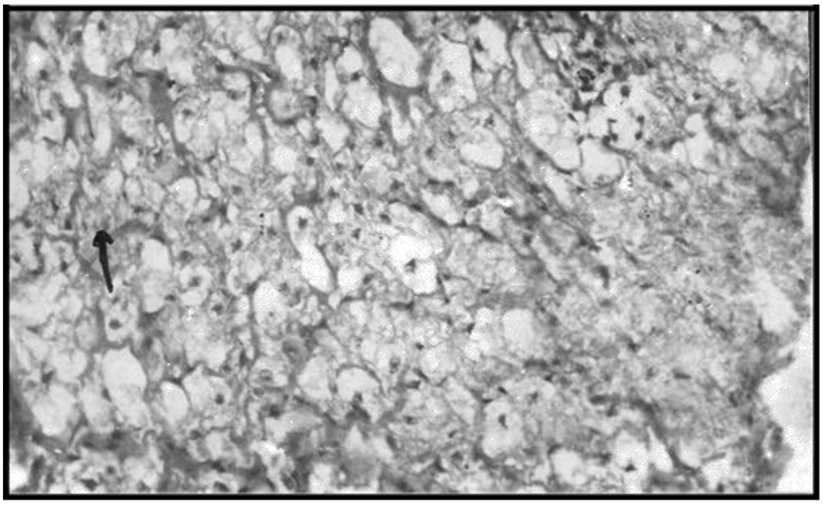

Fig 1: Liver of fish exposed to 0.38 mg/ml fenthion showing vacuolated, cloudy swollen, disintegrated and extremely ruptured hepatic cells.

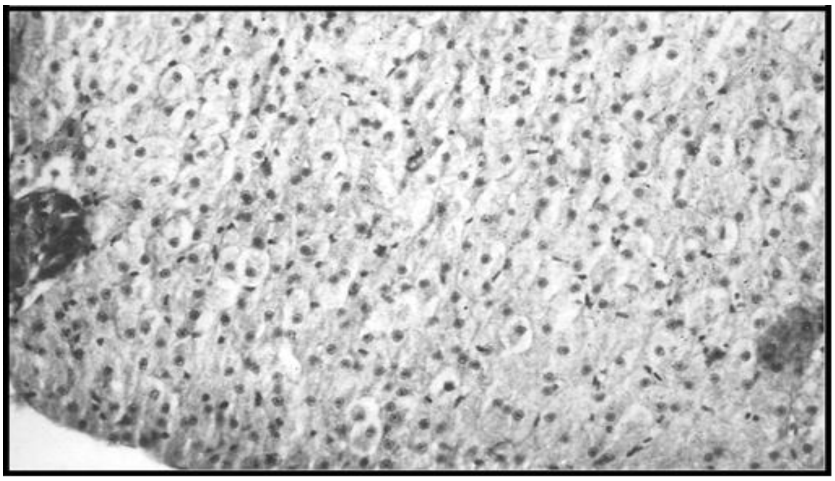

Fig 2: Liver of fish exposed to 0.096 mg/ml showing pycnosis and large number of necrotic regions.

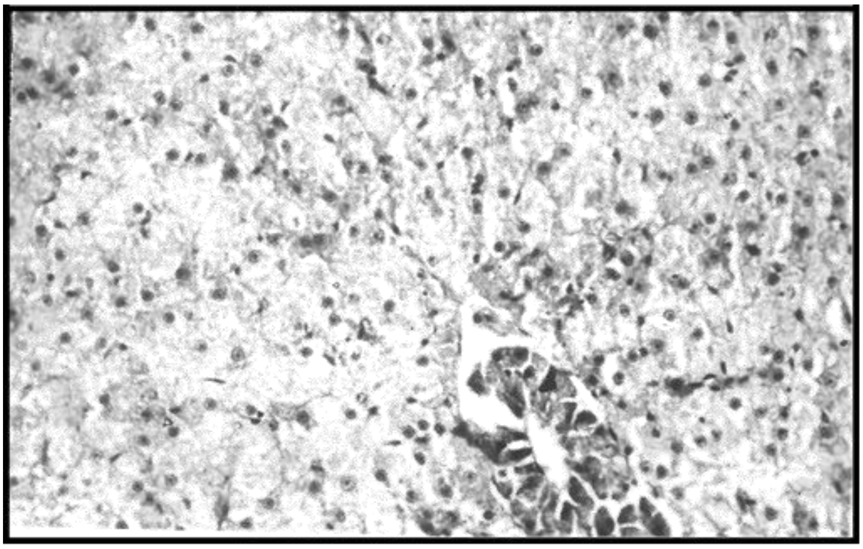

Fig 3: Liver of fish exposed to 0.096 mg/ml showing large no of fatty degeneration and disturbed cordal arrangement of hepatocytes.

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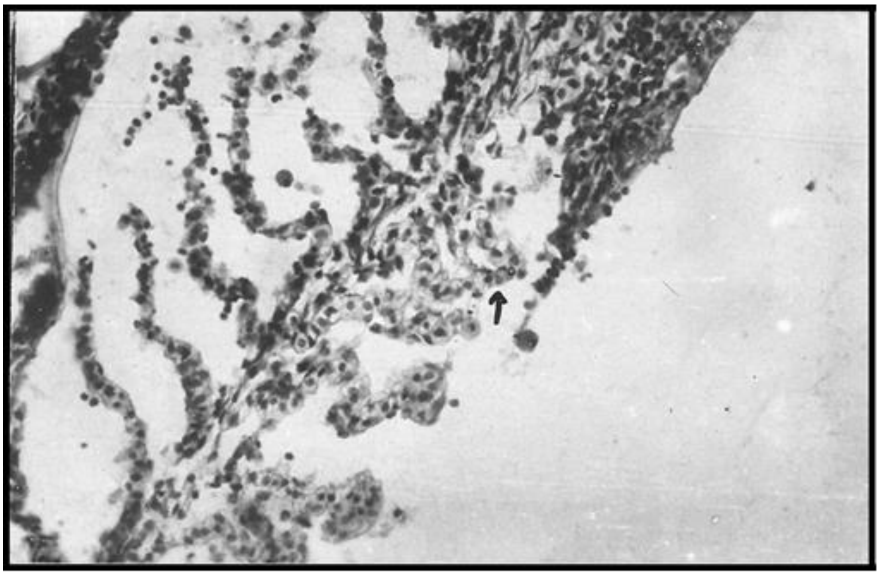

Fig 4: Gill of fish exposed to 0.38 mg/ml. Fenthion for 60 days exposure showing vacuolated, deformed and shortened secondary lamellae (arrow mark)

A significant increase in lipid content was observed in muscle and liver tissues of Fenthion exposed fish and can be read from table 1 &.2. [12, 7] Observed similar increase in lipid content in DDT and Dieldrin exposed *S. gairdneri* and in *T. mossambica* on exposure to Thiodan. [31] noticed increase in lipid content in *C. fasciata* when exposed to insecticide Lebaycid. [9, 10], Also reported increase in lipid content of fresh water fishes exposed to Endrin and Lebaycid, respectively. According to [4] lipids are formed as the end product of carbohydrate metabolism especially in anaerobic and sluggish fish. [16] Suggested that tissue hypoxia might have played significant role in synthesis of lipid for carbohydrate precursors in fish exposed to DDT. In the present study the significant increase in lipid content could be due to tissue hypoxia and it could also be attributed to fall in glycogen and protein contents which in turn are compensated with rise in lipid content so as to withstand the stress of toxicant.

The increase in the moisture content of the muscle and liver of the fish exposed to all the concentrations 0.38, 0.193, 0.096 mg/1 of Fenthion was maintained till the end of 60 days experimental period (table 1 & 2). [14] postulated that the fish may at first consume lipid from the liver and start to mobilize muscle proteins, only after this source of energy depletes subsequent to the utilization of muscle proteins, water moves in to take its place and thus resulting in the increase in moisture content. The increased levels in moisture content were observed by [7] in *Tilapia mossambica* exposed to Thiodan and PMA. [31] also noted similar observation in *C. fasciata* exposed to Lebaycid 1000. Hence, in the present study the cause for rise in moisture content could be due to the subsequent utilization of muscle proteins.

In the present investigation a significant drop in LSI was observed in Fenthion exposed *C. carpio* (Table 3). According to [7] low LSI value in *T. mossambica* exposed to Thiodan Could be due to the damage caused to liver by the pesticide or by the pollutant. [26] studied change in LSI value in the liver and reported that LSI value reduced when *T. mossambica* was exposed to Dichlorvos and further discussed that it could be due to loss of cells of respective tissue. Hence, in the present study taking the support of above workers, the reduction in somatic index of liver of exposed fish *C. carpio*, could be attributed to the loss of somatic cells, bio- chemical changes and may also be due to histopathological lesion of liver cells fig 1, 2, 3 and 4.

4. Conclusion
Reduction in protein and carbohydrate contents of Fenthion treated *C. carpio* indicates fall in nutritional value or quality of food. The increase in moisture content may possibly be as a result of the change occurred in the energy sources while the increase in lipid content indicates the alternative mechanism induced to compensate the toxicity stress. Decrease in LSI value probably denotes the damage caused to liver cells in response to stress induced by Fenthion.

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