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# Toxic Effects of Mercury Pertaining to Glycogen Content of Muscle and Liver of The Fish, Puntius Sophore (ham.)

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## Abstract

Glycogen content of liver and muscle alongwith survival and growth rate is being reported in the paper after exposure to mercuric chloride and methyl mercury chloride for six weeks followed by two weeks of recovery. Methyl mercury chloride was found to be more toxic as it caused greater mortality and slower growth rate as compared to mercuric chloride. A steady decrease in liver and muscle glycogen content was also observed, but the same was found more significant for methyl mercury chloride in comparison to mercuric chloride at identical exposure levels, i.e. 1/4, 1/6, 1/9 of $LC_{50}$ values

**Keywords:** Mercuric Chloride - Methyl Mercury Chloride-Fish - Puntius Sophore.

**Introduction**

Mercury used in the manufacture process of caustic chlorine, dye and pigment industries has been found to be acutely toxic (Goldwater, 1971;, Shaffi, 1981; Beena and Viswaranjan, 1987 and Aruna and Gopal, 1987). It is concentrated through food chain in the animal body (Sharma and Davi's 1980) causing various physiological abnormalities. It also interferes with biochemical functioning of the living system through its ligand binding property (Itano and Sasaki, 1983). There are only few reports on the biochemical aspect of mercury toxicity (Gill and Pant, 1981; Rana and Sharma, 1982; Dutta etal. 1983; Hossain and Dutta, 1986 and Verma and Chand, 1986). In the present study comparative toxicity of mercuric chloride and methyl mercury chloride was investigated in the muscle and liver of a fresh water fish Puntiussophore (Ham.)

**Review of literature**

Heavy metals and their compounds are generally found dissolved in natural waters through soil leaching and runoff, erosion and break down of mineral deposits. Trace metals such as vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, selenium, etc. are essential for the growth of organisms, whereas, others such as lead, cadmium, mercury, arsenic, etc. are considered not only biologically non-essential but toxic even at very low concentrations.

There is ample evidence that excesses or deficiencies of trace metals may result in severe physiological disorders.

Heavy metals like zinc and selenium, in traces, are essential and beneficial for metabolism (Ogino and yang, 1979; Zeigler et al., 1961 and Vellee, 1957). Zinc deficiency leads to growth retardation, whereas, high concentrations cause adverse changes in the morphology and physiology of various animals (Crandall and goodnight, 1962 and savage et al., 1964). Similarly, selenium is required in traces and becomes toxic when ingested in amounts ranging from 0.1 to 10mg/kg of food (Lindberg, 1968).

Mercury is very toxic and relative accessibility of this metal makes it a major environmental pollutant (Wood, 1974). Mercury occurs in natural waters and is added progressively to the environment through regular mining. Mercury and its salts are more soluble in water in comparison to other metals and their derivatives. Fungicide- producing factories use large amounts of mercury and a part of the factory as fault products. One such chemical factory was established in 1953 and started its production in 1956 in Denmark. In 1962, the factory was demolished and the rubble kept lying at the factory site. After a period of nearly 20 years sticking chemicals were found to be leaching out at the factory site, affecting the aquatic fauna (Kiorbee et al., 1983) Several other alarming incidents of mercurial

Poisoning were also reported from other continents and thus, it was concluded that mercury can also produce hazards in aquatic systems. The investigations revealed abnormally high concentrations of mercury compounds in fresh and salts water fish and other aquatic organisms, which in 1967 led to a ban by the Swedish medical board on the sale of fish from approximately 40 lakes and rivers (Goldwater, 1971).

Although, there is sparse data of toxic concentration of mercury ions in Indian waters high concentrations have been reported in waters of the south west coast of India. Wide uses of metals in the industrial, agricultural and domestic sectors have a great deal of concern regarding the release of metals in the Indian environment (Borgaonkar and Gokhle, 1992). The heavy metals pollution including mercury has also been reported in the state of Rajasthan due to both mining and processing of zinc (Udaipur region) and copper (Jaipur region) and other industrial activities (particularly in and around Kota region). Therefore, it is necessary to investigate the state of mercury pollution to prevent large scale chemical pollution in India.(Araújo CVM, Cedeño- Macias LA. 2016) Studied heavy metals in yellowfin tuna (Thunnus albacares) and common dolphinfish (Coryphaena hippurus), and on the other hand, (Arcagni M, Rizzo A, Juncos R, Pavlin M, Campbell LM, Arribere MA, Horvat M, Ribeiro Guevera S. 2017)Studied mercury and selenium in the food web of Lake Nahuel Huapi, Patagonia, Argentina

**Aim of the study**

Aim is to study the toxic effects of organic and inorganic forms of mercury on the biochemistry of some organs of a fish which is food for poor people in our country. These fishes come in contact with mercury from the effluents coming from various industries.

**Objective of the study**

The most important aspect in the study of toxicology is to find the permissible limits of the toxicants for the organisms in relation to various environmental compartments.

**Material and methods**

Four hundred and fifty healthy fish (average weight 3.9 + 0.48g and length 8.42 + 0.29 cm) of 1 + age group were selected for each experiment. These were kept in large glass aquaria of 570 capacity and acclimatized to laboratory conditions for a week on a special type of selected diet, prepared by mixing 1 part wheat flour fed at the rate of 2% of body weight daily at 11.30 h. Bioassays were conducted (Ipsen and Feigl, 1970) for 24 h, 48 h and 96 h. Fish were divided into four groups of 100 each for both forms of mercury viz: mercuric chloride and methyl mercury chloride separately. One group was used as control and the other three were kept on different sublethal dose levels viz: (1/4, 1/6, 1/9 of 96 h $LC_{50}$ value) 190 $ugl^{-1}$, 120 $ugl^{-1}$ and 80 $ugl^{-1}$ for mercuric chloride and 1.7 $ugl^{-1}$, 1.2 $ugl^{-1}$ and 0.7 $ugl^{-1}$ for methyl mercury chloride. The exposure medium was changed daily restoring the mercury level at each change. Dechlorinated water used in the experiment was analysed for physicochemical characteristics (USDA, 1954). The range of values was as follows: Chlorides - 2.4 m. eg/1, Sulphates - 0.12 m eq/1, Calcium and Magnesium - 4.5 m. eq/1, Sodium - 1.6 m. eq/1, Potassium - 0.02 m. eq/1, Carbonates - 0.8 m. eg/1, Bicarbonates - 2.8 m. eq/1; Ph - 7.8; Temperature - 24°C - 28°C. Liver and muscle glycogen content was estimated as per Montogomery's (1957) method. Statistical comparisons of values for each sample were made (Bancroft's, 1957) using students' t-test.

**Result**

Initially fish exposed to mercury showed disturbed swimming movements but later they sank to the bottom motionless. Profuse mucus secretion with a slow opercular movement showed respiratory distress.

**Growth and mortality**

Data presented here (Table 5 and 6 ) showed that both mercuric chloride and methyl mercury chloride affected growth. The loss of weight and percent mortality depended upon the form of mercury, its level and duration of exposure. A distinct gain in weight was observed on restoration of the fish to control conditions during the final two weeks

**Biochemical Changes**

Liver and muscle glycogen content showed a steady decrease when fish were exposed to mercury. The decrease was highly significant (P 0.001) for liver glycogen content (Table 3) when methyl mercury chloride was used at 1.7 $ugl^{-1}$, 1.2 $ugl^{-1}$ and 0.7 $ugl^{-1}$ concentrations. Muscle glycogen content (Table 4) for methyl mercury chloride was found highly significant (P 0.001) at concentrations 1.7 $ugl^{-1}$ and 1.2 $ugl^{-1}$, whereas for mercuric chloride (Table 2) it was significant (P 0.001) at 190 $ugl^{-1}$ and 120 $ugl^{-1}$ concentrations.

**Discussion**

Results presented above showed that the fish Puntiussopore is sensitive to both mercuric chloride and methyl mercury chloride, but it shows greater depletion of glycogen content both in liver and muscle (Tables 3 and 4) when exposed to 1.7 $ugl^{-1}$ of methyl mercury chloride in comparison to mercuric chloride. It is reported that glycogen being the energy reserve of the body show depletion at the slightest stress. Gill and Pant (1961) also observed depletion in liver glycogen content in a telecast Puntiusconchonius. Mercury has been found to inhibit enzymic action in liver and gills (Verma and Chand, 1986). Inhibition of enzymic action disregulates blood glucose concentration within hepatic cells and the blood concentration of the two pancreatic hormones, insulin- to indicate the adequate glucose (Robert, 1983).

Pollutants affect normal gas exchange and interfere with oxygen uptake by gills (Verma and Chand, 1986). Mucus formation around the operculum and the body may reduce the uptake of oxygen necessary for metabolic activities and the breakdown of gas exchange at the gills, leading to tissue hypoxide and the glycogen depletion of the viscera (Shaffi, 1981). Results of the present study indicate that all stress conditions induce depletion of energy reserves, retardation of growth and loss of body weight.

**Conclusion**

Highly significant changes were observed in muscle and liver glycogen contents of the Puntius sophore. Organic form of mercury was found to be more toxic. As the time of exposure increased recovery became more difficult. When the fishes were returned to mercury free water after an incisal exposure, no significant recovery was observed in fishes exposed to organic mercury toxicants

**Table - 1. Effects of different concentrations of mercuric chloride on the level of liver glycogen (mg/gm) of Puntius sophore (Ham.). 7th and 8th week represent the recovery period when normal control conditions were restored. Significance as students' t-test, P < 0.05 slightly significant (+), P < 0.01 significant (++) P < 0.001 highly significant (++1).**

| Weeks | Control (Ambient water) | 190ugl$^{-1}$ | 120ugl$^{-1}$ | 80ugl$^{-1}$ |
|---|---|---|---|---|
| 1 | 18.21 + 0.20 | 17.31+ 0.34 | 17.49 + 0.20$^{+}$ | 17.49 + 0.20$^{+}$ |
| 2 | 18.21 + 0.20 | 16.06 + 0.46$^{++}$ | 16.42+ 0.29$^{++}$ | 16.60+ 0.34$^{++}$ |
| 3 | 18.39 + 0.18 | 15.17 + 0.60$^{++}$ | 15.17 + 0.44$^{+++}$ | 15.89 + 0.73$^{+}$ |
| 4 | 18.39 + 0.18 | 14.10 + 0.34$^{+++}$ | 14.99 + 0.29$^{+++}$ | 15.17 + 0.44$^{+++}$ |
| 5 | 18.21 + 0.35 | 12.85 + 0.29$^{+++}$ | 14.28 + 0.29$^{+++}$ | 13.03 + 0.34$^{+++}$ |
| 6 | 18.03 + 0.34 | 11.06 + 0.46$^{+++}$ | 12.85 + 0.29$^{+++}$ | 13.03 + 0.34$^{+++}$ |
| 7 | 18.03 + 0.18 | 13.03 + 0.44$^{+++}$ | 13.92 + 0.46$^{+++}$ | 14.28 + 0.29$^{+++}$ |
| 8 | 17.58 + 0.41 | 14.99 + 0.29$^{++}$ | 15.35 + 0.20$^{++}$ | 16.06 + 0.20$^{++}$ |

**Table - 2. Effects of different concentrations of mercuric chloride on the level of muscle glycogen (mg/gm) of Puntius sophore (Ham.). 7th and 8th week represent the recovery period when normal control conditions were restored. Significance as students' t-test, P < 0.05 slightly significant (+), P < 0.01 significant (++), P < 0.001 highly significant (+++).**

| Weeks | Control (Ambient water) | 190ugl-1 | 120ugl-1 | 80ugl-1 |
|---|---|---|---|---|
| 1 | 3.21+ 0.20 | 3.03+0.17 | 3.21+0.20 | 3.21+0.20 |
| 2 | 3.39+0.44 | 2.85+0.29 | 3.21+0.20 | 3.21+0.20 |
| 3 | 3.21+0.20 | 2.49+0.20+ | 2.85+0.29 | 3.03+0.34 |
| 4 | 3.57+0.29 | 1.78+0.20++ | 2.49+0.20+ | 2.67+0.17+ |
| 5 | 3.39+0.34 | 1.24+0.17++ | 1.60+0.34++ | 2.32+0.17+ |
| 6 | 3.57+4.41 | 0.89+0.17+++ | 1.42+ 0.29++ | 1.78+0.20++ |
| 7 | 3.39+ 0.34 | 1.78+0.20 | 2.14+0.29+ | 2.67+0.34 |
| 8 | 3.39+0.44 | 2.14+0.29 | 2.85+0.29 | 3.03+0.34 |

**Table - 3. Effects of different concentrations of methyl mercury chloride on the level of liver glycogen (mg/gm) of Puntius sophore (Ham.). 7th and 8th week represent the recovery period when normal control conditions were restored. Significance as students' t-test, P < 0.05 slightly significant (+), P < 0.01 significant (++) P < 0.001 highly significant (+++).**

| Weeks | Control (Ambient water | 190ugl-1 | 120ugl-1 | 80ugl-1 |
|---|---|---|---|---|
| 1 | 18.92+ 0.20 | 14.10+ 0.34+++ | 14.99+ 0.29+++ | 15.17+ 0.44+++ |
| 2 | 18.92+ 0.20 | 13.03+0.44++ | 14.28+0.29+++ | 14.28+0.29+++ |
| 3 | 18.57+0.41 | 12.85+0.29+++ | 13.03+ 0.44+++ | 14.10+ 0.34+++ |
| 4 | 18.92+ 0.20 | 10.53+ 0.17+++ | 11.78+0.20+++ | 13.92+0.46+++ |
| 5 | 18.57+ 0.41 | 9.46+0.18+++ | 10.35+0.20+++ | 11.60+0.18+++ |
| 6 | 18.39+0.18 | 8.21+0.20+++ | 9.46+0.18+++ | 10.35+0.28+++ |
| 7 | 18.21+0.20 | 8.21+0.20+++ | 9.46+0.18+++ | 10.35+0.20+++ |
| 8 | 18.21+0.20 | 8.74+0.17+++ | 9.64+ 0.20++ | 10.53+0.17+++ |

**Table - 4. Effects of different concentrations of methyl mercury chloride on the level of liver glycogen (mg/gm) of muscle sophore (Ham.). 7th and 8th week represent the recovery period when normal control conditions were restored. Significance as students' t-test, P < 0.05 slightly significant (+), P < 0.01 significant (++) P < 0.001 highly significant (+++).**

| Weeks | Control (Ambient water | 190ugl-1 | 120ugl-1 | 80ugl-1 |
|---|---|---|---|---|
| 1 | 3.92+ 0.20 | 2.49+0.20++ | 2.67+0.17++ | 2.85+ 0.29+ |
| 2 | 3.92+0.20 | 3.92+0.33++ | 2.49+0.20++ | 2.49+0.20++ |
| 3 | 3.74+0.17 | 1.96+0.17+++ | 2.32+0.17++ | 2.32+0.33++ |
| 4 | 3.57+0.41 | 1.42+0.29+++ | 1.96+0.17+ | 1.96+0.17+ |
| 5 | 3.74+0.17 | 1.07+0.20+++ | 1.42+ 0.29+++ | 1.60+ 0.39+++ |
| 6 | 3.57+0.29 | 0.89+0.17+++ | 1.24+0.34++ | 1.42+0.29++ |
| 7 | 3.39+0.34 | 0.89+0.17+++ | 1.24+0.34++ | 1.42+0.29++ |
| 8 | 3.39+ 0.34 | 0.89+0.17+++ | 1.42+0.29++ | 1.60+0.17++ |

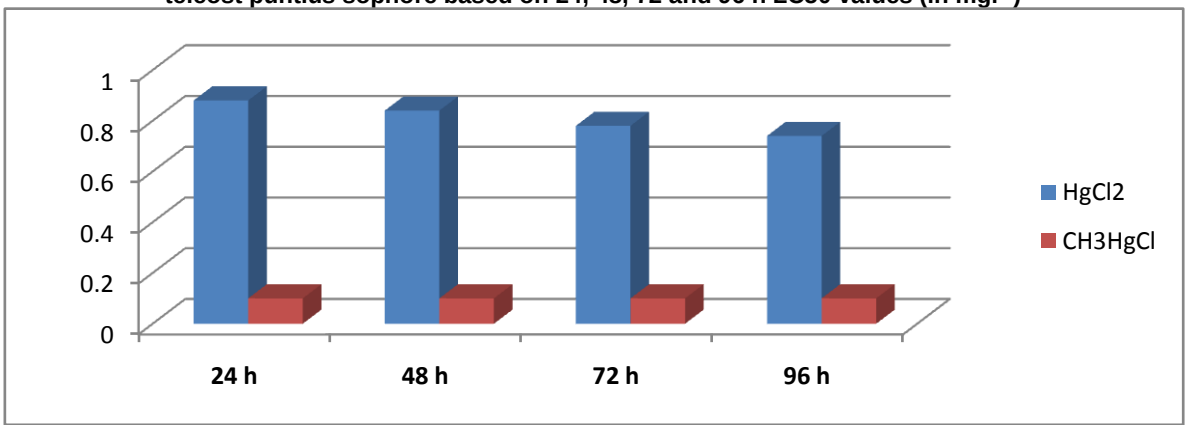
**Table - 5.Effects of different concentrations of mercuric chloride and methyl mercury chloride on gain (+) or loss (-) in body weight (%). 7th and 8th week represent the recovery period when normal control conditions were restored.**

| WEEKS | CONTROL (Ambient water) HgCl2 | CH3HgCl | HgCl2 190 ugl-1 | CH3HgCl 1.7 ugl-1 | HgCl2 120 ugl-1 | CH3HgCl 1.2 ugl-1 | HgCl2 80 ugl-1 | CH3HgCl 0.7 ugl-1 |
|---|---|---|---|---|---|---|---|---|
| 1 | + 0.48 | + 0.50 | - 0.23 | - 0.36 | - 0.14 | - 0.23 | - 0.07 | - 0.20 |
| 2 | + 0.96 | + 1.01 | - 0.46 | - 0.73 | - 0.29 | - 0.47 | - 0.15 | - 0.40 |
| 3 | + 1.44 | + 1.52 | - 0.70 | - 1.09 | - 0.44 | - 0.70 | - 0.22 | - 0.60 |
| 4 | + 1.92 | + 2.03 | - 0.93 | - 1.86 | - 0.59 | - 0.94 | - 0.30 | - 0.80 |
| 5 | + 2.40 | + 2.53 | - 1.76 | - 1.83 | - 0.73 | - 1.17 | - 0.37 | - 1.00 |
| 6 | + 2.88 | + 3.04 | - 1.40 | - 2.19 | - 0.88 | - 1.41 | - 0.45 | - 1.20 |
| 7 | + 3.36 | + 3.55 | - 1.63 | - 2.56 | - 1.03 | - 1.64 | - 0.52 | - 1.40 |
| 8 | + 3.85 | + 4.06 | - 1.87 | - 2.93 | - 1.18 | - 1.88 | - 0.60 | - 1.60 |

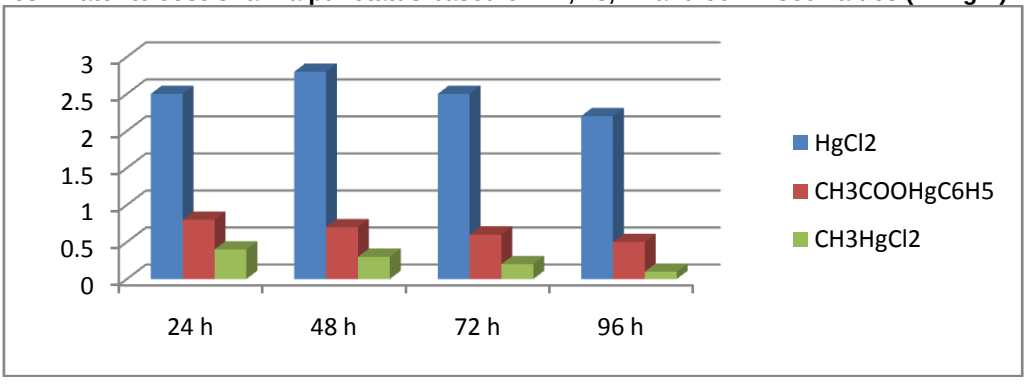
**Table - 5.Effects of different concentrations of mercuric chloride and methyl mercury chloride on cumulative (%)mortality. 7th and 8th week represent the recovery period when normal control conditions were restored.**

| Weeks | Control (Ambient water) $HgCl_2$ | $CH_3HgCl$ | $HgCl_2$ 190 $ugl^{-1}$ | $CH_3HgCl$ 1.7 $ugl^{-1}$ | $HgCl_2$ 120 $ugl^{-1}$ | $CH_3HgCl$ 1.2 $ugl^{-1}$ | $HgCl_2$ 80 $ugl^{-1}$ | $CH_3HgCl$ 0.7 $ugl^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| 1 | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| 2 | Nil | Nil | Nil | 1.0 | Nil | 1.0 | Nil | Nil |
| 3 | 1.0 | 1.0 | 3.0 | 5.04 | 1.0 | 3.02 | Nil | 1.0 |
| 4 | 1.0 | 1.0 | 5.06 | 9.25 | 3.02 | 5.08 | 1.0 | 3.02 |
| 5 | 2.01 | 1.0 | 9.27 | 14.75 | 6.11 | 8.23 | 3.02 | 5.08 |
| 6 | 2.01 | 2.01 | 14.76 | 21.71 | 9.30 | 12.57 | 4.05 | 8.23 |
| 7 | 2.01 | 2.01 | 17.08 | 25.46 | 10.39 | 14.84 | 4.05 | 9.31 |
| 8 | 2.01 | 2.01 | 18.27 | 29.35 | 11.50 | 17.16 | 4.05 | 10.40 |

**Graph - 1. Histograms showing relative toxicity of mercuric chloride and methyl chloride on a fresh water teleost puntius sophore based on 24, 48, 72 and 96 h LC50 values (in $mgl^{-1}$)**




**Graph -2. Histograms showing relative toxicity of mercuric chloride, phenyl mercuric acetate and methyl chloride on a fresh water teleost Channa punctatus based on 24, 48, 72 and 96 h LC50 values (in $mgl^{-1}$)**



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