Effect of Organochlorine pesticide, Mythoxychlor on biochemical profile of common carp *Cyprinus carpio* L.

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Abstract

The present investigation deals with the effect of Methoxychlor on biochemical Profile of Cyprinus carpio. Methoxychlor is an organochlorine pesticide that is extensively used as a broad-spectrum insecticide on a variety of crops in India and Maharashtra. Methoxychlor creates serious threat to the environment as well as non-target organisms like aquatic and land dwelling animals. This pesticide may enter the water bodies via spray drift, run off, rains, soil leaching, sewage discharges and affect non-target organisms like fishes. Due to its lipophilic nature, it is persistent in environment and has tendency to bio accumulate in fish tissues. Methoxychlor's impact on total carbohydrates, proteins and lipids was studied in the gills, liver and brain of Cyprinus corpio after a 21-day sub-lethal toxicity exposure

Keywords: Methoxychlor, Organochlorine, Carbohydrate, Protein, Lipid, Biochemical.

1. Introduction:

Fish's physiological and health condition may be seriously harmed by pesticides. Organochlorines are a class of chlorinated chemicals that are extensively employed as insecticides. These compounds are classified as persistent organic pollutants because of their long-term environmental persistence. Organochlorines insecticides were formerly effective in the prevention of malaria and typhus, but they are now prohibited in the majority of developed nations [1]. According to data on the usage of various pesticides, the organochlorine class of chemicals accounts for 40% of all pesticides used [2], [3]. Organochlorine insecticides such as DDT, hexachlorocyclohexane, aldrin, and Methoxychlor are among the most commonly used pesticides in Maharashtra, India [2] because to its cheap cost and need against different pests.

Changes in biochemical parameters such as carbohydrates, proteins, and lipids are significant indicators of organ systems' vulnerability to pollutants such as pesticides by changing their function, as Verma et al [4] have shown. In fish, pesticides have been shown to affect glucose metabolism. Several researchers investigated the effects of various pesticides on glucose metabolism in fish, looking at different aspects of the process [5], [6]. Mayes [7] found that when animals are exposed to pesticide contamination, carbohydrates from their energy stores are altered and reduced.

As a result, pesticides tests may be used to detect acute or chronic toxicity of pesticides [8], such as the organochlorine pesticide Methoxychlor, and can be a helpful tool for diagnosing toxicity effects in target

organs and determining fish physiological condition. A biochemistry test can reveal the changes that have occurred in the bodies of fish exposed to pesticides. Because of the severity of the damage to the tissues, especially the liver, cellular production of several biochemical compounds may be reduced, resulting in a reduction in certain biochemical components in the pesticides exposed fish. These alterations were found in monocrotophos, bifenthrin, cypermethrin, diazinon, and malathion-exposed *Channa punctatus* [9], *Oncorhynchus mykiss* [10], *Clarias batrachus* [11] and *Cyprinus carpio* [12].

Because fishes are essential suppliers of proteins and fats in the form of food, the health of these creatures is critical for humans. Fish are especially vulnerable to water pollution due to environmental factors. As a result, when pollutants such as pesticides reach the organs of fish, they may cause substantial harm to specific physiological and biochemical processes [13]. Under stable environmental circumstances, fish have a generally consistent basal or standard metabolism. pesticides have been shown to interfere with cellular metabolism's at various stages. *C. carpio* is an ecologically and economically important species and it is used in toxicity assays as a bio-indicator due to its sensibility and easy maintenance under laboratory conditions. Therefore, this fish is being selected for this study. The goal of this research is to see how non-target animals like fish are impacted by the organochlorine pesticide (Methoxychlor) by monitoring chosen biochemical reactions in the selected test species *Cyprinus carpio,* a typical species from the aquatic environment, under laboratory settings.

2. Materials and Methods

2.1 Collection of Test Organism and Their Maintenance:

The freshwater fish *Cyprinus carpio*, with a length of 6–8cm and a weight of 6.5–7.5g, regardless of sex, has been selected as the test organisms for this study. For three weeks, the fish were acclimatised to laboratory settings in big plastic water tanks at a room temperature of 28 °C. Every day, with a 12+12 hour dark and light cycle, the water was replenished. The fish were given groundnut oil cake and rice bran ad libitum throughout the acclimation phase. One day before the acute toxicity test, the feeding was discontinued. All of the precautions set forth by the committee on aquatic organism toxicity studies [14] were followed, and only such acclimatised fish were utilised for toxicity testing. If any batch of fish died more than 5% of the time during acclimation, the whole batch was discarded.

2.2 Methoxychlor preparation:

Methoxychlor, an organochlorine pesticide produced by Agri Life India Private Limited in Maharashtra, was utilised in the research. Methoxychlor was purchased directly from the manufacturer. The stock solution of Methoxychlor was made in acetone, which was proven to be harmless to fish. This emulsified concentration was used to extract the required amount of Methoxychlor.

2.3 Acute toxicity test:

Methoxychlor's acute toxicity (96-hour LC50) for the freshwater fish *Cyprinus carpio* was established in the laboratory following the OECD's 1998 standards [15]. Concentrations of the test compound used in short term definitive tests were between the lowest concentration at which there was 0% mortality (<2µg/L) and the highest concentration at which there was 100% mortality (>3µg/L). Without aeration, the test medium was replaced every 24 hours with their corresponding toxicant test concentrations.

Every 24 hours, mortality was recorded, and dead fish were removed when discovered, with the number of fish deaths at each dose noted up to 96 hours for estimate of acute toxicity (LC50). If there was no apparent movement (e.g. gill movement) and touching the caudal peduncle produced no response, the fish was deemed dead.

Duncan's multiple range test [17] was used to compare mean mortality values after repeated measurements ANOVA was used to estimate residual variance. The repeated measure component was time of exposure, while the second element was treatment (concentration and control). Probit analysis [17], which is suggested by OECD standards as an acceptable statistical technique for toxicity data analysis, was used to determine the LC_{50}. To provide a consistent presentation of the toxicity data, the concentration response curve was linearized by logarithmic transformation of concentrations. The 96h LC_{50} with 95 percent confidence limits and slope function were calculated after the concentration response curve was linearized by logarithmic transformation of concentrations.

2.4 Fixation of sub-lethal concentrations:

Methoxychlor was chosen as the fatal dose to investigate the biochemical reactions of the fish, *Cyprinus carpio*, based on the fact that the impact of organochlorine pesticides on fish becomes consistent with 96 hours of exposure for LC_{50} (2.3 µg/L). However, knowing the toxicant concentration that kills 50% of test animals in a certain amount of time may be inadequate to evaluate the animal's different reactions to the toxicant. Furthermore, acute toxicity studies have major limitations, such as the possibility of test animals adapting to the imposed toxicity. Sub-lethal investigations are required because different changes involving a series of events in the responses of test animals may occur at sub-lethal concentrations. For future research, 1/10th of the 96h LC_{50} (0.23 µg/L) was chosen as the sublethal concentration of Methoxychlor. Because the length of exposure has a significant impact on a pesticide's toxicity to an organism. To further understand the impact of toxicity, the effects of a sub-lethal dose of Methoxychlor were examined for 21 days.

2.5 Experimental design:

After acclimation, healthy *Cyprinus carpio* (95±5g) were selected and divided into two groups of 20 fish each. Group 1 was the control group, whereas Group 2 was the experimental group. For 21 days, the fish in group 2 were exposed to 1/10th of the LC_{50} value of Methoxychlor (0.23 µg/L). Methoxychlor was administered to the fish at their respective sub-lethal doses, which they were kept at for the duration of the experiment. The test medium was changed on a daily basis, allowing for the elimination of nitrogenous waste emitted by the test fishes as well as unconsumed food.

The fish were killed 24 hours following the exposure period, and the main organs, gills, brain, and liver, were dissected from each animal. The tissue was then processed right away for biochemical analysis.

2.6 Biochemical estimations:

Nicholas et al. [18] used the Anthrone reagent with minor modifications to measure total carbohydrates. Brain tissues were homogenised in 10% TCA to estimate total carbohydrates at 10 mg/ml liver and 20 mg/mL gill. The homogenate was centrifuged for 15 minutes at 3000 rpm. Total carbohydrates were calculated directly from one mL of clear supernatant. In each tube with 1 mL of clear supernatant, 5 mL of Anthrone reagent was added in an inclined position. The tubes were all sealed and allowed to cool to ambient temperature. In a UV–visible spectrophotometer, the colour produced was compared to a blank at 620 nm. The results were reported as mg of glucose per gram of wet tissue weight.

The total protein content was measured using Lowry et al [19] technique with minor adjustments. Briefly, aliquots of tissue extracts were collected and mixed with distilled water to make a final volume of 1ml. 5mL alkaline copper reagent was added and left to sit for 10 minutes at room temperature. The Folin-Ciocalteau reagent (0.5mL) was then added. After 20 minutes, the blue colour produced was measured at 720nm in a spectrophotometer against a reagent blank. Using the standard curve produced, the quantity of protein contained in the aliquot of the sample was determined. The total protein content is measured in milligram per gram of tissue.

The technique of Frings et al [20] was used to determine total lipids, with minor changes. 0.2 mL lipid extract, 0.2 mL concentrated sulphuric acid was used to calculate total lipids. All of the ingredients were cooked for 10 minutes in a boiling water bath before chilling for 5 minutes in cold water. The phosphovanillin reagent (ten millilitres) was then added. For 15 minutes, the contents were incubated at 37°C in a water bath. Within 30 minutes, the colour produced was quantified at 540nm against a reagent blank. The standards and sample were both run at the same time. The standard curve was used to determine the sample's total lipid concentration. The total lipid concentration is measured in milligram per gram of tissue.

Statistical analysis was performed on all of the data collected using Microsoft Office Excel programme. One-way ANOVA was employed as the test. At the 0.05 level of significance, all findings are given as mean standard deviation.

3. Results and Discussion:

3.1 Effect of Methoxychlor on Total carbohydrates.

Table-1 shows the carbohydrate content of control and experimental animals' gills, liver, and brain. Carbohydrate concentration was highest in the brain of control fishes, followed by liver and gills. In comparison to the control group, carbohydrate content in the gills, liver, and brain reduced substantially following exposure to Methoxychlor. The most significant reductions were observed in the gills (23%), liver (20%), and brain (14%).

Table-1. Total carbohydrate content in gills, liver and brain of *C. carpio* on exposure to sub-lethal dose of Methoxychlor.

Sl.No	Tissue	Carbohydrate content in control and experimental animals (mg/gr tissue)	
		Control	Methoxychlor
1	Gill	18.4	15.3
2	Liver	22.5	18
3	Brain	25.8	22.6

Values are expressed as Mean±standard deviation. *p<0.05.

3.2 Effect of Methoxychlor on Total proteins:

Table-2 illustrates the total protein composition of control and experimental animals' gills, liver, and brain. The liver had the highest total protein concentration in control fishes, followed by the brain and gills. When fish were exposed to Methoxychlor, the total protein content of their gills, liver, and brain reduced substantially compared to the control group. Methoxychlor exposure causes a 27 percent decrease in total protein content in the brain, 23 percent in the gills, and 17 percent in the liver.

Table-2. Total protein content in gills, liver and brain of *C. carpio* on exposure to sub-lethal dose of Methoxychlor.

Sl.No	Tissue	Protein content in control and experimental animals (mg/gr tissue)	
		Control	Methoxychlor
1	Gill	6.10	5.7
2	Liver	6.80	5.9
3	Brain	6.40	4.9

Values are expressed as Mean±standard deviation. *p<0.05.

3.3 Effect of Methoxychlor on Total lipids:

Table 3 shows the lipid content of control and experimental animals' gills, liver, and brain. The liver had the highest lipid level in control fishes, followed by the brain and gills. When compared to the comparable group of control animals, lipid levels in the liver, gills, and brain reduced substantially following exposure to Methoxychlor. The liver showed the greatest decrease (25%) followed by the brain (12%) and the gills (11%).

Table-3. Total lipid content in gills, liver and brain of *C. carpio* on exposure to sub-lethal dose of Methoxychlor.

Sl.No	Tissue	Total lipid content in control and experimental animals (mg/gr tissue)	
		Control	Methoxychlor
1	Gill	3.70	3.24
2	Liver	4.25	3.27
3	Brain	3.70	3.35

Values are expressed as Mean±standard deviation. *p<0.05.

Methoxychlor was shown to have a continuous reduction in protein, carbohydrate, and lipid content in various organs of common carp exposed to organochlorine pesticide, implying enhanced proteolysis and potential product consumption and degradation for metabolic reasons. Proteins are essential and distinctive components of living stuff [21]. The decrease in protein content in the gills, liver, and brain of *Cyprinus carpio* exposed to the organochlorine pesticide Methoxychlor may be related to their participation in the energy producing process through inter conversion metabolism, according to the current research. The finding is consistent with earlier findings that revealed a substantial reduction in protein concentration in muscle, liver, and gut in *Cyprinus carpio* exposed to monocrotophos [22]. Jha and Verma observed a decrease in protein content in the stomach and intestine of *Clarias batrachus* treated to the insecticides Endosulfan, Malathion, and agrofen.

Under the influence of Methoxychlor, protein may be broken down into free amino acids, resulting in a decrease in total protein concentration. When an organism is exposed to toxic stress, it diversifies its energy sources to meet the anticipated energy needs, which may result in protein depletion. Protein loss in the gills, liver, and brain tissues may potentially be attributed to degradation and the potential use of degraded products for metabolic functions.

Carbohydrates are a crucial organic component of animal tissues. Carbohydrates are the most direct and main source of energy [21]. They not only function as cell building components, but also as a chemical energy store that may be raised or reduced depending on the needs of the organism. Several authors have

found lower glucose levels in different fish tissues. Shrivastava et al. [24] found that glucose levels in the brain of *Heteropneustes fossilis* exposed to carbaryl were lower. Tilak and Yacobu [25] found that the glucose content in the different tissues of fenvalerate-exposed *Ctenopharyngodon idellus* decreased.

Lipid is an essential component of animal tissue that plays a key function in energy metabolism and is one of the living system's components. They play a role in cellular and subcellular membranes as well. It is a significant fuel reserve found in mammals, including energy-dense reserves with calorific values double those of carbohydrates or proteins [26]. The lipid content of Methoxychlor-exposed *Cyprinus carpio* gills, liver, and brain tissue were reduced, according to the current research. The reduction in lipid content may be related to increased lipid hydrolysis to meet the higher energy requirement brought on by Methoxychlor. A reduction in lipid content was also seen in many additional investigations in response to various pesticide toxicities. The lipid content in fish tissues was shown to be lower, which may be related to the use of lipid for energy requirement under stressful situations [27]. When *Labeo rohita* was exposed to the heavy metal cadmium, Hameed and Muthukumaravel [28] found a significant reduction in lipid content.

4. Conclusion:

Proteins, carbohydrates, and lipids content in various organs of common carp exposed to Methoxychlor exhibited a continuous reduction in the current study, implying enhanced proteolysis, lipid hydrolysis, and potential product consumption and degradation for metabolic reasons. Finally, our findings provide direct proof of Methoxychlor toxicity in *Cyprinus carpio* based on biochemical markers

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