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Metabolic Adjustments in Glycogen Profiling of Cirrhinus Mrigala under Pirimicarb-Induced Stress

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Abstract: *Aquatic toxicology is a multidisciplinary field that not only measures contaminant levels to assess hazards in aquatic ecosystems but also evaluates how these contaminants affect aquatic life and, ultimately, human health. It is broadly defined as the study of the adverse effects of synthetic and natural chemicals, as well as other materials, on aquatic organisms at cellular, tissue, organ, population, community, and ecosystem levels. Historical incidents have clearly demonstrated the harmful consequences of anthropogenic chemical contaminants on aquatic environments and human populations.Among these contaminants, Pirimicarb, a selective carbamate insecticide, has been widely used as an effective aphicide since the 1990s in India. Chemically, Pirimicarb ($C_{11}H_{18}N_4O_2$) is N, N,4,5-tetramethylpyrimidin-2-amine substituted with oxygen dimethyl carbamoyl at position 4. It belongs to the class of synthetic amino-pyrimidines and tertiary amino compounds derived from dimethyl carbamic acid. This compound is a colourless solid with a molecular weight of 238.29 g/mol, solubility of 2700 mg/L in water, density of 1.21 g/cm^3, and a vapour pressure of 7.28 × 10^{-6} mmHg.Due to its higher aphicidal efficacy compared to other carbamates, Pirimicarb is used extensively worldwide. However, its continuous application raises concern about non-target toxicity, particularly in aquatic systems. The present investigation was therefore undertaken to evaluate the toxicological impact of Pirimicarb on glycogen metabolism in freshwater fish* Cirrhinus mrigala *under acute exposure, providing insights into its role as a metabolic biomarker of pesticide-induced stress*

Keywords: Aquatic toxicology, pirimicarb, Cirrhinus *mrigala,* Glycogen metabolism, acute exposure, biomarker

I. INTRODUCTION

The quantification of toxicity is inherently complex, as toxic effects may be either acute or chronic and vary depending on the organism, genetic makeup, physiological and environmental conditions, age, gender, and overall health status. Unlike experimental animals, which are often inbred and genetically uniform, humans represent a highly outbred population with substantial genetic variability, making genetic differences a critical factor in human toxicological responses.The simplest and most widely used measures of toxicity are based on organismal mortality and morbidity. From a quantitative perspective, these endpoints are expressed as lethal dose 50 (LD_{50}), effective dose 50 (ED_{50}), lethal concentration 50 (LC_{50}), and effective concentration 50 (EC_{50}) (Lorke, 1983). LD_{50} and ED_{50} represent the dose of a toxicant required to cause death or a defined effect in 50% of a treated population (Bruce, 1985; Lipnick et al., 1995). In contrast, LC_{50} and EC_{50} refer to the concentration of a toxicant in the environment that results in mortality or a specified effect in 50% of the exposed organisms.While LD_{50} and ED_{50} values are dose-dependent and normalized to body weight expressed as milligrams of chemical per kilogram of body weight, LC_{50} and EC_{50} values are concentration-dependent and determined by the exposure medium e.g., milligrams of chemical per litre of water for aquatic organisms.

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Toxicity assessment is far from simple, as toxic effects can be acute or chronic and vary widely with species, genetic background, physiology, environment, age, sex, and overall health. Unlike laboratory animals that are largely inbred and genetically uniform, humans represent a highly diverse population, making genetic variability a key factor in toxicological responses. To simplify this complexity, toxic effects are often quantified using standard measures of mortality and morbidity. The most common indices include the lethal dose 50 (LD_{50}), effective dose 50 (ED_{50}), lethal concentration 50 (LC_{50}), and effective concentration 50 (EC_{50}) (Lorke, 1983). LD_{50} and ED_{50} represent the dose of a toxicant required to induce death or a defined effect in 50% of a treated population (Bruce, 1985; Lipnick et al., 1995), whereas LC_{50} and EC_{50} reflect the concentration of a toxicant in the environment that produces mortality or a defined effect in 50% of exposed organisms.In practice, LD_{50} and ED_{50} are dose-based valuesnormalized to body weight mg/kg, while LC_{50} and EC_{50} are concentration-based values determined by the exposure medium e.g., mg/L in aquatic environments. Together, these metrics provide a standardized yet powerful framework for quantifying the hazards posed by toxicants.

The measurement of toxicity is inherently complex, as toxic effects may manifest acutely or chronically and vary with species, genetic makeup, physiology, environmental conditions, age, gender, and health status (Bahamonde et al., 2016). Unlike laboratory animals that are highly inbred and genetically uniform, humans are an outbred population with remarkable genetic diversity, making genetic variation a critical factor in human toxicological responses (Festing, 2010).To simplify these complexities, toxicity is often assessed through basic endpoints such as mortality and morbidity (Alewu and Nosiri, 2011). From a quantitative standpoint, these are expressed as lethal dose 50 (LD_{50}), effective dose 50 (ED_{50}), lethal concentration 50 (LC_{50}), and effective concentration 50 (EC_{50})(Lorke, 1983). LD_{50} and ED_{50} denote the dose of a toxicant required to cause death or a defined effect in 50% of a test population (Bruce, 1985; Lipnick et al., 1995). In contrast, LC_{50} and EC_{50} represent the concentration of a toxicant in the environment that produces mortality or a specific effect in 50% of exposed organisms.In practice, LD_{50} and ED_{50} values are dose-dependent, calculated relative to body weight (mg/kg), while LC_{50} and EC_{50} values are concentration-dependent**,** determined by the exposure medium—for example, milligrams of chemical per litre of water in aquatic toxicity studies.

II. MATERIALS AND METHODS

Aquatic animals serve as valuable models in toxicological assessments, offering a crucial link between laboratory investigations and real-world environmental challenges (Couch et al., 1984). Various fish and aquatic invertebrates are particularly suitable for both laboratory- and field-based studies of acute and chronic toxicity (Hill, 1989). Investigations into the impact of natural and anthropogenic stressors, as well as toxicants on specific organs, can be effectively carried out using aquatic organisms that exhibit morpho-physiological characteristics closely associated with such functions (Nebeker et al., 1984). Small fish species, in particular, are advantageous for toxicity research due to their ease of availability, simple maintenance, short life cycle, distinct behavioural patterns, and ability to thrive in confined environments (Chapman and Wang, 2001).

In the present study, fingerlings of freshwater fish *Cirrhinus mrigala*, measuring 4.0 ± 1.0 cm in length and weighing approximately 4–5 g, were procured from a local source. The fish were transported to the laboratory in well-oxygenated polythene bags and treated with 0.05% potassium permanganate solution to eliminate fungal, bacterial, and dermal infections. Healthy and active fingerlings were acclimatized in glass aquaria containing laboratory tap water at 26 ± 2°C for 15 days, with continuous aeration. Fish were fed twice daily with commercial feed TAIYO Pet Products Pvt. Ltd., India at approximately 2% of their body weight. Aquarium water was renewed daily, and physicochemical parameters were monitored regularly to ensure optimal water quality.Following acclimatization, fingerlings were transferred to a departmental water tank for further growth. Once they attained an average weight of 6–8 g, healthy fish were reintroduced into laboratory aquaria and maintained under controlled conditions for 15 days. To assess biological capacity and ensure survival, the fishes were maintained under natural, non-toxic conditions, minimizing stress and avoiding premature mortality.

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The acute toxicity of the insecticide Pirimicarb was assessed using 20-liter plastic troughs. Acclimatized fish were divided into 10 groups (nine experimental and one control) and transferred into troughs containing 20 litres of water. Test troughs were treated with graded concentrations of Pirimicarb 0.0175, 0.019, 0.0205, 0.022, 0.0235, 0.025, 0.0265, 0.028, and 0.0295 ppm, prepared by diluting a 1 ppm stock solution. The control group was maintained under identical conditions in aerated, toxicant-free water. During the experimental period, neither control nor test groups were fed.

Water in both control and experimental troughs was renewed every 24 h with freshly prepared solutions at the respective concentrations. Mortality was recorded at 24, 48, 72, and 96 h, and dead fish were removed immediately to prevent water quality deterioration. After 96 h, mortality ranged between 0% and 50%, depending on concentration. The toxicity experiment was replicated five times under identical conditions. The relationship between Pirimicarb concentration and mortality was analysed using linear regression, and LC_{50} values were calculated by probit analysis following the method of Finney (1971).

The interaction between toxicants and biomolecules represents the primary step in eliciting toxic effects. Toxicant exposure can disrupt biochemical pathways involving carbohydrates, proteins, and lipids, which serve as key metabolic substrates. Under toxic stress, these macromolecules undergo significant alterations, reflecting changes in energy metabolism and cellular function. In the present study, biochemical analyses were performed to evaluate total protein, total lipid, and glycogen content under conditions of acute and chronic exposure.For biochemical assays, after 96 h of Pirimicarb exposure, fish from the control, LC_0, and LC_{50} groups were sacrificed. Fresh tissues of gill, liver, muscle, and brain were carefully excised, weighed, and homogenized for biochemical estimation.

Estimation of Glycogen (DeZwaan and Zandee, 1972)

The total glycogen content in various tissues of freshwater fish *Cirrhinus mrigala* was estimated following the method of DeZwaan and Zandee (1972) after exposure to the insecticide Pirimicarb. After 96 hours of exposure, fresh tissues from vital organs, including liver, muscle, brain, and gill, were collected from both control and experimental groups subjected to acute toxicity.Tissues were pooled and homogenized in 30% KOH solution, boiled in a water bath for 5 minutes, and then cooled to room temperature. Glycogen was precipitated by adding 2 ml of absolute ethanol, followed by overnight incubation at 4°C. The homogenate was centrifuged at 3000 rpm for 15 minutes, and the supernatant was discarded. The resulting pellet was suspended in 20 ml distilled water and incubated at 70°C for 5 minutes. This homogenate extract was used for glycogen estimation.

For the assay, 0.1 ml of the homogenate was mixed with 0.9 ml distilled water in test tubes. Tubes were cooled in ice water, and 3 ml of enthrone reagent was added. Samples were then boiled for 10 minutes in a water bath and cooled under running tap water. The optical density was measured at 660 nm against a blank containing 0.1 ml distilled water, 3 ml Lowry's C solution, and 0.5 ml of 50% Folin–Ciocalteu reagent.A standard curve was constructed using D-glucose according to DeZwaan and Zandee (1972), and total glycogen content was calculated from the standard curve. Results were expressed as µg of glycogen per mg of wet tissue weight µg/mg wet weight.

III. RESULT

The health, distribution, abundance, movement, production, and diversity of fish are influenced by a combination of biotic and abiotic factors. Among these, the physicochemical parameters of water play a critical role in regulating biological activity in aquatic organisms. In the present study, water quality parameters like temperature, pH, dissolved oxygen, dissolved carbon dioxide, hardness, nitrate, and phosphatewere analysed following the standard methods of APHA (1985).The effect of Pirimicarb on total glycogen content in various tissues (liver, muscle, brain, and gill) of freshwater fish *Cirrhinus mrigala* was evaluated after 96 hours of exposure in control, LC_0, and LC_{50} concentration groups (Table 1, Graph 1). In control fish, glycogen content followed the order: Liver > Muscle > Brain > Gill.

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In the liver, glycogen content was highest in the control group (78.24 ± 1.52 µg/mg wet weight), decreased in the LC_0 group (50.25 ± 2.45 µg/mg), and was lowest in the LC_{50} group (33.41 ± 1.85 µg/mg). Muscle glycogen similarly decreased from 50.66 ± 3.16 µg/mg in controls to 41.82 ± 1.20 µg/mg in LC_0 and 35.24 ± 2.11 µg/mg in LC_{50} fish. Brain glycogen declined from 47.46 ± 1.22 µg/mg in controls to 40.10 ± 1.13 µg/mg in LC_0 and 32.15 ± 1.01 µg/mg in LC_{50}. In gill tissues, glycogen content decreased from 47.46 ± 1.22 µg/mg in controls to 40.13 ± 1.13 µg/mg in LC_0 and 32.15 ± 1.01 µg/mg in LC_{50}.

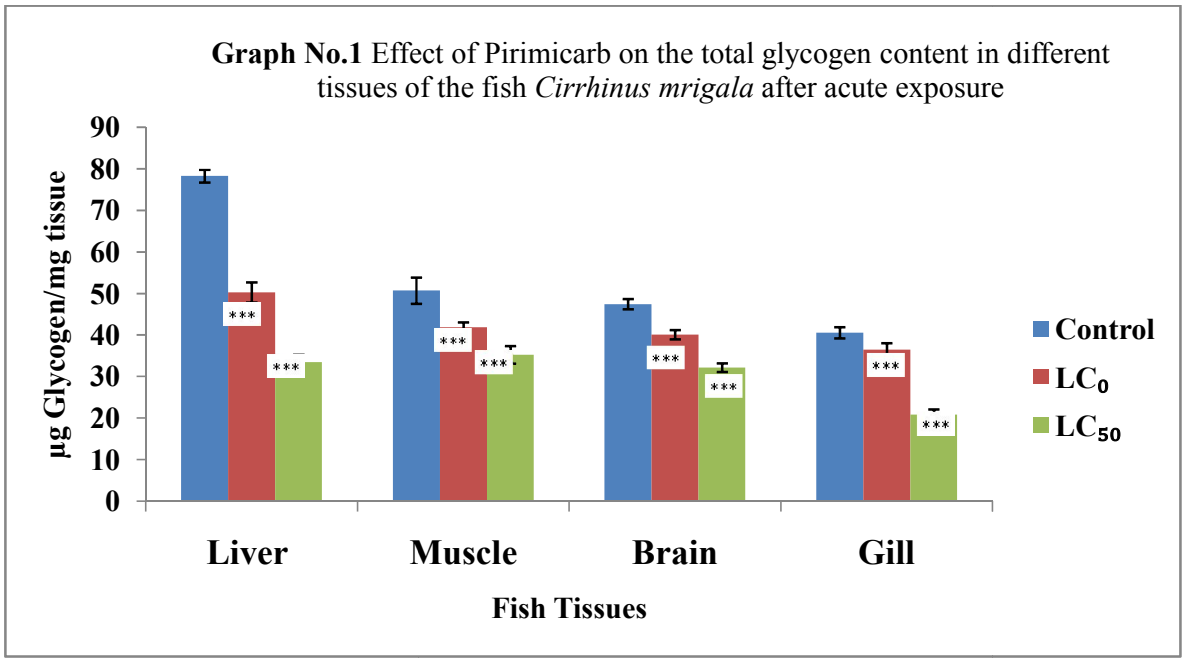
Overall, total glycogen content in liver, muscle, brain, and gill tissues of *Cirrhinus mrigala* was significantly reducedin both LC_0 and LC_{50} Pirimicarb-exposed groups compared to controls, with the differences being highly significant $p < 0.001$. These results indicate that Pirimicarb induces a dose-dependent depletion of glycogen, reflecting altered carbohydrate metabolism under acute toxic stress.

1)

Table No. 1 Effect of Pirimicarb on the total glycogen content in different tissues of the fish *Cirrhinus mrigala* after acute exposure.

Groups	Amount of total glycogen (µg glycogen/mg wet wt. of tissue)			
	Liver	Muscle	Brain	Gill
Control Group	78.24±1.52	50.66±3.16	47.46±1.22	41.54±1.34
LC_0	50.25±2.45 ***	41.82±1.20 ***	40.10±1.13 ***	36.47±1.58 ***
LC_{50}	33.41±1.85 ***	35.24±2.11 ***	32.15±1.01 ***	20.85±1.22 ***

(Values expressed as Arithmetic Mean of (n=5); ±SD) *=P<0.05; **=P<0.01; ***=P<0.001; NS= > 0.05).



(Values expressed as Arithmetic Mean of (n=5); ±SD, *** indicate P<0.001).

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IV. DISCUSSION

Water is the most abundant and essential compound in the environment, critical for the survival, growth, and physiological functioning of all organisms. However, increasing global population, industrialization, and the extensive use of chemical fertilizers in agriculture have led to significant contamination of natural water resources (Ali, 2010). Monitoring the chemical composition of water is therefore essential to prevent waterborne diseases and mitigate the adverse effects of toxicants on biological systems (Sen et al., 2010). Understanding biological activity in aquatic environments is challenging, as chemical parameters influence ecosystem functionality and provide insight into hydro-biological interactions (Zainudin, 2005).

Carbohydrate-based glycogen, stored in tissues, serves as a vital energy reserve that can be mobilized during stress (Stellingwerff and Boit, 2007). Glucose and glycogen are primary sources of energy for essential body functions (Jequier, 1994), and carbohydrates are considered the first organic nutrient to be depleted under stress conditions (Surai et al., 2019). Animals store glucose in the form of glycogen, a branched polysaccharide, which contributes to the glucose cycle and can be rapidly mobilized to meet urgent energy demands (Meurant, 2012; Dienel and Cruz, 2015).Glycogen plays a central role in carbohydrate metabolism, and alterations in glycogen levels are among the most notable biochemical effects induced by insecticide exposure (Cruz et al., 2017). In animals, glycogen is primarily stored in the liver and muscle (Flatt, 1995). Liver glycogen functions to store and export hexose units for blood glucose regulation (Hers, 1990), whereas muscle glycogen provides an accessible source of hexose units for glycolysis within muscle tissue (Akram et al., 2011). Brain tissue, although metabolically active, lacks significant glycogen stores and relies almost entirely on blood glucose (Mizock, 1995).A reduction in glycogen levels under toxic stress indicates rapid utilization to meet elevated energy demands through glycolysis or the hexose monophosphate pathway. Additionally, insecticides may inhibit hormones involved in glycogen synthesis, contributing further to the observed depletion (Radziuk and Pye, 2001).

The observed decline in glycogen levels in fish tissues is consistent with previous reports. Verma and Tonk (1983) documented reduced glycogen content in the liver and muscle of fish exposed to mercury. Jagadeesan (1994) reported significant decreases in glycogen in the muscle, gut, brain, and kidney tissues of *Labeorohita* following exposure to mercuric chloride. Similarly, Borah (2005) observed a continuous reduction in glycogen levels in *Heteropneustesfossilis* tissues exposed to petroleum oil. Palanismy et al. (2011) reported decreased glycogen content in the liver, brain, muscle, and gill tissues of *Mystuscavasius* following brief exposure to chromium-containing electroplating effluent. Thenmozhi (2011) noted a progressive decline in liver, gill, and muscle glycogen in *Labeorohita* during malathion exposure.

DeZwaan and Zandee (1972) suggested that reduced tissue glycogen levels may result from both inhibited synthesis and enhanced breakdown under toxic stress. Reddy et al. (1993) further noted that carbohydrate depletion reflects increased glycogenolysis and glycolytic activity, generating additional energy under stress. Endosulfan exposure also caused decreased glycogen levels in multiple tissues of *Clariasbatrachus* (Asifa and Vasantha, 1994). Carbamate insecticides were shown to significantly reduce glycogen in the liver, muscle, kidney, and gills of *Oreochromismossambicus*, likely due to enhanced glycogenolysis and decreased tissue glucose (Arockia Rita and John Milton, 2006). Additional mechanisms include conversion of carbohydrates to amino acids for protein synthesis (Ezemonye et al., 2007) and increased energy demand for cellular metabolism under toxic stress (Martin and Arivoli, 2008). Enzymatic disruption in glucose metabolism has also been implicated in glycogen depletion across critical tissues (Nagaraju and VenkataRathnamma, 2013). Tripathi and Yadav (2015) further emphasized that rapid glycogen reduction signals activation of stress-responsive pathways in response to elevated toxicant levels.

In the present study, exposure of *Cirrhinus mrigala* to lethal and sub-lethal concentrations of Pirimicarb resulted in significant alterations in total glycogen levels, reflecting toxic stress and disruption of enzymes involved in carbohydrate metabolism.

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Summary

The present study investigated the effect of acute and sub-lethal exposure to the insecticide Pirimicarb on glycogen metabolism in the freshwater fish *Cirrhinus mrigala*. Total glycogen content was measured in key tissues, including liver, muscle, brain, and gill. Results demonstrated a significant, dose-dependent decline in glycogen levels in all tissues of exposed fish compared to controls. The liver exhibited the highest glycogen content, followed by muscle, brain, and gill, consistent with the primary storage sites of carbohydrate reserves. Glycogen depletion under Pirimicarb stress reflects enhanced glycogenolysis and glycolytic activity to meet increased energy demands. These findings align with previous reports showing reduced glycogen in fish tissues under exposure to various toxicants, including heavy metals, pesticides, and industrial effluents, indicating that glycogen serves as a sensitive biochemical marker of toxic stress.

V. CONCLUSION

Exposure to Pirimicarb induces significant alterations in carbohydrate metabolism in *Cirrhinus mrigala*, as evidenced by marked reductions in glycogen levels across liver, muscle, brain, and gill tissues. The observed glycogen depletion likely results from enhanced energy utilization, disrupted enzymatic activity and impaired glycogen synthesis under toxic stress. These results highlight glycogen as a reliable biomarker for assessing sub-lethal and acute toxic effects of insecticides in freshwater fish. The study emphasizes the potential ecological risks posed by Pirimicarb contamination in aquatic environments and underscores the importance of monitoring biochemical responses to better understand pesticide-induced metabolic stress in non-target organisms.

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