Haematological and Biological Response in the Freshwater Fish Cyprinus Carpio Exposed to Chlorpyrifos

Anil Kumar
Deptt of Zoology, Janta Vedic College,Barant (Baghpat) 250611

Abstract
Exposure of *Cyprinus carpio* (common carp) to sublethal concentrations of the organophosphate insecticide chlorpyrifos produces measurable haematological and biological perturbations. Reported responses across laboratory studies include significant alterations in red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV), and white blood cell (WBC) profiles, together with suppression of acetylcholinesterase (AChE), increased oxidative stress (lipid peroxidation), changes in antioxidant enzymes, and histopathological lesions in gill, liver and kidney. The direction and magnitude of blood- and tissue-level changes are exposure- and time-dependent, and frequently correlate with AChE inhibition and markers of oxidative injury. These integrated endpoints make haematology a sensitive tool for sublethal chlorpyrifos effects and for ecological risk assessment in freshwater systems.

Keywords:
chlorpyrifos;Cyprinuscarpio;haematology;
oxidative stress; acetylcholinesterase

Introduction

Chlorpyrifosis a broad-spectrum organophosphate insecticide extensively applied in agriculture to control a wide range of insect pests. Owing to its widespread use, chlorpyrifos frequently enters freshwater ecosystems through agricultural runoff, spray drift and leaching, posing a significant risk to non-target aquatic organisms, particularly fish (Singh & Sharma, 2017; Zhang et al., 2023). Even at low concentrations, chlorpyrifos has been reported to induce sublethal physiological disturbances that compromise fish health and survival.

The primary mechanism of chlorpyrifos toxicity involves inhibition of acetylcholinesterase (AChE), an enzyme essential for terminating nerve impulses by hydrolyzing acetylcholine at synaptic junctions. Inhibition of AChE leads to excessive accumulation of acetylcholine, resulting in continuous nerve stimulation, neuromuscular dysfunction and behavioral abnormalities in exposed fish (WHO, 1997; Ural et al., 2013). However, growing evidence suggests that chlorpyrifostoxicity extends beyond neurotoxicity to include oxidative stress, metabolic imbalance and tissue damage.

Freshwater fish are particularly vulnerable to pesticide contamination because of their intimate and continuous contact with the aquatic environment. Fish readily absorb dissolved pesticides through gills, skin and gastrointestinal tract, leading to bioaccumulation and physiological stress even when environmental concentrations are below lethal levels (Mishra & Mohanty, 2008). Sublethal effects, though not immediately fatal, may impair vital functions such asrespiration, immunity, growth and reproduction.

The common carp, *Cyprinus carpio*, is one of the most widely distributed freshwater teleosts and holds immense ecological and economic importance due to its role in capture fisheries and aquaculture. Its hardiness, omnivorous feeding habit and tolerance to varying environmental conditions make it a preferred model organism in ecotoxicological investigations (Talwar & Jhingran, 1991; Banerjee et al., 2016). Consequently, *C. carpio* is frequently used to assess pesticide-induced physiological and biochemical alterations.

Haematological parameters are widely recognized as sensitive indicators of environmental stress in fish. Variables such as red blood cell count, haemoglobin concentration, packed cell volume and leukocyte profile are directly linked to oxygen transport, immune competence and metabolic efficiency. Alterations in these parameters provide early warning signals of toxic stress before the manifestation of visible pathological symptoms or mortality (Wedemeyer & Yasutake, 1977; Blaxhall & Daisley, 1973).

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Several studies have reported chlorpyrifos-induced changes in haematological indices of freshwater fishes. Reductions in red blood cell count, haemoglobin content and packed cell volume have often been attributed to haemolysis, suppression of erythropoiesis or oxidative damage to erythrocyte membranes, leading to anaemia-like conditions (Natarajan, 1984; Yonar et al., 2018). Conversely, some investigations have reported increased haematocrit values, interpreted as compensatory responses to hypoxic stress caused by gill damage.

Leukocyte responses to chlorpyrifos exposure are equally significant, as white blood cells play a crucial role in immune defense. Both leukocytosis and leukopenia have been documented in exposed fish, reflecting stress-induced immune activation or immunosuppression depending on exposure concentration and duration (Singh & Srivastava, 2010; Sinha et al., 2022). Such immunological alterations may increase susceptibility to pathogens and reduce overall fitness.

In addition to haematological effects, chlorpyrifos exposure is known to induce oxidative stress by generating reactive oxygen species that overwhelm antioxidant defense systems. Elevated lipid peroxidation levels and altered activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase have been reported in chlorpyrifos-exposed carp, linking oxidative damage to cellular and tissue-level dysfunction (Ince et al., 2013; Ural et al., 2013).

Histopathological studies further support the systemic toxicity of chlorpyrifos. Structural alterations in gills, liver and kidney—including lamellar fusion, hepatic vacuolation and renal tubular degeneration—have been documented in *C. carpio*, impairing respiration, detoxification and osmoregulation and thereby contributing to observed haematological disturbances (Altun et al., 2017; Hossain et al., 2022).

The severity of chlorpyrifos-induced effects is strongly influenced by exposure concentration and duration. Acute exposures typically elicit rapid neurochemical and haematological changes, while subacute and chronic exposures lead to progressive biochemical imbalance, tissue damage and compromised growth performance (Sprague, 1973; Verma et al., 2014). These time-dependent responses underscore the importance of evaluating both short- and long-term effects.

Integration of haematological parameters with biochemical and histopathological endpoints provides a comprehensive understanding of pesticide toxicity in fish. Such multi-biomarker approaches enhance the reliability of ecological risk assessment and help elucidate mechanisms underlying sublethal toxicity (Sinha et al., 2022).

Despite extensive research, gaps remain regarding standardized dose–response relationships, recovery potential and long-term ecological implications of chlorpyrifos exposure in freshwater fish. Understanding these aspects is essential for establishing environmentally safe pesticide application practices. Therefore, the present study focuses on synthesizing available information on haematological and biological responses of *Cyprinus carpio* exposed to chlorpyrifos, with emphasis on their relevance to freshwater ecosystem health and environmental monitoring.

Methodology

The methodology adopted to evaluate the haematological and biological responses of the freshwater fish *Cyprinus carpio*to chlorpyrifos exposure is based on standardized laboratory bioassay procedures widely employed in fish ecotoxicology. Healthy and active specimens of *C. carpio* of nearly uniform size and weight are procured from local hatcheries or uncontaminated freshwater bodies and transported to the laboratory under minimal stress conditions. Fish are acclimatized for a period of 15–30 days in large glass aquaria containing dechlorinated tap water, during which unhealthy or injured individuals are removed. Only disease-free fish showing normal swimming and feeding behavior are selected for experimentation (APHA, 2017; Banerjee et al., 2016).

During acclimatization, fish are maintained under controlled physicochemical conditions, including temperature, dissolved oxygen, pH and natural photoperiod. Water is renewed regularly to prevent accumulation of metabolic wastes, and fish are fed a standard commercial or natural diet. Feeding is discontinued 24 hours prior to the start of exposure to avoid post-feeding variations in haematological parameters, as recommended in earlier toxicological studies (Natarajan, 1984; Sinha et al., 2022).

Chlorpyrifos, either in analytical grade or commercial formulation, is used as the test toxicant. A stock solution is prepared by dissolving a known quantity of chlorpyrifos in distilled water, and appropriate dilutions are made to obtain the desired experimental concentrations. Sublethal concentrations are selected based on previously reported 96-hour LC_{50} values for *C.*

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carpio or related freshwater fishes, commonly using fractions such as 1/10 or 1/20 of the LC_{50} value to ensure non-lethal exposure while inducing physiological stress (Sprague, 1973; Verma et al., 2014).

Exposure experiments are conducted using static or semi-static bioassay systems. In static systems, the test solution is maintained throughout the exposure period, whereas in semi-static systems, the test medium is renewed every 24 hours to maintain pesticide concentration and water quality. Exposure durations typically range from 24 to 96 hours for acute sublethal studies, while subacute experiments may extend up to 14–21 days to assess prolonged effects (Ural et al., 2013; Hossain et al., 2022).

Each experimental group generally consists of 6–10 fish per aquarium, with at least three replicates per treatment. A control group is maintained simultaneously under identical conditions without the addition of chlorpyrifos. Fish are observed daily for behavioral changes such as erratic swimming, reduced feeding and surfacing behavior, as well as for any mortality, to confirm that the selected concentrations remain sublethal (APHA, 2017).

At the end of the exposure period, fish are anesthetized using approved anesthetics such as MS-222 or clove oil to minimize handling stress. Blood samples are collected either from the caudal vein or by cardiac puncture using sterile disposable syringes. For haematological analysis, blood is transferred into vials containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant to prevent clotting (Blaxhall & Daisley, 1973).

Haematological parameters analyzed include total erythrocyte count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV) and total leukocyte count (WBC). RBC and WBC counts are performed using a haemocytometer, haemoglobin concentration is estimated by Sahli's or spectrophotometric methods, and PCV is determined using the microhaematocrit method. These parameters provide reliable indicators of oxygen transport efficiency and physiological stress in fish (Blaxhall & Daisley, 1973; Wedemeyer & Yasutake, 1977).

Derived erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are calculated using standard formulae based on RBC, Hb and PCV values. These indices help characterize the nature of haematological disturbances such as hypochromic or macrocytic anemia induced by chlorpyrifos exposure (Natarajan, 1984; Sinha et al., 2022).

Differential leukocyte counts are carried out by preparing thin blood smears, staining them with Giemsa or Wright's stain, and examining them under a light microscope. Percentages of lymphocytes, neutrophils, monocytes, eosinophils and basophils are recorded to assess immunological alterations resulting from pesticide-induced stress (Singh & Srivastava, 2010; Sinha et al., 2022).

To complement haematological findings, biochemical parameters such as plasma glucose, total protein and selected antioxidant enzymes may also be analyzed following standard protocols. These parameters help establish links between metabolic stress, oxidative damage and blood profile alterations in chlorpyrifos-exposed fish (Ince et al., 2013; Ural et al., 2013).

Statistical analysis of the data is performed using appropriate statistical software. Results are expressed as mean ±standard deviation, and differences between control and treated groups are evaluated using one-way analysis of variance (ANOVA) followed by suitable post hoc tests. A probability level of $p < 0.05$ is considered statistically significant (Zar, 2014).

All experimental procedures are conducted in accordance with institutional and national guidelines for the care and use of laboratory animals. Ethical considerations such as humane handling, use of anesthesia and minimization of animal suffering are strictly followed, ensuring the scientific reliability and ethical acceptability of the study (CPCSEA, 2018; Sinha et al., 2022).

Results

Across the literature up to 2024, chlorpyrifos exposure consistently alters haematological and biological endpoints in *Cyprinus carpio*. The following paragraphs synthesize reported trends and illustrate them with two literature-synthesis tables.

Many studies report **decreased RBC counts, haemoglobin concentration and PCV** after sublethal or acute chlorpyrifos exposure—findings interpreted as haemolytic damage, impaired erythropoiesis, or blood loss secondary to tissue damage. Yonar and co-workers and other authors documented such reductions alongside evidence for oxidative damage and AChE inhibition.

White blood cell responses are variable: some experiments show **leukocytosis** (stress-related

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mobilisation), while others report **leukopenia** or reductions in specific leukocyte classes consistent with immunosuppression. Differential counts often indicate lymphocyte decreases and neutrophil or monocyte changes, reflecting inflammatory responses or immune compromise.

Erythrocyte morphology and indices (MCV, MCH, MCHC) reveal chlorpyrifos-associated changes: increased erythrocyte abnormalities (membrane blebbing, nuclear irregularities), decreased MCHC (hypochromia) and sometimes increased MCV (macrocytosis) during recovery phases. Blood smear examinations provide direct evidence of erythrocyte membrane and nuclear damage.

Acetylcholinesterase activity is robustly and dose-dependently inhibited in brain and plasma of carp following chlorpyrifos exposure; AChE inhibition often correlates with behavioral signs (reduced feeding, erratic swimming), and with downstream biochemical and haematological disruptions. Measurement of AChE therefore remains a cornerstone mechanistic assay.

Oxidative stress biomarkers are frequently elevated: tissue MDA (a marker of lipid peroxidation) increases while SOD/CAT activities often change (either compensatory increases or later declines), and GSH is depleted with prolonged exposures. These patterns support oxidative membrane damage as a contributor to erythrocyte fragility and organ pathology.

Histopathological studies report gill lamellar fusion, epithelial lifting, hepatic vacuolation, inflammatory infiltration and renal tubular degeneration in chlorpyrifos-exposed carp. Such lesions compromise respiration and detoxification, plausibly contributing to hypoxia and haematological compensatory responses.

Growth and condition indices decline in some subchronic exposures: reduced weight gain and feed conversion efficiency have been documented at environmentally relevant sublethal chlorpyrifos concentrations, indicating energetic costs of detoxification and repair. These biological effects complement blood and biochemical markers in indicating reduced fitness.

Recovery studies show partial to full restoration of some endpoints (AChE, some haematological values) after termination of exposure, but the recovery timeline is variable and dependent on exposure level and duration—supporting the need for chronic and post-exposure follow-up in risk assessment.

Table 1 — Summary of selected haematological findings in *Cyprinus carpio* after chlorpyrifos exposure (literature synthesis)

Study (year)	Exposure (conc, duration)	RBC	Hb	PCV	WBC	Notes
Yonar et al. (2018)	sublethal (varied), 7–14 d	↓	↓	↓	variable	↓ RBC/Hb; oxidative stress measured
Ural et al. (2013)	sublethal, 7 d	↓	↓	↓	↓	AChE inhibition; altered SOD/CAT
Hossain et al. (2022)	sublethal, 21 d	↓	↓	↓	↑/↓	histopathology (gill, liver) reported
Mişe Yonar (2022)	sublethal + ameliorant trials	↓	↓	↓	variable	ellagic acid ameliorated oxidative effects

(Table compiled from cited studies; directional arrows indicate net reported change relative to controls.)

Table 2 — Biochemical, oxidative and histopathological responses associated with chlorpyrifos exposure (literature synthesis)

Endpoint	Typical response	Representative studies
AChE activity	Strong dose-dependent ↓	Ural et al. (2013); Xing et al. (2012) summarized.
Lipid peroxidation (MDA)	↑ (marker of oxidative damage)	Yonar et al. (2018); Nunes et al. (2018).

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Antioxidant enzymes (SOD/CAT/GPx)	**Early ↑ (compensatory) → later ↓ with prolonged damage**	**Ural et al. (2013); Mişe Yonar (2022).**
Histopathology	**Gill lamellar damage; hepatic vacuolation; renal tubular changes**	**Altun et al. (2017); Hossain et al. (2022).**

(Table based on synthesis of experimental reports cited above.)

Discussion and Conclusion

The compiled evidence up to 2024 shows that chlorpyrifosproduces reproducible haematological and biological disturbances in *Cyprinus carpio*. Decreases in RBC, haemoglobin and PCV are among the most frequently reported haematological outcomes and are consistent with erythrocyte membrane damage, impaired erythropoiesis, or blood loss secondary to tissue pathology. These blood changes often occur alongside AChE inhibition and increased lipid peroxidation, linking neurotoxicity with oxidative and systemic injury.

Variability among studies (direction of WBC change, magnitude of erythron shifts) underscores the influence of experimental design: concentration, exposure duration, life stage, fish condition, water chemistry and use of commercial vs analytical formulations. This heterogeneity argues for standardized protocols (acclimatization length, sampling times, consistent haematology methods) to make inter-study comparisons more robust.

The frequent co-occurrence of AChE inhibition and oxidative stress indicates multiple interacting pathways of chlorpyrifos toxicity. AChE inhibition causes behavioral and physiological stress that increases metabolic demand, while oxidative damage undermines cellular membrane integrity (including erythrocytes), together producing the observed haematological outcomes. Measuring a suite of biomarkers (AChE, LPO, SOD/CAT, haematology, histopathology) therefore provides a mechanistic and sensitive approach for sublethal toxicity detection.

From an ecological and management standpoint, sublethal blood and biochemical effects can impair growth, reproduction and disease resistance in carp populations exposed to chlorpyrifos in agricultural landscapes. Regulatory frameworks should consider sublethal biomarker-based thresholds (not only mortality-based LC50) when evaluating pesticide application near freshwater habitats.

Research priorities include standardized dose-response datasets across life stages, longer-term recovery studies to determine reversibility of effects, and field studies that link measured environmental concentrations to biomarker responses in wild carp populations. Such efforts will strengthen ecological risk assessments and inform mitigation (buffer zones, application timing) to protect freshwater fish.

In conclusion, haematological assays combined with biochemical and histopathological measures provide a sensitive, integrative framework to detect and interpret sublethal chlorpyrifos impacts on *Cyprinus carpio*. Adoption of standardized protocols and incorporation of these biomarkers into monitoring programs will improve early detection of contamination effects and support more protective pesticide management in freshwater systems.

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