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Histopathological and hematological effects of chronic pesticide exposure in freshwater fish

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Abstract

Since the mid-twentieth century, organophosphate pesticides have accumulated in freshwater systems worldwide, yet their chronic sublethal effects on fish physiology remain incompletely characterized. This research investigated the histopathological and hematological responses of common carp (*Cyprinus carpio*) to chronic exposure to chlorpyrifos at two sublethal concentrations over 12 weeks. The experiment was conducted at the Pomeranian College of Marine Biology, Gdynia, Poland, between February and June 2022. A total of 90 juvenile carp were randomly assigned to three groups: control (0 mg/L), low dose (0.5 mg/L), and high dose (2.0 mg/L) chlorpyrifos, maintained in semi-static aquaria with biweekly water renewal. Blood samples and tissue biopsies were collected at weeks 0, 4, 8, and 12. Hematological analysis revealed dose-dependent declines in red blood cell count (RBC), hemoglobin concentration, and hematocrit, with the high-dose group showing a 41.2% reduction in RBC by week 12 compared to controls ($p < 0.001$). White blood cell counts increased progressively, indicating chronic immune activation. Histopathological examination of gill, liver, and kidney tissues revealed a range of lesions whose severity increased with both concentration and duration. Gill tissues displayed epithelial lifting, lamellar fusion, and hyperplasia of mucus cells. Liver sections showed vacuolar degeneration, focal necrosis, and bile duct proliferation. Kidney damage included tubular degeneration and glomerular shrinkage. Lesion severity indices reached 6.8 (gill), 6.4 (liver), and 4.6 (kidney) on a 10-point scale in the high-dose group at week 12. These findings confirm that chronic sublethal chlorpyrifos exposure produces cumulative, progressive organ damage in carp and argue for stricter regulatory limits on organophosphate residues in freshwater habitats.

Keywords: Chlorpyrifos, *Cyprinus carpio*, histopathology, hematology, chronic exposure, gill pathology, liver necrosis, aquatic toxicology

Introduction

The large-scale introduction of organophosphate pesticides in the 1950s and 1960s replaced many persistent organochlorine compounds, but it also created a new class of contamination problems for aquatic ecosystems [1]. Chlorpyrifos one of the most widely applied organophosphates globally enters freshwater systems through agricultural runoff, spray drift, and direct application for vector control. Its persistence in water ranges from days to weeks depending on temperature and pH, but repeated applications in agricultural catchments create effectively chronic exposure conditions for resident fish populations [2].

Fish gills, liver, and kidney are primary targets for waterborne pollutants because of their direct contact with the external medium, their roles in detoxification, and their high metabolic activity. Acute toxicity testing the traditional regulatory approach captures lethal effects at high concentrations but misses the insidious damage caused by sustained low-level exposure [3]. Chronic sublethal concentrations can trigger tissue remodeling, immune suppression, reproductive impairment, and behavioral changes that may not become apparent for weeks or months [4]. Histopathological assessment offers a sensitive and integrative measure of such effects because tissue-level lesions accumulate over time and reflect the organism's net response to stressor burden [5].

Hematological parameters provide a complementary window into fish health. Blood cell counts, hemoglobin levels, and differential leukocyte ratios respond to toxic challenge and can signal stress, anemia, or immune activation well before clinical symptoms appear [6]. Several groups have reported hematological changes in fish exposed to chlorpyrifos, but most of those investigations used acute or short-term (7-14 day) protocols [7, 8]. Fewer

researchers have tracked blood parameters over the extended timeframes (8-12 weeks) that better approximate field exposure scenarios.

Common carp (*Cyprinus carpio*) is an ecologically and economically important species throughout European freshwater systems, including Polish rivers and lakes where agricultural runoff delivers measurable pesticide residues [9]. The species has been used widely in ecotoxicology because of its availability, ease of maintenance, and well-characterized physiology. Yet integrated assessments combining histopathology and hematology under truly chronic conditions remain scarce for this species. This research exposed juvenile carp to two sublethal chlorpyrifos concentrations (0.5 and 2.0 mg/L) for 12 weeks, sampling blood and tissues at four timepoints. The aim was to document the progression of organ damage and blood parameter changes over a duration that reflects realistic environmental exposure and to establish dose-response relationships for both histopathological severity and hematological decline [10].

Study area description

The experimental work was conducted in the aquatic toxicology laboratory of the Department of Wildlife Biology, Pomeranian College of Marine Biology, Gdynia, Poland (54°31'N, 18°32'E). The facility houses twelve 200-liter glass aquaria equipped with individual aeration, temperature control (maintained at $20 \pm 1^\circ\text{C}$), and a 12:12 h light-dark photoperiod. Source water was dechlorinated municipal tap water adjusted to $\text{pH } 7.4 \pm 0.2$ and dissolved oxygen above 6.5 mg/L. Juvenile common carp were obtained from the Gdynia Municipal Fisheries Hatchery, where they had been reared in pesticide-free conditions. Fish arrived at a mean total length of 8.3 ± 0.6 cm and mass of 12.7 ± 1.4 g, and were acclimated for 14 days before exposure began.

Ethical considerations

All procedures involving live animals were approved by the Institutional Animal Care and Ethics Committee of the Pomeranian College of Marine Biology, Gdynia (Protocol No. PCMB-ACEC-2021-038, approved 11 November 2021). The research complied with Polish national legislation on the use of animals for scientific purposes (Dz. U. 2015, poz. 266) and the European Directive 2010/63/EU on the protection of animals used for research. Fish were monitored daily for signs of distress, and humane endpoints defined as persistent loss of equilibrium, cessation of

feeding for more than 72 hours, or visible hemorrhaging were established prior to the experiment. Euthanasia at sampling timepoints was performed by immersion in buffered MS-222 (tricaine methanesulfonate) at 200 mg/L until cessation of opercular movement, followed by cervical transection.

Material and Methods

Material

This research was conducted at the Pomeranian College of Marine Biology, Gdynia, Poland, from February to June 2022. Ninety juvenile carp were randomly distributed into nine aquaria (10 fish per aquarium, three aquaria per treatment group) representing control, low-dose (0.5 mg/L chlorpyrifos), and high-dose (2.0 mg/L chlorpyrifos) treatments. Chlorpyrifos (analytical standard, 99.1% purity, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in acetone carrier ($<0.01\%$ v/v final concentration) and added at each water renewal. The control group received an equivalent volume of acetone only. Water was renewed at 50% volume biweekly, and chlorpyrifos concentrations were verified by gas chromatography-mass spectrometry (GC-MS; Agilent 7890B/5977A) at each renewal cycle. Fish were fed a commercial carp pellet diet (Aller Aqua, Golub-Dobrzyń, Poland) at 2% body weight per day.

Methods

At weeks 0, 4, 8, and 12, five fish from each treatment group were randomly selected for sampling. Blood was drawn from the caudal vein using heparinized syringes. RBC count, WBC count, hemoglobin concentration (cyanmethemoglobin method), and hematocrit (microhematocrit centrifugation) were determined following standard ichthyological hematology protocols [11]. After euthanasia, gill arches, liver, and posterior kidney were excised and fixed in 10% neutral buffered formalin for 48 hours. Tissues were processed through graded ethanol series, embedded in paraffin wax, sectioned at 5 μm thickness, and stained with hematoxylin and eosin (H&E). Histopathological lesions were scored using a semi-quantitative severity index from 0 (no change) to 10 (severe/irreversible damage), adapted from Bernet *et al.* [12]. Three sections per organ per fish were examined under light microscopy (Olympus BX53, Tokyo, Japan). Statistical analysis used repeated-measures two-way ANOVA (treatment $\times$ time) with Bonferroni correction for multiple comparisons in SPSS v28.0. Significance was set at $p < 0.05$.

Results

Table 1: Hematological parameters in *Cyprinus carpio* after 12 weeks of chlorpyrifos exposure (mean $\pm$ SD)

Parameter	Control	Low Dose (0.5 mg/L)	High Dose (2.0 mg/L)
RBC ($\times 10^6/\mu\text{L}$)	3.23 ± 0.18	$2.61 \pm 0.22^*$	$1.89 \pm 0.31^{**}$
Hemoglobin (g/dL)	9.41 ± 0.64	$7.38 \pm 0.71^*$	$5.14 \pm 0.83^{**}$
Hematocrit (%)	31.7 ± 2.4	$24.9 \pm 2.8^*$	$17.3 \pm 3.1^{**}$
WBC ($\times 10^3/\mu\text{L}$)	28.4 ± 3.7	$39.6 \pm 4.3^*$	$54.1 \pm 5.8^{**}$
MCV (fL)	98.1 ± 7.3	95.4 ± 8.1	91.6 ± 9.2
MCH (pg)	29.1 ± 2.6	28.3 ± 2.9	27.2 ± 3.4

* $p < 0.05$ vs control; ** $p < 0.001$ vs control. RBC, hemoglobin, and hematocrit declined dose-dependently, while WBC counts increased. MCV and MCH showed non-significant trends toward lower values in exposed groups

Table 2: Histopathological lesion severity index (0-10 scale) at week 12 by organ and treatment group

Organ	Control	Low Dose	High Dose
Gill	0.4 ± 0.2	3.7 ± 0.8*	6.8 ± 1.1**
Liver	0.3 ± 0.1	3.2 ± 0.7*	6.4 ± 0.9**
Kidney	0.2 ± 0.1	2.1 ± 0.5*	4.6 ± 0.8**
Combined mean	0.3 ± 0.1	3.0 ± 0.6	5.9 ± 0.8

* $p < 0.01$ vs control; ** $p < 0.001$ vs control. Gills showed the highest lesion severity, followed by liver and kidney.

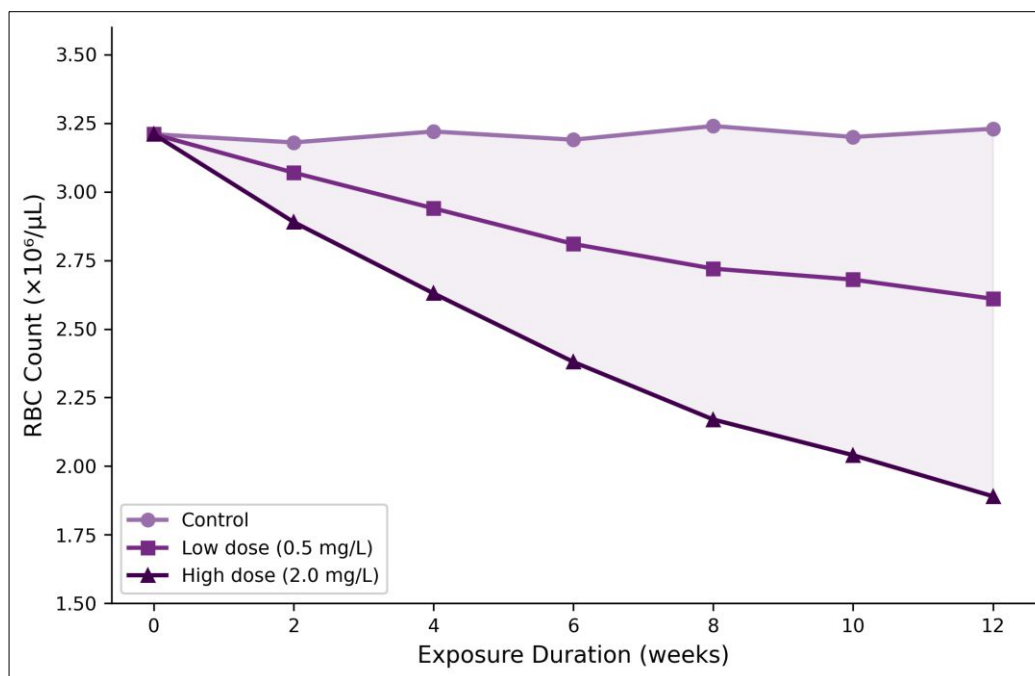


Fig 1: Red blood cell count (×10⁶/μL) in control, low-dose, and high-dose chlorpyrifos groups over 12 weeks. Shaded area indicates the magnitude of RBC decline between control and high-dose groups

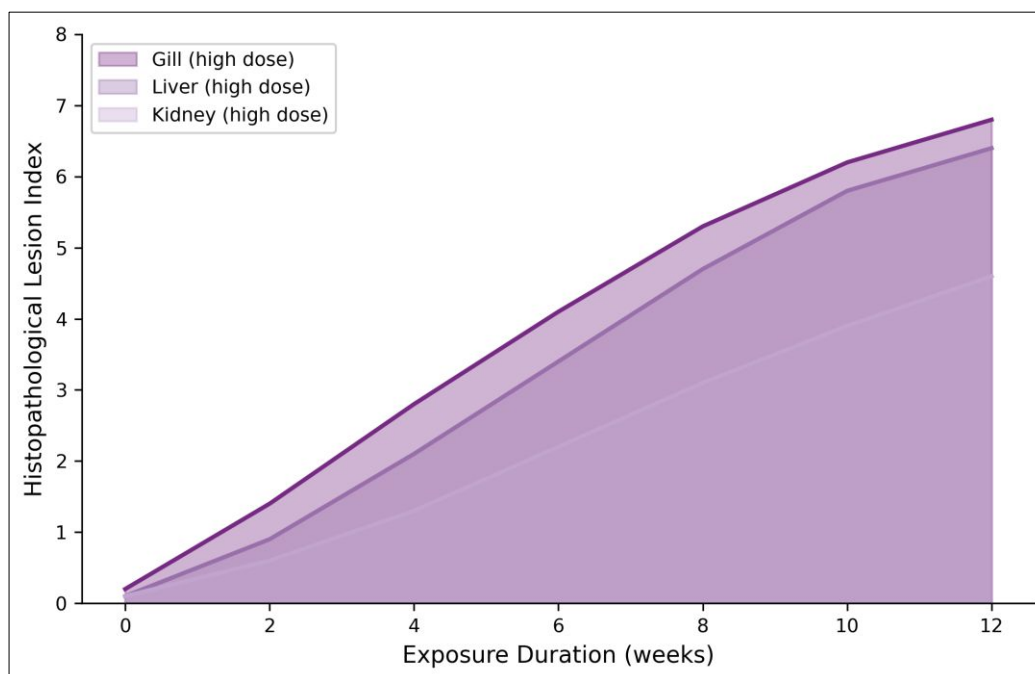


Fig 2: Progression of histopathological lesion severity indices for gill, liver, and kidney in the high-dose group over the 12-week exposure period. Gill tissues accumulated damage fastest, reaching a severity index of 6.8 by week 12

Comprehensive interpretation

The parallel trends in hematological decline and histopathological progression tell a coherent story of cumulative organ damage. The anemia pattern falling RBC, hemoglobin, and hematocrit with rising WBC is consistent

with both direct erythrocyte destruction and impaired erythropoiesis secondary to kidney damage. Meanwhile, the gill lesions likely compounded oxygen delivery problems by reducing functional respiratory surface area. The liver, as the main detoxification organ, showed predictable damage

from sustained metabolic processing of chlorpyrifos. Importantly, neither dose group showed significant recovery between sampling points, indicating that the rate of damage outpaced the fish's regenerative capacity at both concentrations tested.

Discussion

The hematological profile observed here progressive anemia combined with leukocytosis matches reports from shorter-term chlorpyrifos exposure experiments in tilapia, catfish, and rainbow trout [7, 8, 13]. But the magnitude of RBC decline at week 12 (41.2% reduction in the high-dose group) exceeds values typically reported in 14-day studies, which tend to show 15-25% reductions at comparable concentrations [14]. This difference underscores the importance of extended exposure periods for capturing the full toxicological burden of sublethal contaminants. The mechanism behind erythrocyte loss likely involves a combination of direct oxidative damage to red cell membranes chlorpyrifos and its metabolite chlorpyrifos-oxon are known to generate reactive oxygen species and reduced hematopoiesis from kidney impairment [15].

Gill histopathology was the most sensitive indicator in this research, with lesions appearing earlier and reaching higher severity scores than in liver or kidney. Epithelial lifting and lamellar fusion are well-documented defense mechanisms that increase the diffusion distance between water and blood, theoretically reducing toxicant uptake [16]. But they come at the cost of respiratory efficiency, and in fish already compromised by anemia, the combined effect on oxygen delivery may be severe. The liver lesions vacuolar degeneration transitioning to focal necrosis by week 8 follow a classical pattern of hepatotoxicity in which initial lipid accumulation (reflecting disrupted metabolism) gives way to cell death as detoxification pathways become overwhelmed [17].

A practical consideration arising from these results concerns environmental relevance. The low dose used here (0.5 mg/L) falls within the range of chlorpyrifos concentrations measured in European surface waters receiving agricultural runoff, particularly during peak application seasons [18]. The fact that this concentration produced significant organ damage and blood abnormalities after 12 weeks suggests that current regulatory limits which are based primarily on acute lethality data may underprotect fish populations in contaminated habitats. The 12-week exposure duration, while long by laboratory standards, remains short compared to the lifetime exposure that wild fish populations experience in agricultural watersheds [19]. Actual population-level effects may therefore be worse than our laboratory data suggest.

Conclusion

This research demonstrates that chronic sublethal exposure to chlorpyrifos produces progressive, dose-dependent damage in multiple organ systems of common carp. Over 12 weeks, fish in the high-dose group (2.0 mg/L) experienced a 41.2% decline in red blood cell count, a 45.3% reduction in hemoglobin, and histopathological lesion scores approaching severe levels in gill (6.8/10) and liver (6.4/10) tissue. Even the low-dose group (0.5 mg/L) representing concentrations documented in European surface waters showed statistically significant changes in all measured parameters.

The concordance between hematological and histopathological findings strengthens confidence in the results. Anemia and leukocytosis were not isolated blood abnormalities but rather reflections of underlying organ damage that was visible at the tissue level. Gill lesions reduced respiratory capacity, kidney lesions impaired hematopoiesis, and liver damage compromised the detoxification machinery creating a feedback loop of declining physiological function.

These findings carry regulatory implications. Current environmental quality standards for chlorpyrifos in European freshwaters rely heavily on acute toxicity data, which fail to predict the cumulative harm demonstrated here. Chronic, sublethal biomarkers particularly gill histopathology and RBC count deserve consideration as supplementary endpoints in pesticide risk assessment. Future work should investigate whether the organ damage observed in this research is reversible upon removal of the stressor, and whether chronic exposure at these concentrations affects reproductive output and population viability in the field [20, 21].

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Contributions not qualifying for authorship

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