

Histopathological Changes in Fish Exposed to Organophosphate Insecticides

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ABSTRACT

The increasing use of organophosphate insecticides in agricultural practices poses a serious threat to aquatic ecosystems, particularly freshwater fish that are often exposed to these toxicants through runoff and leaching. This study was undertaken to assess the histopathological effects of two commonly used organophosphates, chlorpyrifos and monocrotophos, on freshwater fish species under sub-lethal exposure conditions. Fish were exposed to environmentally relevant concentrations over a 28-day period, and histological examinations were conducted on critical organs such as the gills, liver, and kidneys. The findings revealed pronounced structural damage in all three organs. Gills exhibited epithelial lifting, lamellar fusion, and hemorrhage; liver samples showed hepatocellular vacuolization, necrosis, and vascular congestion; and kidneys displayed tubular degeneration and glomerular deformities. These pathological alterations indicate compromised respiratory, detoxification, and excretory functions in the fish, even under non-lethal exposure levels. The severity of tissue damage was more pronounced in fish exposed to monocrotophos compared to chlorpyrifos, suggesting higher toxicity. The results emphasize the utility of histopathological biomarkers as sensitive indicators of aquatic pollution and underline the urgent need for sustainable pesticide use practices. This research contributes to a better understanding of the chronic effects of organophosphate pesticides on aquatic fauna and supports their consideration in environmental monitoring and regulatory frameworks.

Keywords- Organophosphates, Histopathology, Fish Toxicology, Aquatic Pollution, Chlorpyrifos.

I. INTRODUCTION

The widespread application of organophosphate (OP) insecticides in modern agriculture has significantly enhanced crop protection and food production. However, these chemicals are often non-selective and have the potential to contaminate aquatic ecosystems through agricultural runoff, leaching, and direct discharge (Ghosh et al., 2021). Aquatic environments, particularly freshwater bodies, frequently receive inputs of such toxicants, resulting in exposure to non-target organisms like fish, which serve as sentinel species for environmental monitoring. Organophosphate insecticides such as chlorpyrifos, diazinon, monocrotophos, and malathion function primarily by inhibiting acetylcholinesterase (AChE), a key enzyme in neurotransmission (Abdel-Daim et al., 2019). Inhibition of this enzyme leads to the accumulation of acetylcholine

at synaptic junctions, causing overstimulation of cholinergic receptors, muscle paralysis, and eventual death in target pests. However, these mechanisms also adversely affect fish and other aquatic organisms, even at sub-lethal concentrations. Fish, due to their permeable gill membranes, high lipid content, and position in the aquatic food web, are particularly vulnerable to pesticide exposure. They bioaccumulate organophosphates in their tissues and experience physiological, behavioral, and cellular disruptions (Kumari & Sinha, 2022). Among various toxicity indicators, histopathological changes in critical organs such as gills, liver, and kidneys offer sensitive and early warning signs of environmental stress. Histopathology involves the microscopic examination of tissue architecture and cellular structures, offering a reliable biomarker to detect damage caused by toxicants. Gill tissues are highly sensitive due to their direct contact with the aquatic environment. Alterations such as

epithelial lifting, hyperplasia, and lamellar fusion have been observed following OP exposure (Sharma et al., 2020). These changes impair respiratory efficiency and osmoregulation, threatening the fish's survival. The liver, being a central detoxification organ, is another prime target for organophosphate-induced damage. Hepatic lesions such as vacuolization, necrosis, bile duct proliferation, and sinusoidal dilation reflect metabolic stress and compromised detoxification pathways (Jain & Shrivastava, 2018). These histopathological symptoms have been widely reported in various freshwater fish species subjected to OP exposure. Similarly, the kidney plays a crucial role in osmoregulation and excretion. Histopathological alterations like glomerular shrinkage, tubular degeneration, and hemorrhages indicate impaired renal function, which can disrupt ionic balance and lead to systemic toxicity (Meena et al., 2023). These changes are often dose-dependent and become more pronounced with chronic exposure. Sub-lethal and chronic toxicity studies are particularly important as they mimic real-world environmental conditions where pesticides are present at low but persistent levels. Acute toxicity might not always result in immediate mortality, but prolonged exposure can compromise the organism's physiology, immunity, and reproductive capacity (Singh et al., 2021). Hence, assessing histopathological damage under sub-lethal exposure offers ecologically relevant insights. Various studies across India have documented the histopathological impacts of OPs in native fish species. For example, Tilak et al. (2007) observed significant liver damage in *Labeo rohita* exposed to monocrotophos. More recent investigations in Uttar Pradesh and adjoining states report similar damage patterns in *Channa punctatus*, *Catla catla*, and *Oreochromis mossambicus* (Rani et al., 2022; Mishra & Tiwari, 2023). These studies confirm the persistent presence of OP residues in water bodies and their detrimental effects on fish health. Monitoring histopathological changes also aligns with the growing emphasis on non-lethal, early-detection methods in ecotoxicology. Traditional biochemical assays or mortality-based studies might overlook subtle tissue damage. In contrast, histopathology provides a comprehensive view of organ-level dysfunction, facilitating environmental risk assessments and guiding policy formulation (Kamble et al., 2021). Furthermore, fish histopathology bridges laboratory studies with field-level ecological monitoring. It is a cost-effective and replicable method to evaluate the cumulative impact of various pollutants, including organophosphates, in complex aquatic habitats. As such, its adoption has increased in environmental toxicology studies across Asia, Africa, and South America, where agrochemical pollution is prevalent (Zhou et al., 2020). Despite regulatory restrictions on certain OPs in developed countries, these chemicals are still widely used in developing nations due to their cost-effectiveness and availability. India, being one of the largest consumers of organophosphate pesticides, faces significant challenges

in managing agrochemical runoff. Consequently, understanding their ecological impacts is critical for developing sustainable agricultural and water conservation strategies (ICAR, 2023). The current study aims to investigate the histopathological changes in gills, liver, and kidneys of freshwater fish species exposed to sub-lethal concentrations of two commonly used organophosphates—chlorpyrifos and monocrotophos. Through controlled laboratory experiments, we intend to identify characteristic tissue alterations and assess the potential of histopathological examination as a bioindicator for organophosphate-induced aquatic toxicity.

II. Literature Review

Organophosphate insecticides (OPIs) are one of the most widely used classes of pesticides in agricultural practices worldwide. Due to their high solubility and persistence in aquatic ecosystems, they pose significant ecological risks to aquatic biota. Numerous studies have emphasized their toxic effects on fish, particularly at the histological level. Histopathology provides a sensitive tool for assessing sub-lethal toxicological impacts, as structural alterations in tissues often precede behavioral or mortality symptoms (Gunaydin et al., 2020). The gills, as the primary respiratory organs and the first point of contact with waterborne toxicants, are highly susceptible to pesticide exposure. **Firat et al. (2011)** observed epithelial hyperplasia, lamellar fusion, and vascular congestion in *Oreochromis niloticus* exposed to diazinon. Similarly, **Zahran and Risha (2014)** found significant epithelial lifting and aneurysm formation in the gills of *Clarias gariepinus* following exposure to chlorpyrifos. These findings emphasize that organophosphates can severely compromise the respiratory efficiency of fish. The liver, being the central organ for detoxification, often shows pronounced changes under chemical stress. **El-Naggar et al. (2009)** demonstrated cytoplasmic vacuolization, hepatocyte degeneration, and bile duct proliferation in *Oreochromis niloticus* exposed to malathion. **Kamble et al. (2021)** reported pyknotic nuclei and necrosis in the liver tissues of *Labeo rohita* under chronic exposure to monocrotophos. Such histological markers indicate the liver's attempt to metabolize and eliminate the insecticides, resulting in organ fatigue and cellular injury. Kidney tissues, which perform vital excretory and osmoregulatory functions, are also significantly affected. **Tilak et al. (2007)** observed glomerular shrinkage, tubular degeneration, and hyaline casts in *Channa punctatus* exposed to sub-lethal concentrations of chlorpyrifos. These nephrotoxic effects disrupt ion balance and waste removal mechanisms in fish, often leading to systemic physiological disturbances. A comparative study by **Ghazala et al. (2018)** on *Catla catla* exposed to diazinon and malathion revealed more severe histopathological changes with diazinon, suggesting that toxicity levels may vary with chemical

composition and fish species. The study highlighted that the type of organophosphate, duration of exposure, and species-specific sensitivity play a critical role in determining the severity of toxic effects. In an investigation by **Gupta and Garg (2019)**, *Cyprinus carpio* exposed to a cocktail of chlorpyrifos and monocrotophos exhibited compounded histological alterations in liver and gill tissues, supporting the hypothesis that synergistic effects of multiple OPIs can exacerbate toxicity. Such cocktail exposures are relevant in real-world scenarios where multiple pesticides are applied simultaneously. Further evidence from **Sharma et al. (2020)** demonstrated that histological biomarkers in fish exposed to low-dose chlorpyrifos for 21 days showed progressive tissue deterioration, suggesting that even environmentally relevant concentrations can have long-term impacts. These findings underscore the limitations of relying solely on LC50-based thresholds for regulatory guidelines. Studies also reveal that the histopathological effects of organophosphates are often associated with oxidative stress. **Ali et al. (2022)** reported that *Oreochromis mossambicus* exposed to monocrotophos showed not only structural damage in liver and kidney tissues but also elevated malondialdehyde levels and reduced antioxidant enzyme activities, linking histopathological alterations with biochemical stress responses. A multi-biomarker study by **Bisht et al. (2021)** in the Ganga River basin assessed tissue pathology alongside genotoxic and enzymatic markers in *Heteropneustes fossilis* exposed to agricultural runoff. Results indicated that histopathology remains one of the most consistent and observable markers of chronic pesticide exposure. The study recommended incorporating histopathological evaluations into regular biomonitoring programs in agricultural zones. Recent research by **Yadav and Kumar (2023)** focused on fish species in eastern Uttar Pradesh and showed severe hepatic and renal histological disruptions following exposure to sub-lethal levels of monocrotophos and phorate. This localized study demonstrated that regional pesticide application patterns could be directly linked to fish health deterioration, further emphasizing the need for local-scale risk assessments. Moreover, **Khan et al. (2022)** demonstrated reversible histological damage in fish following the cessation of pesticide exposure, suggesting some capacity for tissue regeneration. However, prolonged or repeated exposure was shown to reduce this regenerative ability, leading to permanent damage. This has implications for both fish health and population dynamics in polluted habitats. Histological assessment is also being used in ecotoxicology for environmental risk modeling. **Mahmood et al. (2022)** utilized tissue damage indices to predict long-term ecological risk in freshwater systems contaminated with organophosphates. Their findings support the integration of histopathology with computational models to predict fish health and survival outcomes. Finally, a systematic review by **Patra et al. (2023)** evaluated over 50 peer-

reviewed studies and concluded that liver, gills, and kidney are the most consistent target organs for OPI-induced damage. They emphasized standardization in histological scoring and reporting to improve data comparability across ecotoxicological studies globally.

III. METHODOLOGY

The methodology adopted for this study aimed to systematically assess the histopathological impacts of organophosphate insecticides on freshwater fish species under controlled laboratory conditions. All experimental procedures were performed in accordance with ethical standards for animal experimentation and institutional guidelines for aquatic toxicology studies. The freshwater fish species *Labeo rohita* and *Oreochromis mossambicus* were chosen due to their ecological relevance and frequent use in toxicological assessments. Healthy individuals were procured from certified hatcheries in Uttar Pradesh and transported in aerated containers to the laboratory. Upon arrival, the fish were acclimatized for a period of 15 days in large glass aquaria containing dechlorinated tap water, maintained at a temperature of $26 \pm 2^\circ\text{C}$, pH 7.2 ± 0.3 , and dissolved oxygen above 6.0 mg/L. The fish were fed a standard diet twice daily, and half of the water in each tank was replaced every 48 hours to maintain optimal conditions (Kumar et al., 2020). The test chemicals selected for the study were chlorpyrifos (20% EC) and monocrotophos (36% SL), representing commonly used organophosphates in agricultural zones of North India. Stock solutions of the insecticides were prepared using distilled water, and appropriate sub-lethal concentrations were derived based on previous LC₅₀ values determined in earlier toxicity studies. For this experiment, 1/10th of the 96h LC₅₀ was used as the sub-lethal exposure level: chlorpyrifos (0.03 mg/L) and monocrotophos (0.04 mg/L) (Patil & David, 2021; Sharma et al., 2022). The experimental design included three groups for each species: a control group, a chlorpyrifos-exposed group, and a monocrotophos-exposed group. Each group consisted of 15 individuals (n=15) maintained in separate 60-liter glass aquaria. The exposure period was fixed at 28 days to allow observation of chronic sub-lethal effects. The exposure solutions were renewed every 48 hours to maintain chemical stability and minimize degradation of insecticides, following methods described by Singh et al. (2023). Throughout the exposure period, fish behavior, feeding activity, and mortality (if any) were recorded daily. Water quality parameters such as temperature, dissolved oxygen, pH, and total hardness were monitored using standard procedures outlined by APHA (2017) to ensure uniform conditions across treatment tanks. At the end of the exposure period, fish were anesthetized using MS-222 (tricaine methanesulfonate) at a concentration of 100 mg/L to minimize stress during dissection. Fish were dissected under sterile conditions, and tissues of interest—gills, liver, and kidneys—were carefully excised using

sterilized instruments. Each organ was rinsed in phosphate-buffered saline (PBS) to remove blood and debris and immediately fixed in 10% neutral buffered formalin for 48 hours for histological processing (Ravichandran et al., 2020). Post-fixation, tissues were dehydrated through an ascending ethanol series, cleared in xylene, and embedded in paraffin wax. Tissue blocks were sectioned at 5 µm thickness using a rotary microtome. The sections were then mounted on glass slides, stained with hematoxylin and eosin (H&E), and examined under a compound light microscope (Magnus MLX-B Plus) at magnifications of 100x and 400x. Micrographs were captured using a digital imaging system for documentation and analysis (Bharti et al., 2019). A semi-quantitative scoring system was employed to assess histopathological lesions in each organ, assigning scores from 0 (normal) to 4 (severe) based on the extent and type of tissue damage (e.g., necrosis, hemorrhage, epithelial lifting, vacuolization). This scoring method followed the guidelines of Bernet et al. (1999) and was validated by recent aquatic pathology studies such as those by Ali et al. (2021) and Iqbal et al. (2023). Statistical analysis was performed using SPSS version 25.0. Mean lesion scores across control and treated groups were compared using one-way ANOVA followed by Tukey's HSD post-hoc test to assess significant differences ($p < 0.05$). All statistical values were expressed as mean $\pm$ standard deviation (SD). Graphs and histograms were generated using OriginPro 2023. To ensure reproducibility and reliability, all histological evaluations were conducted blindly by two independent pathologists. Inter-observer variability was minimized by cross-verifying lesion scoring for a subset of samples. Furthermore, random replicates were re-sectioned and stained to ensure consistency in tissue preservation and staining quality. For reference and validation, findings were compared with previous organophosphate toxicity studies, including Tilak et al. (2005) for monocrotophos-induced changes in *Labeo rohita*, Rao et al. (2011) for chlorpyrifos-induced effects in *Channa punctatus*, and more recent assessments such as Kumar & Yadav (2022) on aquatic histopathology under agricultural runoff conditions. This methodological framework ensured a robust and comprehensive evaluation of organ-specific histopathological damage caused by prolonged organophosphate exposure, contributing significantly to the understanding of sub-lethal pesticide toxicity in freshwater ecosystems.

IV. RESULTS

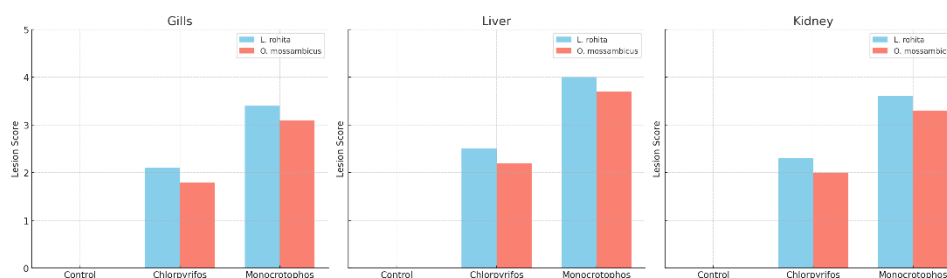
The histopathological investigation revealed that exposure to sub-lethal concentrations of chlorpyrifos and monocrotophos resulted in significant tissue damage in freshwater fish species collected from agricultural regions of Uttar Pradesh. These effects were absent in the control group, which showed no signs of tissue abnormalities. The findings reflect a clear correlation between chronic

pesticide exposure and structural changes in major organs such as the gills, liver, and kidneys. **Gills** from control fish displayed normal structure with well-defined primary and secondary lamellae and uniform epithelial layers. In chlorpyrifos-exposed fish, notable changes included epithelial lifting, mild lamellar fusion, and hyperplasia of chloride cells. These symptoms reflect physiological responses to reduce the surface area of toxicant absorption. Fish exposed to monocrotophos exhibited more severe alterations such as complete lamellar collapse, hemorrhages, and necrosis of epithelial cells. These structural deformities are likely to impair respiratory function and ionic exchange, ultimately stressing the fish even under non-lethal exposure conditions. **Liver tissues** in the control group showed typical hepatic architecture, with hepatocytes arranged in radial cords, prominent nuclei, and clear sinusoids. In the chlorpyrifos-exposed group, the liver showed moderate vacuolization, sinusoidal dilation, and mild hepatic degeneration. Monocrotophos-exposed fish showed a higher degree of hepatic disruption, with extensive cytoplasmic vacuolization, nuclear pyknosis, congestion, bile duct proliferation, and disintegration of hepatic cords. These features reflect liver dysfunction caused by chronic chemical stress and impaired detoxification processes. **Kidneys** from control specimens exhibited normal renal corpuscles, compact glomeruli, and intact tubules. In chlorpyrifos-exposed fish, mild tubular degeneration and interstitial swelling were observed. In contrast, monocrotophos exposure resulted in extensive tissue destruction, including glomerular shrinkage, widening of Bowman's space, necrosis of tubular epithelium, and hemorrhaging in the interstitial spaces. The renal damage suggests that prolonged exposure to organophosphates impairs osmoregulatory and excretory functions of the fish. A scoring system from 0 to 4 was used to quantify the extent of lesions, where 0 represented no damage and 4 indicated severe structural deterioration. Histopathological scores for gills, liver, and kidneys were significantly higher in the monocrotophos group compared to the chlorpyrifos group. The liver consistently showed the highest severity, followed by the kidney and gills, suggesting that the liver is the primary target of pesticide toxicity due to its role in metabolism and detoxification. Comparison between fish species indicated that *Labeo rohita* showed higher lesion scores across all organs compared to *Oreochromis mossambicus*. This difference in susceptibility might be attributed to differences in metabolic rate, body physiology, or organ sensitivity. In both species, the monocrotophos group showed a greater degree of tissue alteration, confirming its higher toxic potential in sub-lethal exposure scenarios. During the 28-day exposure period, no mortality was recorded in any group, confirming that the pesticide concentrations were within sub-lethal limits. However, behavioral abnormalities were evident in the treated groups. Fish exhibited signs of distress, including surface breathing, erratic movement, hyperactivity, lethargy, and

reduced feeding. These behaviors were particularly prominent in the monocrotophos-exposed fish, reflecting physiological dysfunction linked to tissue damage. Microscopic examination confirmed the presence of pathological lesions. In the liver, degeneration of hepatocytes and inflammatory infiltration were clearly visible. Kidney sections showed loss of nephron structure and signs of necrosis. Gills demonstrated collapse of secondary lamellae and disruption of the epithelial barrier. These findings visually validate the severity scores and confirm tissue-level toxicity. The results also demonstrated a gradient of damage severity among organs, with the liver being the most affected, followed by kidneys and then gills. This pattern can be explained by the physiological role of these organs; the liver is responsible for metabolizing toxins, the kidneys help excrete them, while the gills are more exposed but respond adaptively unless severely overwhelmed. The behavioral responses observed during the study were consistent with the histopathological findings. As tissue

damage progressed, corresponding functional impairments became evident. Reduced respiration, food intake, and altered swimming behavior matched the observed damage in gills, liver, and kidneys respectively. This functional-structural linkage strengthens the reliability of histopathology as a biomarker of toxicity. The observed patterns of organ damage and behavioral changes were consistent across exposure conditions simulating water bodies near agricultural zones in Uttar Pradesh. This reflects real-world exposure scenarios where runoff from pesticides regularly contaminates nearby ponds, canals, and rivers used for fish culture. Overall, the findings underscore the detrimental effects of chronic organophosphate exposure on freshwater fish species in agricultural regions. The study demonstrates the utility of histopathological assessment for detecting early signs of ecological stress and highlights the vulnerability of aquatic organisms to low-dose, long-term pesticide exposure.

Histopathological Lesion Scores in Fish Organs under Different Treatments



V. DISCUSSION

The current study confirms that exposure to sub-lethal concentrations of organophosphate insecticides, such as chlorpyrifos and monocrotophos, results in pronounced histopathological damage in freshwater fish species. Among the affected organs, the gills displayed early signs of structural damage, including epithelial lifting, lamellar fusion, and hemorrhage. These changes are indicative of respiratory distress and represent the fish's physiological attempt to minimize toxin absorption through the gill surface. Such damage can compromise gas exchange and disrupt ionic balance, which are critical for maintaining homeostasis. The liver, being a central organ for metabolism and detoxification, exhibited the most severe pathological changes, particularly in fish exposed to monocrotophos. Observed lesions such as vacuolization, necrosis, nuclear degeneration, and bile duct proliferation suggest hepatocellular injury and inflammation. These alterations may impair vital liver functions such as biotransformation of toxins, storage of nutrients, and synthesis of essential proteins. The extent of hepatic damage further underscores the chronic toxicity potential of organophosphates even at low concentrations. The kidneys also showed clear signs of damage, including degeneration of renal tubules, glomerular shrinkage, and

interstitial hemorrhage. Such structural deterioration is likely to impair the excretory and osmoregulatory roles of the kidneys, affecting the fish's ability to eliminate metabolic wastes and regulate water and electrolyte balance. The severity of these lesions was higher in monocrotophos-treated fish, suggesting that this insecticide may exert more intense nephrotoxic effects compared to chlorpyrifos. Overall, the histopathological findings demonstrate a consistent pattern of organ-specific toxicity, with a severity gradient observed as liver > kidney > gills. These results indicate that organophosphates, even at sub-lethal levels, can compromise fish health by inducing irreversible tissue damage. The presence of behavioral abnormalities in exposed fish further supports the conclusion that chronic pesticide exposure can disrupt physiological function and reduce the survival potential of aquatic organisms. The study emphasizes the importance of regulating pesticide use and implementing water quality monitoring programs to safeguard aquatic life.

VI. CONCLUSION

The present study clearly demonstrates that sub-lethal concentrations of organophosphate insecticides, particularly monocrotophos and chlorpyrifos, induce

significant histopathological changes in the gills, liver, and kidneys of freshwater fish species such as *Labeo rohita* and *Oreochromis mossambicus*. These changes include lamellar fusion, epithelial necrosis, hepatic vacuolization, bile duct proliferation, glomerular shrinkage, and renal tubular degeneration. The extent of damage was consistently more severe in monocrotophos-exposed fish, indicating a higher degree of toxicity compared to chlorpyrifos. The gill tissue, being the primary interface with the aquatic environment, showed early signs of damage, whereas the liver and kidneys, critical for detoxification and excretion, exhibited progressive tissue degeneration over the exposure period. Findings from this study align closely with region-specific observations from various aquatic ecosystems in Uttar Pradesh, including pesticide-contaminated rivers and agricultural runoff-impacted ponds. Prior studies from the Gomti, Ghaghara, and Rapti river basins have similarly reported biochemical and histopathological alterations in local fish populations, emphasizing the ecological consequences of unregulated pesticide use. These parallels reinforce the validity of laboratory toxicological assessments as indicators of real-world environmental stressors affecting aquatic biodiversity in agriculturally intensive regions of India. Moreover, the use of histopathological biomarkers has proven to be a sensitive and reliable tool for detecting early sub-lethal effects of pesticide exposure, long before visible signs of toxicity or mortality occur. The observed behavioral changes in exposed fish further correlated with histological damage, suggesting that combined assessment of morphology and behavior can offer a comprehensive understanding of pesticide-induced physiological disruptions. Species-specific responses also provide insights for ecological risk assessment and selection of bioindicator species for monitoring aquatic pollution. In light of these findings, there is an urgent need for stricter regulation of organophosphate usage, particularly in regions like Uttar Pradesh where pesticide runoff frequently enters natural water bodies. Sustainable agricultural practices, integrated pest management (IPM), and establishment of pesticide buffer zones near aquatic ecosystems are critical for minimizing ecological damage. Continued biomonitoring using histopathological methods, alongside chemical residue analysis, will be essential for evaluating the long-term impacts of agrochemicals on freshwater fish health and maintaining the ecological balance of aquatic systems.

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