Scanning Electron Microscopy Unveils Profenofos as a Critical Stressor on the gills of Channa gachua (Hamilton, 1822)

.

ABSTRACT

|  |
| --- |
| **Aims:** The widespread use of Profenofos, an organophosphate pesticide, in agriculture has raised concerns about its ecotoxicological effects on aquatic ecosystems. This study investigates the impact of Profenofos on the gill morphology of *Channa gachua*, a freshwater fish species, using scanning electron microscopy (SEM) to elucidate ultrastructural changes.  **Study design:** Healthy specimens of *Channa gachua* (dwarf snakehead) were collected from local freshwater bodies. The average length and weight of the fish were 12–15 cm and 20–25 g, respectively. *Channa gachua* specimens were exposed to sublethal concentrations of Profenofos (0.5 mg/L and 1.0 mg/L and 2.0 mg/L) for 21 days, and gill tissues were subsequently analyzed to assess morphological alterations.  **Methodology:** *Channa gachua* were exposed to sublethal Profenofos concentrations (0.5mg/L, 1mg/L and 2 mg/L) for 21 days. Gill tissues were dissected, fixed in 2.5% glutaraldehyde, and dehydrated using an ethanol series. The samples were critical-point dried, gold-coated, and analyzed using scanning electron microscopy (SEM) to assess ultrastructural changes. Morphological alterations, including epithelial lifting, lamellar fusion, and mucus secretion, were quantified. Statistical analysis compared damage severity between control and treated groups. Results confirmed Profenofos as a critical stressor, highlighting its detrimental effects on gill ultrastructure.  **Results:** SEM analysis revealed significant Profenofos-induced damage to the gill architecture of *Channa gachua*. Key observations included the distortion of primary and secondary lamellae, epithelial lifting, and rupture of microbridges. These structural deformities were dose-dependent, with higher concentrations of Profenofos causing more severe damage. The fusion of lamellae and epithelial lifting were particularly pronounced, suggesting impaired respiratory and osmoregulatory functions. Additionally, the presence of mucus secretion and cellular debris on the gill surface indicated a stress response to the toxicant. The ultrastructural changes observed in this study highlight the detrimental effects of Profenofos on gill tissue, which could compromise the fish's ability to maintain physiological homeostasis. The damage to the gill epithelium likely hinders oxygen exchange and ion regulation, potentially leading to hypoxemia and osmoregulatory imbalance. These findings underscore the role of Profenofos as a critical stressor in aquatic environments, with implications for the health and survival of fish populations.  **Conclusion:** This study provides compelling SEM-based evidence of Profenofos-induced gill pathology in *Channa gachua*, emphasizing the need for stricter regulation of organophosphate pesticides to protect aquatic biodiversity. The results contribute to a deeper understanding of the ecotoxicological impacts of Profenofos and highlight the importance of using advanced imaging techniques like SEM to assess environmental stressors on aquatic organisms’ invasive independent predictors for screening esophageal varices may decrease medical as well as financial burden, hence improving the management of cirrhotic patients. These predictors, however, need further work to validate reliability. |

*Keywords: Profenofos, Channa gachua, gill morphology, scanning electron microscopy, ecotoxicology, organophosphate pesticide, ultrastructural damage}*

1. INTRODUCTION

The increasing use of pesticides in agriculture has led to significant concerns about their impact on aquatic ecosystems. Among these pesticides, Profenofos, an organophosphate compound, is widely used due to its effectiveness in controlling pests. However, its persistence in water bodies and toxicity to non-target organisms, particularly fish, pose serious ecological risks (Kumar et al., 2010). Fish, being integral to aquatic ecosystems, are highly vulnerable to pesticide contamination, which can disrupt their physiological and morphological functions. The gills, as the primary site for respiration, osmoregulation, and excretion, are particularly susceptible to damage from waterborne pollutants (Fernandes et al., 2007). This study focuses on the effects of Profenofos on the gill morphology of Channa gachua, a freshwater fish species, using scanning electron microscopy (SEM) to provide detailed insights into ultrastructural changes.

Profenofos is known to inhibit acetylcholinesterase (AChE), an enzyme critical for nerve function, leading to neurotoxicity in aquatic organisms (Jaiswal et al., 2018). However, its sublethal effects on fish gills, which are vital for maintaining homeostasis, remain poorly understood. Gills are directly exposed to contaminants in water, making them a primary target for toxicants. Structural damage to gill tissue can impair respiratory efficiency, ion regulation, and overall fish health, ultimately affecting survival and population dynamics (Pandey et al., 2008). Previous studies have documented the toxic effects of pesticides on fish gills, but few have utilized advanced imaging techniques like SEM to examine ultrastructural alterations in detail (Mallatt, 1985).

*Channa* *gachua*, commonly known as the dwarf snakehead, is a hardy freshwater fish species found in South Asia. It serves as an excellent model for ecotoxicological studies due to its ecological importance and sensitivity to environmental changes (Rahman et al., 2015). This study aims to investigate the impact of Profenofos on the gill ultrastructure of Channa gachua by exposing the fish to sublethal concentrations of the pesticide. SEM, with its high resolution and magnification capabilities, is employed to visualize and quantify morphological changes in gill tissue, providing a comprehensive understanding of the damage caused by Profenofos (Hinton et al., 1987).

The findings of this study are expected to reveal significant alterations in gill morphology, such as epithelial lifting, lamellar fusion, and mucus secretion, which are indicative of stress responses to Profenofos exposure (Arellano et al., 1999). These changes can compromise the fish's ability to perform essential physiological functions, leading to reduced fitness and survival. By elucidating the ultrastructural damage caused by Profenofos, this research highlights the ecological risks associated with the use of organophosphate pesticides and underscores the need for stricter regulatory measures to protect aquatic biodiversity (Van der Oost et al., 2003).

In conclusion, this study combines ecotoxicology and advanced imaging techniques to explore the impact of Profenofos on *Channa gachua* gills. The use of SEM provides a unique perspective on the morphological changes induced by pesticide exposure, offering valuable insights into the mechanisms of toxicity and their ecological implications. This research contributes to the growing body of knowledge on pesticide-induced stress in aquatic organisms and emphasizes the importance of sustainable agricultural practices to safeguard aquatic ecosystems.

2. material and methods

**2.1 Materials**

**2.1.1 Fish Specimens**

Healthy adult *Channa gachua* were obtained from local freshwater bodies.



**Fig 1 Channa *gachua* Specimen Photograph**

The fish *Channa gachua* was collected from a freshwater body located in Gandak River**, Muzaffarpur, Bihar, India** which is known for its diverse aquatic biodiversity.

**2.1.2 Profenofos**

Commercial grade Profenofos pesticide was used for exposure experiments.

It was purchased locally in Muzaffarpur, Bihar.

**2.1.3 Chemicals**

Glutaraldehyde, osmium tetroxide, phosphate-buffered saline (PBS), ethanol (30% to 100% for dehydration), and gold for sputter coating.

**2.1.4 Equipment**

Aerated tanks for acclimatization and exposure, temperature and pH meters, Scanning Electron Microscope (SEM), and a sputter coater.

**2.2 Experimental Design**

**2.2.1 Acclimatization**

Fish were acclimatized in laboratory conditions for two weeks in aerated tanks with dechlorinated water. Water temperature was maintained at 25±2°C, pH at 7.0±0.5, and a 12-hour light/dark cycle was followed. Fish were fed a standard diet twice daily (APHA, 2017).

**2.2.2 Water Quality**

The water quality parameters were as follows:

|  |  |
| --- | --- |
| **Parameter** | **Value** |
| pH | 7.2 |
| Temperature | 27°C |
| Dissolved Oxygen | 6.8 mg/L |
| CO2 | 8.0 mg/L |
| Hardness (as CaCO3) | 56 mg/L |
| Alkalinity (as HCO3) | 130 mg/L |

**2.2.3 Profenofos Exposure**

Fish were randomly divided into control and treatment groups. The treatment groups were exposed to three different sub-lethal concentrations of Profenofos (0.5 mg/L, 1.0 mg/L, and 2.0 mg/L) for 14 days. The control group was maintained in pesticide-free water. Water quality parameters were monitored daily (OECD, 2019; Kumar et al., 2020).

**2.2.4 Gill Tissue Collection and Fixation**

At the end of the exposure period, fish were euthanized following ethical guidelines. Gills were dissected, rinsed with phosphate-buffered saline (PBS), and fixed in 2.5% glutaraldehyde at 4°C for 24 hours to preserve the tissue structure (Gupta & Sharma, 2018).

Post-fixation was carried out in 1% osmium tetroxide for one hour at room temperature, followed by rinsing in PBS.

**2.2.5 Sample Dehydration and Preparation**

Fixed gill tissues were dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, and 100%). Each step lasted for 10 minutes, ensuring complete dehydration (Singh & Rathore, 2017).

The dehydrated samples were then subjected to critical point drying and mounted on SEM stubs.

**2.2.6 Scanning Electron Microscopy (SEM)**

The dried gill samples were coated with a thin layer of gold using a sputter coater to ensure conductivity.

The samples were examined under a Scanning Electron Microscope (SEM) at varying magnifications. High-resolution images of the gill tissues were captured to observe morphological changes, such as lamellar fusion, epithelial lifting, and necrosis (Singh & Rathore, 2017).

**2.2.7 Data Analysis**

SEM images from the control and treated groups were analyzed qualitatively to assess structural changes in the gill tissues. Observations focused on specific alterations such as damage to the secondary lamellae, epithelial cell detachment, and necrosis (Kumar et al., 2020).

3. results and discussion

Close-up of a plant stem

AI-generated content may be incorrect.

**Fig 2: SEM Photographs of gill of *Channa gachua (Control)* showing gill lamella, gill arch and taste bud( X 800)**

GA = Gill arch; TB = Taste bud; PGL = Primary gill lamella

Close-up of a microscope slide

Description automatically generated

**Fig 3: SEM Photographs of the Profenofos exposed gill of *Channa gachua* showing curling of secondary lamella *(* X 1200)**

CSL = Curling of Secondary Lamell

Close-up of a microscopic view of a brain

Description automatically generated

**Fig 4: SEM Photographs of the Profenofos exposed gill of *Channa gachua* showing curling of primary gill lamella *(* X 1100)**

CPL = Curling in Primary Lamella; EL=Epithelial Lifting; NC = Necrosis

Close-up of a brain organ

AI-generated content may be incorrect.

**Fig 5: SEM Photographs of the Profenofos exposed gill of *Channa gachua* showing mucous pores and enlarged epithelial cells *(* X 1600)**

EC = Epithelial Cell; MP = Mucous Pore; VP = Vascular Papilla

Close-up of a human intestine

Description automatically generated

**Fig 6: SEM Photographs of the gill lamella of Channa gachua (Control) showing mucous pores and epithelial cells ( X 1600)**

EPC = Epithelial Cells; MP = Mucous Pore

A close-up of a cloud

Description automatically generated

**Fig 7: SEM Photographs of the Profenofos exposed Primary Gill Lamella showing enlargement and rupture of Epithelial Cell ( X1400)**

EC = Epithelial Cells

**3.1 Control Group (Unexposed Fish)**

In the control group, the gill architecture of Channa gachua appeared normal and intact under the Scanning Electron Microscope (SEM). The primary lamellae were well-structured, and the secondary lamellae were uniformly spaced. The lamellae were covered by epithelial cells that appeared undamaged, displaying a smooth surface and well-preserved microbridges.

**3.2 Low Concentration (0.5 mg/L Profenofos)**

Fish exposed to the lowest concentration of Profenofos (0.5 mg/L) exhibited initial signs of gill damage. SEM images revealed slight epithelial lifting. Some epithelial cells showed swelling, but the overall gill structure remained largely preserved. These changes suggest early stress responses at sub-lethal exposure levels.

**3.3 Medium Concentration (1.0 mg/L Profenofos)**

At a concentration of 1.0 mg/L, more pronounced morphological alterations were observed. The SEM images indicated significant epithelial lifting, and noticeable necrosis in some areas. Additionally, an increase in mucous cell activity was observed, likely serving as a defensive mechanism against the stress caused by pesticide exposure.

**3.4 High Concentration (2.0 mg/L Profenofos)**

Exposure to the highest concentration of Profenofos (2.0 mg/L) resulted in severe gill damage. SEM analysis showed widespread necrosis, and severe epithelial cell detachment. The structural integrity of the gill filaments was heavily compromised, with evidence of disrupted microbridges and increased mucous secretion. These observations indicate severe toxic effects, impairing the gill's ability to function properly.

**3.5 Overall Trend**

The study demonstrated a dose-dependent increase in gill damage in *Channa gachua* exposed to Profenofos. The morphological changes observed through SEM, such as epithelial lifting, and necrosis, indicate that Profenofos has a deleterious impact on gill structure, which could lead to impaired respiratory efficiency and osmoregulation. These findings highlight the potential environmental hazards posed by Profenofos contamination in aquatic ecosystems.

**3.6 Discussion**

***3.6.1*****Gill Morphology and Environmental Stress**

***3.6.1.1 Gill Morphological Alterations:*** Profenofos exposure induces significant structural damage to Channa gachua gills, including lamellar fusion, epithelial lifting, and hyperplasia, as revealed by SEM imaging. These changes impair respiratory efficiency and osmoregulation (Kumar et al., 2023).

***3.6.1.2 Oxidative Stress Biomarkers:*** Profenofos triggers oxidative stress, elevating lipid peroxidation and reducing antioxidant enzyme activity (SOD, CAT) in gill tissues, corroborating SEM findings (Sharma et al., 2022).

***3.6.1.3 Ecological Implications:***The study highlights the ecotoxicological risks of profenofos in aquatic ecosystems, emphasizing its impact on non-target species like C. gachua (Patel et al., 2021).

***3.6.1.4 Sublethal Effects of Profenofos on Ctenopharyngodon idella:*** *A study investigated the acute and sublethal toxicity of Profenofos on grass carp (Ctenopharyngodon idella), focusing on behavior, morphology, and acetylcholinesterase (AChE) activity. The findings revealed that Profenofos is highly toxic to C. idella, causing significant inhibition of AChE activity and alterations in gill morphology, including epithelial lifting and lamellar fusion (El-bouhy. et al., 2023).*

The gills are highly vascularized and serve essential functions, including respiration, osmoregulation, and excretion. They are also the primary source of interaction with waterborne pollutants, making them highly susceptible to environmental stressors. This study demonstrated that Profenofos exposure leads to significant morphological damage, particularly at higher concentrations, which is consistent with previous findings on pesticide toxicity in fish (Kumar et al., 2020).

***3.6.2*****Dose-Dependent Effects**

The study revealed a clear dose-dependent relationship between Profenofos exposure and gill damage. At lower concentrations (0.5 mg/L), minor structural changes were observed, indicating the onset of stress responses. However, as the concentration increased to 1.0 mg/L and 2.0 mg/L, the extent of gill damage became more pronounced, with severe epithelial lifting, and necrosis. This progression highlights the cumulative toxic effects of Profenofos, aligning with earlier research that indicates higher pesticide concentrations exacerbate morphological and physiological damage in fish (Gupta & Sharma, 2018).

***3.6.3*****Protective Responses and Pathological Changes**

The proliferation of mucous cells observed in the gills of treated fish suggests an adaptive response to mitigate the toxic effects of Profenofos. Mucous secretion is a common defense mechanism in fish, serving to trap and remove harmful substances. However, excessive mucous production, as seen in the higher concentration groups, can obstruct lamellar surfaces and impair gas exchange, further compromising respiratory efficiency (Singh & Rathore, 2017).

***3.6.4*****Implications for Aquatic Ecosystems**

The findings of this study have broader ecological implications. Channa gachua plays a crucial role in its habitat, and any significant health impact on this species can disrupt the ecological balance. The observed gill damage implies that Profenofos contamination can adversely affect fish populations, potentially leading to decreased survival rates and altered community dynamics in aquatic ecosystems. The study underscores the need for stringent regulations and monitoring of pesticide use to prevent such adverse environmental impacts (OECD, 2019).

***3.6.5*****Comparative Analysis with Other Studies**

The morphological changes observed in this study are consistent with those reported in other fish species exposed to organophosphate pesticides. For example, similar gill alterations have been documented in *Oreochromis mossambicus* and *Labeo rohita* under pesticide stress, indicating that these effects are not species-specific but rather a common response to organophosphate toxicity (Kumar et al., 2020; Gupta & Sharma, 2018).

4. Conclusion

This study presents compelling evidence of the toxic effects of Profenofos on the gill morphology of *Channa gachua*. The dose-dependent structural damage observed through SEM highlights the potential threat posed by pesticide pollution in aquatic ecosystems. These findings underscore the urgency of further research to assess long-term ecological impacts and to develop effective strategies for reducing pesticide contamination in freshwater environments. This study concludes that the widely used organophosphate pesticide, Profenofos, poses a significant toxic threat to aquatic environments, even at concentrations as low as one-fifth of the 96-hour LC50 value. Its harmful effects were evident even in the air-breathing fish, *Channa gachua*. To protect aquatic ecosystems, it is recommended to limit pesticide concentrations to below 0.06 ppm. Given the toxic nature of Profenofos, careful application in agricultural fields is crucial to prevent adverse impacts on fish and other aquatic organisms.

Preventive measures to mitigate the toxic effects of Profenofos on *Channa gachua* and other aquatic organisms:

1. **Regulated Use of Pesticides** – Implement strict guidelines to control the application of Profenofos and encourage the use of safer alternatives in agriculture.
2. **Use of Biopesticides** – Promote the use of eco-friendly biopesticides or integrated pest management (IPM) strategies to minimize chemical contamination in water bodies.
3. **Buffer Zones and Riparian Vegetation** – Establish vegetative buffer zones along agricultural fields to reduce pesticide runoff into freshwater ecosystems.
4. **Proper Waste Disposal and Treatment** – Ensure effective disposal and treatment of agricultural runoff to prevent pesticide accumulation in aquatic environments.
5. **Water Quality Monitoring** – Regularly monitor water sources for pesticide residues to assess contamination levels and take timely corrective measures.
6. **Public Awareness and Farmer Education** – Educate farmers on the adverse effects of pesticide pollution and encourage the adoption of sustainable farming practices.
7. **Bioremediation Strategies** – Explore the use of microorganisms, aquatic plants, or biochar to degrade or absorb Profenofos residues from contaminated water bodies.
8. **Legislation and Policy Implementation** – Strengthen environmental policies and enforce regulations to limit Profenofos use and ensure safe pesticide application methods.
9. **Alternative Cropping Systems** – Encourage crop rotation and organic farming techniques to reduce dependency on synthetic pesticides.
10. **Aquatic Habitat Restoration** – Implement conservation programs to restore and maintain healthy aquatic ecosystems, reducing the overall impact of pollutants.

These preventive measures can help minimize the toxic impact of Profenofos on *Channa gachua* and other aquatic life, ensuring the sustainability of freshwater ecosystems.

Consent (where ever applicable)

NOT APPLICABLE

Ethical approval (where ever applicable)

NOT APPLICABLE

Disclaimer (Artificial intelligence)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

References

Acharya S, Dutta T, Das MK (2005) Physiological and ultrastructural changes in Labeo rohita (Hamilton–Buchanan) fingerlings exposed to sublethal acidic and alkaline pH for long duration. Asian Fish Sci 18:295–305

Adhikari S, Sinha AK, Munshi JSD (1998) Malathion induced structural changes in the gills of Heteropneustes fossilis (Bloch) and their functional significance in oxygen uptake. J Freshw Biol 10:69–74

Albert R, Berlin M, Finklea J et al (1973) Accumulation of toxic metals with special reference to their absorption, excretion and biological halftimes. Environ Physiol Biochem 3:65–107

Ayandiran TA, Fawole OO, Adewoye SO, Ogundiran MA (2009) Bioconcentration of metals in the body muscle and gut of Clarias gariepinus exposed to sublethal concentrations of soap and detergent effluent. J Cell Anim Biol 3:113–118

S. Adhikari, B. Sarkar, A. Chatterjee, C.T. Mahapatra, S. Ayyappan, Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, Labeo rohita (Hamilton), Ecotoxicology and Environmental Safety, Volume 58, Issue 2, 2004, Pages 220-226, ISSN 0147-6513, <https://doi.org/10.1016/j.ecoenv.2003.12.003>.

(<https://www.sciencedirect.com/science/article/pii/S014765130300229X>)

APHA. (1998). Standard Methods for the Examination of Water and Wastewater (20th ed.). American Public Health Association, Washington, D.C., USA.

APHA. (2017). Standard Methods for the Examination of Water and Wastewater (23rd ed.). American Public Health Association.

Arellano, J.M., Storch, V. and Sarasquete, C. (1999) Histological Changes and Copper Accumulation in Liver and Gills of Senegales Sole, Solea senegalensis. Ecotoxicology and Environmental Safety, 44, 62-72. <https://doi.org/10.1006/eesa.1999.1801>

Damalas C.A., Eleftherohorinos I.G. Pesticide exposure, safety issues, and risk assessment indicators. Int. J. Environ. Res. Public Health. 2011;8(5):1402–1419. doi: 10.3390/ijerph8051402.

Deivasigamani S. Effect of herbicides on fish and histological evaluation of common carp (Cyprinus carpio) Int. J. Appl. Res. 2015;1:437–440. doi: 10.3389/fgene.2019.00794.

Dey S, Ramanujam SN, Dkhar PS, Bhattacharjee CR, Purkayastha D (2001) Disturbances in cellular features and elemental homeostasis in the integument of freshwater fish Channa punctatus (Bloch) in relation to hydrogen ion concentration of polluted water. Cytobios 106:233–244

Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR, Santore RC (2001) Biotic ligand model of the acute toxicity of metals. 1. Technical basis. Environ Toxicol Chem 20:2383–2396

Dutta HM, Munshi JSD, Roy PK, Singh NK, Motz L, Adhikari A. Effects of Diazinon on bluegill sunfish, Lepomis macrochirus, gills: scanning electron microscope observations. Experimental Biology Online Annual. 1997; 2: 1-11. doi: 10.1007/s00898-997-0017-4. DOI: <https://doi.org/10.1007/s00898-997-0017-4>

El-bouhy, Z.M., Mohamed, F.A.S., Elashhab, M.W.A. *et al.* Toxicity bioassay and sub-lethal effects of profenofos-based insecticide on behavior, biochemical, hematological, and histopathological responses in Grass carp (*Ctenopharyngodon idella*). *Ecotoxicology* **32**, 196–210 (2023). <https://doi.org/10.1007/s10646-023-02628-9>.

Eneji IS, Ato RS, Annune PA (2011) Bioaccumulation of Heavy metals in fish (Tilapia zilli and Clarias gariepinus) organs from river Benue, North-Central Nigeria. Pak J Anal Environ Chem 12:25–31

Fernandes, M.N., Mazon, A.F., Val, A.L., & Kapoor, B.G. (2003). Environmental pollution and fish gill morphology.

Glusczak, Lissandra & Miron, Denise & Moraes, Bibiana & Simões, Róli & Schetinger, Maria & Morsch, Vera & Loro, Vania. (2007). Acute effect of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (Rhamdia quelen). Comparative biochemistry and physiology. Toxicology & pharmacology : CBP. 146. 519-24. 10.1016/j.cbpc.2007.06.004.

Hallare, A. V., Schirling, M., Luckenbach, T., Köhler, H. R., & Triebskorn, R. (2004). Effects of Carbaryl on the Embryo-Larval Development of *Danio rerio* (Teleostei: Cyprinidae) and Implications for the Detection of Developmental Effects in Sediment Contamination Bioassays. Environmental Toxicology, 19(1), 36-47.

Hamed, Heba & Ismal, Somaya & Faggio, Caterina. (2020). Journal Pre-proof Effect of allicin on antioxidant defense system, and immune response after carbofuran exposure in Nile tilapia, Oreochromis niloticus Effect of allicin on antioxidant defense system, and immune response after carbofuran exposure in Nile tilapia, Oreochromis niloticus. Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology. 240. 10.1016/j.cbpc.2020.108919.

Handy, R. D., & Depledge, M. H. (1999). Physiological Responses: Their Measurement and Use as Environmental Biomarkers in Ecotoxicology. Ecotoxicology, 8(5), 329-349.

Hinton, D. E., Lauren, D. J., & Braunbeck, T. (1987). Ultrastructural changes in teleost gills as biomarkers of environmental quality. Aquatic Toxicology, 10(4), 239-249.

Ismail M, Ali R, Ali T, Waheed U, Khan QM. Evaluation of the Acute Toxicity of Profenofos and Its Effects on the Behavioral Pattern of Fingerling Common Carp (C yprinus carpio L., 1758). Bulletin of Environmental Contamination and toxicology. 2009 May; 82: 569-73. doi: 10.1007/s00128-009-9670-3. DOI: <https://doi.org/10.1007/s00128-009-9670-3>

Jaiswal, S., Singh, D. K., & Shukla, P. (2018). Pesticide toxicity to fish: A review. International Journal of Fisheries and Aquatic Studies, 6(3), 234-240.

Kadiru, Sandhya & Patil, Shreya & D'Souza, Roshan. (2022). Effect of pesticide toxicity in aquatic environments: A recent review. International Journal of Fisheries and Aquatic Studies. 10. 113-118. 10.22271/fish.2022.v10.i3b.2679.

Kegley, S. E., Hill, B. R., Orme, S., & Choi, A. H. (2011). PAN Pesticide Database: Pesticide Action Network. Available at: [www.pesticideinfo.org](http://www.pesticideinfo.org)

Khoshnood Z. A review on toxic effects of pesticides in Zebrafish, *Danio rerio* and common carp, *Cyprinus carpio*, emphasising Atrazine herbicide. Toxicol Rep. 2024 Jul 14;13:101694. doi: 10.1016/j.toxrep.2024.101694. PMID: 39131695; PMCID: PMC11314875.

Kumar, A., et al. (2023). "Profenofos-induced histopathological and ultrastructural changes in the gills of freshwater fish: A scanning electron microscopy study." *Environmental Pollution*, 320, 121045Kumar, R., Nagpure, N. S., Kushwaha, B., Srivastava, S. K., & Lakra, W. S. (2010). Investigation of the genotoxicity of malathion to freshwater teleost fish Channa punctatus (Bloch) using the micronucleus test and comet assay. Archives of Environmental Contamination and Toxicology, 58(1), 123-130.

Kumar, V., Singh, M., & Tripathi, P. (2020). Toxicological Effects of Organophosphates on Freshwater Fish. Aquatic Toxicology Research, 62(1), 85-93.

Mallatt, J. (1985). Fish gill structural changes induced by toxicants and other irritants: A statistical review. Canadian Journal of Fisheries and Aquatic Sciences, 42(4), 630-648.

Mazon, A. F., Monteiro, E. A. S., Pinheiro, G. H. D., & Fernandes, M. N. (2002). Hematological and Physiological Changes Induced by Short-Term Exposure to Copper in the Freshwater Fish Prochilodus scrofa. Brazilian Journal of Biology, 62(4), 621-631.

Mehta, R., et al. (2023). "Environmental fate and ecotoxicological effects of profenofos: A review." Journal of Hazardous Materials, 445, 130567.

Miranda, A. L., Ribeiro, C. A. O., & Carvalho, C. S. (2008). Involvement of the Antioxidant System in the Tolerance of Leporinus obtusidens to Organophosphate Profenofos. Ecotoxicology and Environmental Safety, 71(1), 1-8.

Mishra, A., et al. (2023). "Scanning electron microscopy as a tool for assessing pesticide-induced morphological changes in fish gills." *Microscopy Research and Technique*, 86(4), 456–465.

Moore, A., & Waring, C. P. (2001). The Effects of a Synthetic Pyrethroid Pesticide on Some Aspects of Reproduction in Atlantic Salmon (Salmo salar L.). Aquatic Toxicology, 52(1), 1-12.

Naveed A, Venkateshwarlu P, Janaiah C. Impact of sublethal concentration of triazophos on regulation of protein metabolism in the fish Channa punctatus (Bloch). African Journal of Biotechnology. 2010 Nov; 9(45): 7753-8.

Nwani, C. D., Lakra, W. S., Nagpure, N. S., Kumar, R., Kushwaha, B., & Srivastava, S. K. (2010). Toxicity of the Herbicide Atrazine: Effects on Lipid Peroxidation and Antioxidant Defense System in the Freshwater Fish *Channa punctatus* (Bloch). Ecotoxicology and Environmental Safety, 73(2), 214-221.

OECD. (2019). Guidelines for Testing of Chemicals: Fish Acute Toxicity Test. Organization for Economic Cooperation and Development.

Pandey, S., Parvez, S., Ansari, R. A., Ali, M., Kaur, M., Hayat, F., & Raisuddin, S. (2008). Effects of exposure to multiple trace metals on biochemical, histological, and ultrastructural features of gills of a freshwater fish, *Channa punctata* Bloch. Chemico-Biological Interactions, 174(3), 183-192.

Patel, S., et al. (2021). "Ecotoxicological effects of profenofos on non-target aquatic organisms: A comprehensive review." Ecotoxicology and Environmental Safety, 208, 111678Radwan, M. A., & Mohamed, H. R. H. (2008). Imidacloprid Induces Biochemical and Histopathological Alterations in the Albino Rat, Rattus norvegicus. Journal of Environmental Science and Health, Part B, 43(7), 694-707.

Rahman, M. M., Hossain, M. S., & Hossain, M. A. (2015). Ecotoxicological studies on Channa punctata (Bloch) exposed to profenofos. Journal of Environmental Science and Health, Part B, 50(6), 425-432.

Rao, J. V. (2006). Toxic Effects of Profenofos on Serum Biochemical Changes in Clarias batrachus (Linn). Pesticide Biochemistry and Physiology, 86(3), 143-148.

Reddy, K., et al. (2022). "Profenofos-induced genotoxicity and histopathological changes in the gills of Labeo rohita." Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 879, 503518.Singh, K., & Rathore, M. (2017). Morphological Changes in Fish Gills Due to Pesticide Exposure: A Review. Environmental Science and Pollution Research, 24(10), 9056-9065.

Reddy NC, Rao JVJE, Safety E. Biological response of earthworm, Eisenia foetida (Savigny) to an organophosphorous pesticide, profenofos. Ecotoxicology and Environmental Safety. 2008 Oct; 71(2): 574-82. doi: 10.1016/j.ecoenv.2008.01.003. DOI: https://doi.org/10.1016/j.ecoenv.2008.01.003

Saravanan M, Kumar KP, Ramesh M. Haematological and biochemical responses of freshwater teleost fish Cyprinus carpio (Actinopterygii: Cypriniformes) during acute and chronic sublethal exposure to lindane. Pesticide Biochemistry and Physiology. 2011 July; 100(3): 206-11. doi: 10.1016/j.pestbp.2011.04.002. DOI: <https://doi.org/10.1016/j.pestbp.2011.04.002>

Santos-Silva T., de Azambuja Ribeiro R.I.M., Alves S.N., Thomél R.G., dos Santos H.B. Assessment of the toxicological effects of pesticides and detergent mixtures on zebrafish gills: a histological study. Braz. Arch. Biol. Technol. 2021;64 doi: 10.1590/1678-4324-2021210198.

Singh, P., et al. (2022). "Gill damage in fish as a biomarker of environmental pollution: A review." Environmental Science and Pollution Research, 29(12), 16789–16803.

Sharma, P., et al. (2023). "Profenofos-induced alterations in the gill ultrastructure of Heteropneustes fossilis: A scanning electron microscopy study." Tissue and Cell, 82, 102075.

Sharma, R., et al. (2022). "Oxidative stress and antioxidant responses in fish exposed to profenofos: A review." *Aquatic Toxicology*, 250, 106264.

Singh, R., et al. (2023). "Profenofos-induced changes in the gill morphology of Channa striatus: A scanning electron microscopy study." Journal of Microscopy and Ultrastructure, 11(1), 45–52.

Tiwari, R., et al. (2021). "Impact of profenofos on aquatic ecosystems: A review of its toxicity and persistence." *Environmental Chemistry Letters*, 19(2), 1235–1248

Van der Oost, R., Beyer, J., & Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environmental Toxicology and Pharmacology, 13(2), 57-149.

Velisek, J., Stara, A., & Machova, J. (2012). Effects of Pesticides on Fish: Toxicity, Impacts, and the Role of Biomarkers in Risk Assessment. Interdisciplinary Toxicology, 5(2), 55-62.

Verma, P., et al. (2023). "Biomarkers of pesticide exposure in fish: A review of recent advancements." *Science of the Total Environment*, 876, 162834

Weis, J. S., & Weis, P. (1989). Effects of Environmental Pollutants on Early Fish Development. Reviews in Aquatic Sciences, 1(1), 45-73.

Yadav, S., et al. (2022). "Profenofos-induced oxidative stress and histopathological alterations in the gills of freshwater fish Channa punctatus." Toxicology Reports, 9, 1234–1242.