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A review on evaluation of the toxicological impact of Isoproturon herbicide on fish

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Abstract
Isoproturon, a widely used Phenylurea herbicide in agriculture, poses considerable ecological risks due to its persistence in aquatic ecosystems. When exposed to this chemical, fish, often used as indicator species for environmental health, exhibit diverse biochemical, genotoxic, behavioral, and neurotoxic responses. This review compiles recent research on the impact of Isoproturon on fish, including findings on oxidative stress, DNA damage, Altered Behaviors, and Neurotoxic effects. Studies reveal that Isoproturon exposure negatively affects fish health and survival, underscoring the need for stricter regulations and alternative agricultural practices to mitigate environmental contamination.

Keywords: Behavioral changes, DNA damage, fish toxicity, Isoproturon, neurotoxicity, oxidative stress

Introduction
Isoproturon, a commonly applied herbicide for controlling broadleaf weeds and grasses in agriculture, is increasingly recognized as an environmental concern due to its persistence and accumulation potential in aquatic ecosystems through agricultural runoff. Its chemical stability enables Isoproturon to remain in the environment for extended durations, adversely impacting non-target organisms, particularly fish, which are essential bio-indicators of aquatic health and ecosystem stability. Given the role of fish in reflecting ecosystem health, it is vital to understand the physiological, genetic, and behavioral impacts of Isoproturon exposure on these organisms.
Research indicates that Isoproturon exposure significantly alters physiological functions in fish, particularly affecting enzyme activity and oxidative stress markers. (Sharma *et al.*, 2019) [27] studied *Labeo rohita* and reported notable changes in antioxidant enzyme activity, along with liver tissue histology alterations that suggested hepatic stress. Other studies have identified elevated levels of Malondialdehyde (MDA), a lipid peroxidation marker, in fish exposed to Isoproturon indicating that oxidative stress induced by the herbicide may compromise immune function and overall health, thus potentially affecting biodiversity and ecosystem stability.
The genotoxic effects of Isoproturon fish have also been documented. In a study on *Cyprinus carpio*, (Patel *et al.*,2020) [19] observed dose-dependent increases in DNA damage, using methods like the comet assay that revealed substantial DNA fragmentation. These genotoxic impacts raise concerns not only about the health of individual fish but also about potential risks to genetic diversity and the long-term survival of fish populations.
Isoproturon has also been shown to have Neurotoxic and Behavioral effects. (Kumar and Sahu 2021) [13] Examined neurobehavioral effects in *Danio rerio*, finding notable impairments in swimming activity, stimulus-response, and predator avoidance behaviors linked to the inhibition of acetylcholinesterase activity. This enzyme inhibition leads to acetylcholine accumulation, overstimulating cholinergic pathways, and disrupting survival behaviors crucial for fish fitness and survival, further threatening the stability of aquatic ecosystems.
(Singh and Verma, 2022) [29] conducted a comprehensive review of Isoproturon toxic kinetics, noting its bioaccumulation in aquatic organisms and its chronic toxic effects. Their findings highlight the importance of stricter regulatory oversight and the development of sustainable agricultural practices. Together, these studies emphasize the need for environmentally responsible agricultural approaches and stricter regulations to reduce the harmful impacts of herbicides like Isoproturon aquatic ecosystems.

Biochemical Effects of Isoproturon on Fish
Isoproturon, a widely applied agricultural herbicide, has raised environmental concerns due to its persistence in aquatic ecosystems and toxic effects on non-target species, especially fish. As bio-indicators of ecosystem health, fish are particularly vulnerable to the biochemical disruptions caused by Isoproturon exposure, which impacts oxidative stress responses, liver and kidney function, and essential metabolic processes. These disruptions can weaken fish health and survival, emphasizing the broader ecological consequences of Isoproturon contamination.

- **Oxidative Stress**

One major biochemical response to Isoproturon exposure is oxidative stress, which disturbs cellular equilibrium and elevates reactive oxygen species production. (Jadhav *et al.*, 2017) [9] found that *Labeo rohita* exposed to sub-lethal levels of Isoproturon showed increased lipid peroxidation and reduced antioxidant enzyme activity, particularly superoxide dismutase and catalase. This heightened lipid peroxidation indicates severe cellular damage and reduced antioxidant defense capacity. Similarly, (Singh and Mishra, 2019) [30] observed that *Oreochromis niloticus* exposed to Isoproturon had elevated malondialdehyde levels, an indicator of oxidative damage, alongside reduced glutathione peroxidase activity. Together, these findings indicate that oxidative stress is a primary biochemical effect of Isoproturon, leading to weakened immune defenses and health in fish populations.

- **Liver and Kidney Function**

Isoproturon exposure has also been associated with liver and kidney toxicity, as indicated by elevated biomarkers. (Patel *et al.*, 2018) [20] observed that *Cyprinus carpio* exposed to Isoproturon exhibited higher serum levels of alanine aminotransferase and aspartate aminotransferase, markers indicative of liver cell damage. This liver toxicity impairs the fish's detoxification abilities, making them more vulnerable to additional environmental stressors. (Rao and Khan, 2020) [12, 24] found increased creatinine and urea levels in the serum of Isoproturon-exposed fish, indicating renal toxicity that compromises kidney filtration and waste elimination processes. This dysfunction in liver and kidney activity threatens fish health and demonstrates the toxic effects of Isoproturon on aquatic organisms.

- **Metabolic Disturbances**

Isoproturon exposure also significantly disrupts metabolic processes, especially those essential for energy production. (Das and Kumar, 2021) [5] Observed that *Catla catla* exposed to Isoproturon showed impairment in both glycolytic and mitochondrial pathways, which are crucial for ATP production. Reduced energy metabolism from these disruptions can impact growth, immune function, and reproduction. By affecting these key metabolic processes, Isoproturon lowers overall fitness, reducing the survival and reproductive success of fish within impacted ecosystems.

Genotoxic Effects of Isoproturon on Fish

Isoproturon, due to its chemical stability and widespread use in agriculture, has raised concerns regarding its genotoxic effects on fish, which are important bio-indicators in aquatic ecosystems. Research shows that exposure to Isoproturon can lead to DNA damage, chromosomal abnormalities, and alterations in gene expression, all of which may undermine genetic integrity, affect fish health, and potentially reduce population viability.

1. DNA Damage

The genotoxic effects of Isoproturon on fish DNA have been extensively studied, with findings indicating significant DNA strand breaks and chromosomal instability. (Kaur *et al.*, 2017) [11] utilized the comet assay to assess DNA damage in *Channa punctatus* exposed to Isoproturon, observing considerable DNA strand breaks that pointed to severe genotoxic stress. Similarly, (Banerjee *et al.*,2018) [2] found an increased frequency of micronuclei in *Danio rerio*, which signifies chromosomal damage and a reduction in genetic stability. These studies emphasize the potential of Isoproturon to induce structural DNA damage, compromising cellular integrity and making organisms more vulnerable to additional environmental stressors.

2. Chromosomal Abnormalities

Chromosomal abnormalities are another aspect of the genotoxicity associated with Isoproturon exposure. (Sharma and Gupta, 2019) [27] reported chromosomal aberrations in *Labeo rohita* after exposure to the herbicide, raising concerns about heritable genetic damage. The persistence of these chromosomal abnormalities in fish populations could lead to an increased frequency of mutations passed to offspring, posing long-term risks to genetic diversity and stability within these populations. The cumulative impact of such genetic damage could ultimately jeopardize population resilience in contaminated environments.

3. Gene Expression Changes

Isoproturon exposure has also been shown to affect gene expression, particularly genes involved in stress response and DNA repair. (Pandey *et al.*, 2020) [17] Observed that *Oreochromis mossambicus* exposed to Isoproturon exhibited downregulation of genes responsible for managing stress and repairing DNA damage. This suppression of essential genes may impair the fish's ability to defend itself against environmental stressors, making it more susceptible to both chemical pollutants and natural challenges. The downregulation of DNA repair genes weakens the organism's capacity to counteract genotoxic effects, potentially leading to ongoing DNA damage and a decline in genetic health over time.

Behavioral Changes Due to Isoproturon Exposure

1. Alterations in Feeding, Swimming, and Social Behaviors

Behavioral changes often serve as early warning signs of sub-lethal toxicity in aquatic species. (Reddy and Rao, 2019) [25] observed that *Labeo rohita* exposed to Isoproturon displayed reduced feeding activity and more erratic swimming, indicating potential neurological effects. This decrease in feeding behavior can hinder fish growth and survival, while erratic swimming could increase their vulnerability to predators. Similarly, (Singh *et al.*, 2018) [31] found that *Cyprinus carpio* exposed to Isoproturon showed lower activity levels and impaired swimming coordination, which could affect energy acquisition and overall health.

(Kumar *et al.*, 2020) [14] studied *Oreochromis niloticus* and found that exposure to Isoproturon led to a reduction in social interactions and an increase in escape behaviors. These behavioral changes may disrupt social dynamics in fish populations, influencing breeding success and survival. Further research by (Barman *et al.*, 2019) [3] revealed that *Poecilia reticulata* exposed to Isoproturon exhibited decreased mating behaviors, suggesting potential long-term effects on reproductive success and population sustainability.

2. Effects on Predator Avoidance and Foraging Efficiency

(Sarkar *et al.*, 2021) [26] Reported that *Catla catla* exposed to Isoproturon showed diminished predator avoidance responses, including delayed reactions and poor foraging efficiency. These behavioral impairments could heighten predation risk and reduce food intake, leading to decreased survival rates. In agreement with these findings, (Pires *et al.*, 2022) [22] noted that *Danio rerio* exhibited slower swimming speeds and reduced predator avoidance abilities when exposed to Isoproturon. These changes can hinder their ability to evade predators and forage effectively, which could negatively affect their overall health and survival.

In a similar, (Patel *et al.*, 2023) [21] found that *Gambusia affinis* exposed to Isoproturon experienced decreased foraging efficiency, spending more time searching for food and capturing fewer prey. Such inefficiencies could significantly impact fish populations, particularly in environments with limited food availability.

Neurotoxic Effects of Isoproturon on Fish

1. Alterations in Neurotransmitter Levels

Research on neurotoxicity has shown that Isoproturon exposure affects neurotransmitter levels in fish brains.

(Gupta and Sharma, 2020) [8] discovered that *Cyprinus carpio* exposed to Isoproturon exhibited altered acetylcholinesterase (AChE) activity, an enzyme essential for normal nervous system functioning. A decrease in AChE activity can cause imbalances in neurotransmitters, leading to problems with muscle coordination, response to stimuli, and cognitive processes.

Similarly, (Das and Banerjee, 2021) [6] found changes in dopamine and serotonin levels in *Oreochromis mossambicus* exposed to Isoproturon. Such disruptions in neurotransmitters can influence mood, stress responses, and social behavior, potentially causing abnormal behaviors. In addition, (Li *et al.*, 2022) [16] observed similar effects in *Gambusia affinis*, noting a reduction in dopamine and serotonin levels, which was linked to anxiety-like behavior and social withdrawal. This demonstrates the wide-reaching impact of Isoproturon on neurochemical balance and fish behavior.

2. Brain Tissue Damage and Histopathological Alterations

Histopathological studies help understand the physical damage Isoproturon causes to the brain. (Kumar *et al.*, 2022) [15] found that *Danio rerio* exposed to Isoproturon showed neuronal degeneration, with brain tissues exhibiting signs of inflammation, vacuolization, and necrosis. These changes are clear indicators of direct neurotoxic effects that could impair cognitive and sensory functions. Similarly, (Raza *et al.*, 2023) [23] studied *Heteropneustes fossilis* and found similar brain tissue damage, including necrosis and glial cell proliferation, suggesting a compromised neuroprotective response to the herbicide.

3. Effects on Cognitive and Sensory Abilities

Exposure to Isoproturon has been shown to impair both cognitive and sensory functions in fish. (Ahmed *et al.*, 2019) [1] found that *Oreochromis niloticus* exposed to Isoproturon had reduced cognitive performance, as indicated by poor maze-navigation results. This suggests that chronic exposure may hinder learning and memory, limiting the fish's ability to adapt to environmental changes. (Khan *et al.*, 2020) [12] also found that *Poecilia reticulata* exposed to Isoproturon experienced a reduction in olfactory sensitivity, which could affect their ability to find food and avoid predators, thus reducing their overall survival chances.

Comparative Sensitivity of Fish Species to Isoproturon

Different fish species show varying levels of sensitivity to Isoproturon, which may be influenced by their unique physiological, metabolic, and biochemical traits. (Jain *et al.*, 2021) [10] found that *Catla catla* was more susceptible to the oxidative and neurotoxic effects of Isoproturon compared to *Labeo rohita*. This difference could be attributed to variations in antioxidant defense mechanisms, detoxification processes, and the sensitivity of their nervous systems. *Catla catla* might have a less effective ability to process or neutralize the herbicide, leading to a higher accumulation of toxic compounds in its tissues.

In a similar study, (Singh *et al.*, 2022) [32] discovered that *Danio rerio* was more sensitive to Isoproturon than *Puntius tetrazona*, especially regarding oxidative stress. *Danio rerio* showed significant increases in lipid peroxidation and a reduction in antioxidant enzyme activity, indicating greater oxidative damage. This finding suggests that species with more robust antioxidant systems are better able to handle pollutants like Isoproturon, and this difference in oxidative stress responses could explain why some species are more affected than others.

Furthermore, (Sharma *et al.*, 2023) [28] found that the sensitivity of *Oreochromis niloticus* to Isoproturon varied depending on the developmental stage, with juvenile fish being more vulnerable than adults. The increased sensitivity in juveniles was attributed to their developing metabolic systems, which may have fewer detoxifying enzymes and lower antioxidant capacity. As a result, juvenile fish are less capable of processing toxic substances, making them more at risk during exposure.

These findings highlight the need to consider species-specific differences in Ecotoxicological studies. Multi-species assessments offer a more comprehensive understanding of how different fish species interact with contaminants like Isoproturon, which is essential for developing accurate environmental risk evaluations and management strategies

Mechanisms of Toxicity

Isoproturon toxicity in fish is believed to primarily stem from the generation of reactive oxygen species, leading to oxidative damage, and the inhibition of vital enzymes. (Ghosh *et al.*, 2019) [7] suggested that mitochondrial dysfunction plays a central role in Isoproturon toxicity by causing ATP depletion, which further amplifies oxidative stress and neurotoxic effects. Mitochondria are essential for energy production, and when their function is compromised, the fish cells struggle to repair damage and maintain cellular balance, initiating a cascade of harmful effects, including damage to lipids, proteins, and DNA.

Moreover, fish metabolize Isoproturon into reactive intermediates, intensifying its toxic impact. These metabolites are produced through cytochrome P450 enzyme activity and can bind to various cellular components, disrupting normal functions. As a result, oxidative stress is heightened, leading to cellular membrane damage, inflammation, and tissue damage. (Saha *et al.*, 2020) [34] observed that exposure to Isoproturon in *Labeo rohita* resulted in higher lipid peroxidation levels and decreased activity of antioxidant enzymes, indicating the herbicide's role in inducing oxidative damage through reactive intermediates.

Another key toxic mechanism is the inhibition of essential enzymes. Isoproturon inhibits acetylcholinesterase (AChE), an enzyme critical for normal nervous system function. This inhibition leads to an accumulation of acetylcholine, disrupting nerve signal transmission and causing neurological impairments. Additionally, Isoproturon has been shown to block antioxidant enzymes such as superoxide dismutase and catalase, impairing the fish's ability to counteract ROS and repair oxidative damage. This leaves the fish more susceptible to environmental stress and other toxicants.

Gene expression changes due to Isoproturon exposure are also important in understanding its toxicity. Studies by (Pandey *et al.*, 2021) [18] revealed that exposure to Isoproturon alters the expression of genes involved in stress responses, apoptosis, and DNA repair. These changes impair the fish's ability to address oxidative stress and repair cellular damage, leading to long-term genetic and physiological harm.

Mitigation and Environmental Implications

The harmful effects of Isoproturon herbicide on fish, including oxidative stress, genotoxicity, behavioral changes, and neurotoxicity, pose a significant threat to aquatic ecosystems by endangering fish populations and disrupting the overall health and balance of aquatic biodiversity. (Bhatt *et al.*, 2022) emphasized the importance of creating buffer zones around agricultural areas to prevent Isoproturon runoff from contaminating nearby water bodies, significantly reducing the exposure of aquatic life to the herbicide and its detrimental effects. They also advocated for the adoption of safer, eco-friendly herbicides that pose fewer risks to aquatic environments. Transitioning to such alternatives, coupled with the establishment of stronger regulatory frameworks, can lower the environmental harm caused by chemical herbicides, safeguard freshwater ecosystems from long-term pollution, and promote sustainable farming practices while protecting aquatic biodiversity.

Additionally, (Singh *et al.*, 2021) [33] suggested incorporating integrated pest management (IPM) practices, which combine biological, cultural, and mechanical methods along with reduced chemical usage. This strategy can help minimize the reliance on harmful herbicides like Isoproturon and decrease their environmental impact. By using IPM, farmers can effectively manage pests while lessening the risks of contamination to aquatic ecosystems.

Regular monitoring of pesticide levels in water is also critical for early detection of contamination. Monitoring programs can track the health of aquatic life and identify any adverse changes in the environment caused by Isoproturon exposure. Early intervention and actions such as bioremediation or restoring affected habitats can help mitigate the damage from Isoproturon and reduce its long-term effects on aquatic ecosystems.

Conclusion

This review highlights the detrimental effects of Isoproturon exposure on fish, which occur through biochemical, genotoxic, behavioral, and neurotoxic mechanisms. The herbicide's persistence in aquatic environments and its widespread use in agriculture exacerbate its potential to cause oxidative stress, DNA damage, altered behavior, and neurological impairments in fish, leading to negative impacts on their health and overall ecosystem stability. Studies by (Bhatt *et al.*,2022; Ghosh *et al.*, 2019) [4, 7], and others indicate that Isoproturon exposure disrupts critical physiological processes in fish, making them more vulnerable to other environmental stressors and compromising their survival and reproduction.

The consistent findings of toxicity across multiple fish species underscore the need for stringent regulatory measures and the development of safer, more environmentally friendly weed control methods. (Bhatt *et al.*, 2022; Singh *et al.*, 2021) [4, 33] emphasize the importance of promoting alternative herbicides with lower toxicity profiles and implementing buffer zones to reduce Isoproturon contamination in aquatic ecosystems. Such measures are essential for protecting aquatic biodiversity and maintaining ecosystem health.

Further research is necessary to better understand the long-term, population-level consequences of chronic Isoproturon exposure in fish. Studies should focus on the cumulative impacts across multiple generations and explore how different species adapt to or recover from such toxicity. Investigating the genetic, reproductive, and behavioral impacts over time will provide more comprehensive insights into the herbicide's ecological risks. Moreover, advancements in bioremediation techniques and wastewater treatment options could help mitigate the spread of Isoproturon in contaminated water sources, offering additional avenues for protecting aquatic life.

In conclusion, while the risks associated with Isoproturon are well-documented, continued research and the implementation of effective environmental management strategies are critical for minimizing its harmful effects on aquatic ecosystems. The use of sustainable agricultural practices, coupled with stronger regulations and the development of less harmful herbicides, is key to safeguarding our aquatic resources for future generations.

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