Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue 3 Year 2024 Page 957 - 962

Acute Exposure to Glufosinate Ammonium Induces Tissue Glycogen Depletion in the Freshwater Fish *Labeo rohita*

Manjiri A. More[1] , Narayan R. Mane[2*]

[1]Department of Zoology, Gopal Krishna Gokhale College, Kolhapur-416012, Maharashtra, India
[2]Department of Zoology, Gopal Krishna Gokhale College, Kolhapur-416012, Maharashtra, India, Email: manenarayan.136@rediffmail.com*

***Corresponding author's** E-mail: manenarayan.136@rediffmail.com*

Received: 30/01/2024
Revised: 20/03/2024
Accepted: 20/03/2024
Published: 24/06/2024

Abstract

Herbicides are extensively used in agriculture and frequently contaminate aquatic ecosystems through surface runoff, posing a potential risk to non-target aquatic organisms. The present study evaluated the effect of acute exposure to Sweep Power (Glufosinate Ammonium) on the total glycogen content in different tissues of the freshwater fish *Labeo rohita*. Healthy fingerlings were exposed to LC_0 (0.01 ppm) and LC_{50} (0.05 ppm) concentrations of the herbicide for 96 hours. Total glycogen content in the liver, muscle, brain, and gill tissues was estimated by the method of De Zwaan and Zandee (1972). A significant ($P < 0.001$) reduction in glycogen content was observed in all tissues of the treated groups compared to the control, with a greater decline at the LC_{50} concentration. The depletion of glycogen indicates enhanced glycogenolysis and increased energy demand under herbicide-induced stress. The present findings suggest that tissue glycogen is a sensitive biochemical biomarker for assessing the toxic effects of Glufosinate Ammonium in freshwater fishes.

Keywords: Labeo rohita, Glufosinate Ammonium, Sweep Power, Glycogen, Herbicide, Biochemical Biomarker, Ecotoxicology.

INTRODUCTION

The rapid expansion of modern agriculture has substantially increased the application of herbicides for efficient weed control and enhanced crop productivity. Although herbicides contribute significantly to agricultural production, their indiscriminate and excessive use has emerged as a serious environmental concern (Takano and Dayan, 2020; Geng et al., 2021). Large quantities of these chemicals enter nearby aquatic ecosystems through surface runoff, soil erosion, irrigation drainage, and accidental spillage, ultimately contaminating rivers, lakes, ponds, and reservoirs (Silva et al., 2023; FAO, 2022). Consequently, numerous aquatic organisms, particularly freshwater fishes, become exposed to herbicide residues either directly through contaminated water or indirectly through the food chain, resulting in adverse physiological, biochemical, and ecological effects (Xiong et al., 2019; Tudi et al., 2021; Sharma et al., 2024).

Freshwater fishes are considered excellent bioindicators of aquatic pollution because they readily accumulate environmental contaminants in different tissues and reflect changes in water quality (Authman et al., 2015; Ahmed et al., 2022). Owing to their continuous interaction with the aquatic environment through the gills,

digestive tract, and skin, fishes respond rapidly to chemical pollutants by exhibiting measurable physiological, biochemical, histopathological, and behavioural alterations (Rai et al., 2021; Sharma et al., 2024). Herbicide exposure can disrupt normal metabolic processes, induce oxidative stress, alter enzymatic activities, and impair growth, reproduction, immune function, and survival of aquatic organisms (Takano and Dayan, 2020; Tudi et al., 2021). Therefore, biochemical responses in fishes have become reliable and sensitive biomarkers for monitoring environmental contamination and evaluating the toxic effects of pesticides and other aquatic pollutants (Silva et al., 2023; Kumar et al., 2024).

Glufosinate Ammonium is a broad-spectrum, non-selective herbicide widely used for the control of annual and perennial weeds in economically important crops such as soybean, maize, cotton, canola, orchards, and plantation crops (Takano and Dayan, 2020). The herbicide exerts its phytotoxic effect by irreversibly inhibiting glutamine synthetase, resulting in the accumulation of ammonia and disruption of nitrogen metabolism, ultimately leading to plant death (Dayan et al., 2015; Takano and Dayan, 2020). Due to its extensive agricultural application, residues of Glufosinate Ammonium have been detected in agricultural soils, irrigation channels, groundwater, and surface water bodies, raising concerns about its environmental persistence and ecological impacts (Geng et al., 2021; Silva et al., 2023). Recent studies have demonstrated that Glufosinate Ammonium can adversely affect non-target aquatic organisms by inducing oxidative stress, neurotoxicity, immunotoxicity, mitochondrial dysfunction, and disturbances in energy metabolism, thereby compromising normal physiological functions (Xiong et al., 2019; Takano and Dayan, 2020; Sharma et al., 2024).

The liver, muscle, gills, and brain are among the most metabolically active organs in fishes and are particularly susceptible to herbicide-induced toxicity (Ahmed et al., 2022; Sharma et al., 2024). The liver plays a central role in carbohydrate metabolism, detoxification, and biotransformation of xenobiotics, making it one of the primary target organs for toxicant-induced metabolic disturbances (Takano and Dayan, 2020; Kumar et al., 2024). Skeletal muscle serves as the principal energy reservoir, where glycogen is stored and mobilized to meet increased metabolic demands under stress conditions (Prakash and Verma, 2020). The gills constitute the primary interface between fish and the surrounding aquatic environment and are continuously exposed to dissolved pollutants, rendering them highly vulnerable to herbicide uptake and toxic injury (Silva et al., 2023; Xiong et al., 2019). Similarly, the brain requires a continuous supply of energy to maintain neuronal function, neurotransmission, and cellular homeostasis, and therefore is highly sensitive to disturbances in carbohydrate metabolism induced by environmental contaminants (Takano and Dayan, 2020). Consequently, biochemical alterations in these tissues provide valuable biomarkers for assessing the physiological and toxicological responses of fishes exposed to herbicides and other aquatic pollutants (Tudi et al., 2021; Kumar et al., 2024).

Among various biochemical constituents, glycogen is the primary storage form of carbohydrate in vertebrates and plays a central role in maintaining energy homeostasis. Under normal physiological conditions, glycogen stored mainly in liver and muscle tissues provides glucose to meet metabolic demands. However, exposure to environmental stressors such as pesticides, heavy metals and herbicides often accelerates glycogen breakdown through glycogenolysis to compensate for increased energy requirements. Consequently, depletion of tissue glycogen has been widely recognized as an important indicator of toxic stress in aquatic organisms.

Several studies have reported significant reductions in glycogen content in fishes exposed to different classes of pesticides and herbicides, indicating disruption of carbohydrate metabolism under toxic stress (Somaiah et al., 2014; Prakash and Verma, 2020; Ahmed et al., 2022). Herbicide-induced glycogen depletion has been attributed to accelerated glycogenolysis, increased energy expenditure, activation of stress hormones such as cortisol and catecholamines, and impairment of normal metabolic regulation to meet the elevated energy demands associated with detoxification and cellular homeostasis (Barton, 2002; Takano and Dayan, 2020; Kumar et al., 2024). Consequently, the depletion of glycogen reserves represents an adaptive physiological response that enables fish to maintain energy production under environmental stress. Therefore, tissue glycogen has been widely recognized as a sensitive biochemical biomarker for assessing the metabolic effects of herbicides and other environmental contaminants in aquatic organisms (Silva et al., 2023; Sharma et al., 2024).

Although numerous studies have investigated the toxicological effects of herbicides on oxidative stress, antioxidant defense systems, protein metabolism, enzymatic activities, and histopathological alterations in aquatic organisms, information regarding the effects of Glufosinate Ammonium on tissue glycogen metabolism in freshwater fishes remains limited (Takano and Dayan, 2020; Xiong et al., 2019; Sharma et al., 2024). Since glycogen is a key energy reserve that is rapidly mobilized under toxic stress, understanding herbicide-induced alterations in carbohydrate metabolism is essential for elucidating the physiological responses of fishes and assessing the ecological risks associated with herbicide contamination in freshwater ecosystems (Silva et al., 2023; Kumar et al., 2024). Therefore, studies focusing on tissue glycogen depletion can provide valuable insights into the metabolic disturbances caused by Glufosinate Ammonium and support the development of sensitive biochemical biomarkers for environmental monitoring.

Therefore, the present investigation was designed to evaluate the effect of acute exposure to the commercial

herbicide Sweep Power (Glufosinate Ammonium) on total glycogen content in the gill, liver, muscle and brain tissues of the freshwater fish *Labeo rohita*. The study aims to determine whether herbicide-induced stress results in depletion of glycogen reserves and to assess the suitability of tissue glycogen as a biochemical biomarker for monitoring aquatic herbicide pollution.

MATERIALS AND METHODS

Experimental Fish and Laboratory Conditions

The present investigation was carried out in the Department of Zoology, Gopal Krishna Gokhale College, Kolhapur, Maharashtra, India. Healthy fingerlings of the freshwater fish *Labeo rohita* (Hamilton) measuring 6–8 cm in length and weighing 9–11 g were procured from a local fish supplier in Kolhapur. Prior to acclimatization, the fishes were disinfected by giving a prophylactic bath to minimize the risk of infections and external parasites. Subsequently, the fishes were acclimatized in glass aquaria containing dechlorinated tap water for 15 days under laboratory conditions.

During acclimatization, water temperature was maintained at 26 ± 2°C with a 12 h light:12 h dark photoperiod. Continuous aeration was provided throughout the acclimatization period, and the fishes were fed daily with commercially available pelleted fish feed. The aquarium water was renewed periodically to maintain optimum water quality.

Herbicide

The commercial herbicide Sweep Power, containing Glufosinate Ammonium as the active ingredient, was procured from M/s Super Bio Tech Marketing Company, India. Fresh stock solutions were prepared using distilled water before each experiment.

Experimental Design

Healthy acclimatized fishes were randomly divided into three experimental groups, each consisting of ten individuals maintained in separate 20-L plastic troughs.

1.Group I: Control (without herbicide exposure)

2.Group II: LC_0 concentration (0.01 ppm Glufosinate Ammonium)

3.Group III: LC_{50} concentration (0.05 ppm Glufosinate Ammonium)

The exposure period was 96 hours under static-renewal conditions. The experimental media were renewed after every 24 hours with freshly prepared herbicide solutions to maintain constant exposure concentrations. During the exposure period, the fishes were closely observed for behavioural abnormalities and mortality.

Collection of Tissue Samples

At the end of the 96-hour exposure period, fishes from each experimental group were sacrificed humanely. The liver, gill, muscle and brain tissues were carefully excised, rinsed with ice-cold physiological saline to remove adhering blood, blotted dry using filter paper and immediately processed for biochemical estimation of total glycogen.

Estimation of Total Glycogen

Total glycogen content in the liver, gill, muscle, and brain tissues of *Labeo rohita* was estimated according to the method of De Zwaan and Zandee (1972) using the Anthrone reagent. After 96 hours of exposure, fresh tissue samples from the control, LC_0 (0.01 ppm), and LC_{50} (0.05 ppm) groups were homogenized, and glycogen was extracted by alkaline digestion followed by ethanol precipitation. The glycogen content was determined colorimetrically at 660 nm using D-glucose as the standard and expressed as µg glycogen/mg wet weight of tissue.

Statistical Analysis

The experimental data obtained from all biochemical estimations were expressed as mean ± standard deviation (Mean ± SD) for each experimental group. Statistical comparisons between the control and herbicide-treated groups were performed using Student's t-test. Differences were considered statistically significant at $P < 0.05$, while higher levels of significance ($P < 0.01$ and $P < 0.001$) were also recorded wherever applicable.

RESULTS

The effect of acute exposure to Sweep Power (Glufosinate Ammonium) on the total glycogen content in different tissues of *Labeo rohita* after 96 hours is presented in Table 1 and Figure 1. A significant reduction ($P < 0.001$) in glycogen content was observed in the liver, muscle, brain, and gill tissues of fishes exposed to both LC_0 (0.01 ppm) and LC_{50} (0.05 ppm) concentrations compared to the control group. The reduction was more pronounced in the LC_{50} group than in the LC_0 group, indicating a dose-dependent effect of the herbicide.

Among the tissues studied, the greatest depletion of glycogen was observed in the gills, followed by the liver, muscle, and brain.

Table 1. Effect of acute exposure to Sweep Power (Glufosinate Ammonium) on total glycogen content in different tissues of *Labeo rohita* after 96 hours.

Groups	Liver	Muscle	Brain	Gill
Control	36.58 ± 2.05	40.58 ± 1.46	33.52 ± 1.58	28.58 ± 2.32
LC_0	24.67 ± 1.75***	31.50 ± 1.29***	24.46 ± 1.19***	16.25 ± 1.78***
LC_{50}	16.80 ± 1.89***	20.85 ± 1.25***	18.67 ± 1.47***	9.45 ± 2.37***

Values are expressed as Arithmetic Mean (n = 5) ± SD. ***P < 0.001 compared with the control group.

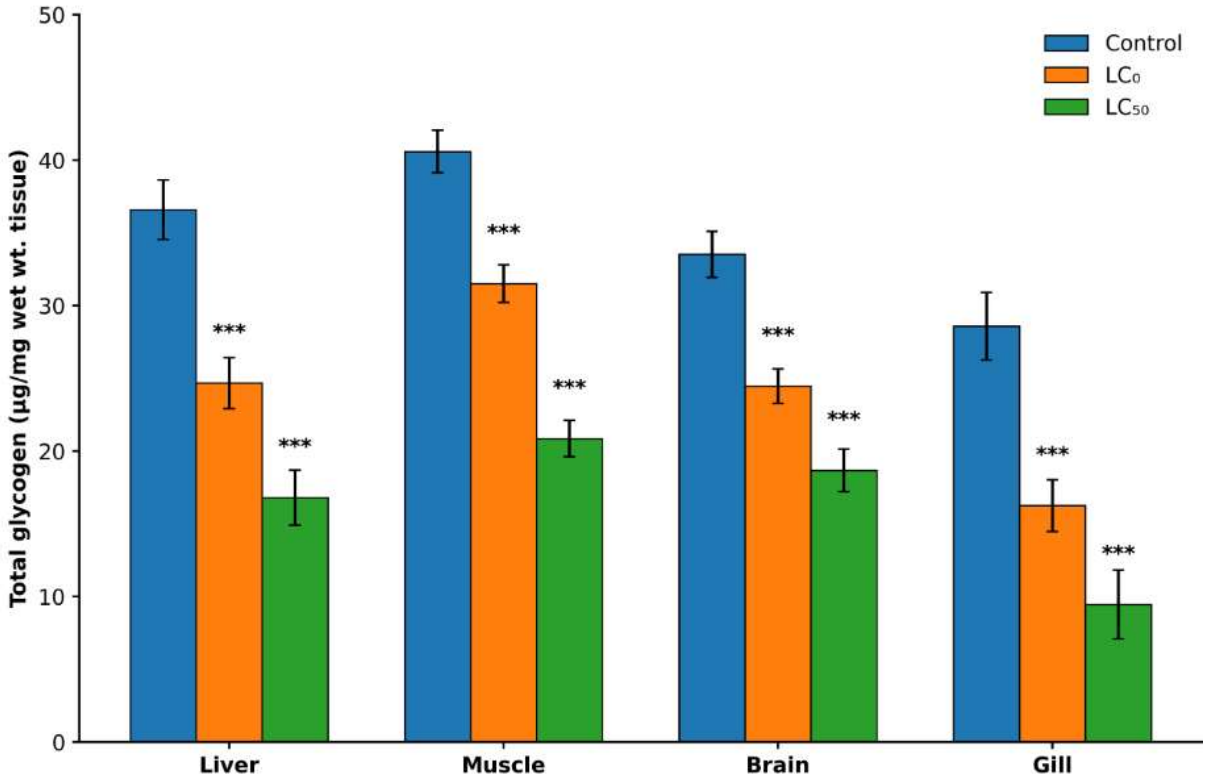

Figure 1. Total glycogen content in different tissues of *Labeo rohita* after acute exposure to Sweep Power (Glufosinate Ammonium). Values are expressed as Arithmetic Mean (n = 5) ± SD. ***P < 0.001 compared with the control group.

DISCUSSION

The present investigation demonstrated that acute exposure to the herbicide Sweep Power (Glufosinate Ammonium) caused a significant depletion of glycogen content in the liver, muscle, brain and gill tissues of *Labeo rohita*. The reduction was observed in both LC_0 and LC_{50} exposure groups, with a greater decline at the LC_{50} concentration, indicating a concentration-dependent effect of the herbicide on carbohydrate metabolism. Similar concentration-dependent biochemical alterations have been reported in fishes exposed to various herbicides and pesticides (Kang et al., 2014; Takano and Dayan, 2020).

Glycogen serves as the primary storage form of carbohydrate and represents the major energy reserve in fishes. Under toxic stress, glycogen is rapidly converted into glucose through glycogenolysis to meet the increased energy demand required for detoxification, osmoregulation, maintenance of cellular homeostasis and repair of damaged tissues (Somaiah et al., 2014). Therefore, depletion of glycogen is considered one of the earliest biochemical indicators of pesticide-induced stress in aquatic organisms (Pazhanisamy and Indra, 2007).

The liver exhibited a marked reduction in glycogen content following Glufosinate Ammonium exposure. Since the liver is the principal organ involved in carbohydrate metabolism and xenobiotic detoxification, increased glycogen breakdown may occur to provide glucose required for enhanced metabolic activity during herbicide stress. Similar observations have been reported in Labeo rohita and other freshwater fishes exposed to arsenic,

organophosphate pesticides and pyrethroids (Prakash and Verma, 2020; Lekeshmanaswamy, 2018; Veeraiah et al., 2014).

A significant decrease in muscle glycogen was also recorded in the present investigation. Muscle tissue acts as an important energy reservoir, and depletion of glycogen reflects increased utilization of stored carbohydrates to sustain muscular activity and physiological adaptation under toxic conditions. Similar reductions in muscle glycogen have been reported following exposure to malathion, glyphosate and other agrochemicals (Somaiah et al., 2014; Pazhanisamy and Indra, 2007).

Brain tissue also exhibited a significant reduction in glycogen content. Although glycogen concentration in the brain is comparatively low, it plays an essential role in maintaining neuronal metabolism during stress. Herbicide-induced depletion of brain glycogen may be associated with increased neuronal energy demand, oxidative stress and disturbance of neurotransmitter metabolism. Glufosinate Ammonium inhibits glutamine synthetase, resulting in ammonia accumulation and altered glutamate metabolism, which may contribute to neurotoxicity (Watanabe and Iwase, 1996; Takano and Dayan, 2020).

Among all tissues examined, the gills showed the greatest depletion of glycogen. Gills are continuously exposed to the external aquatic environment and constitute the primary route for the uptake of dissolved toxicants. Herbicide exposure increases respiratory activity, ionic imbalance and osmoregulatory stress, thereby enhancing energy consumption and accelerating glycogen utilization. Similar biochemical alterations in gill tissues have been reported in freshwater fishes exposed to herbicides and heavy metals (Qian et al., 2008; Kang et al., 2014).

Recent investigations have demonstrated that Glufosinate Ammonium induces oxidative stress by enhancing the production of reactive oxygen species (ROS), causing lipid peroxidation, mitochondrial dysfunction and impairment of antioxidant defence systems (Takano and Dayan, 2020; Xiong et al., 2019). Increased oxidative stress elevates ATP demand for cellular repair and detoxification, thereby accelerating glycogenolysis and reducing tissue glycogen reserves. Thus, the depletion of glycogen observed in the present study may be attributed not only to increased glucose utilization but also to oxidative damage and disruption of carbohydrate metabolism.

Stress hormones such as cortisol and catecholamines are known to stimulate glycogenolysis and gluconeogenesis in fishes during environmental stress. Elevated secretion of these hormones following herbicide exposure enhances glucose mobilization from glycogen stores to maintain metabolic homeostasis (Barton, 2002). Consequently, prolonged depletion of glycogen reserves may adversely affect growth, immune competence, reproduction and survival of fish populations inhabiting contaminated aquatic ecosystems.

The present findings agree with earlier reports that glycogen depletion is a sensitive biochemical response to pesticide exposure and may serve as an early biomarker of aquatic pollution (Somaiah et al., 2014; Prakash and Verma, 2020). The observed concentration-dependent reduction in glycogen clearly indicates that Glufosinate Ammonium disrupts normal carbohydrate metabolism in *Labeo rohita* and imposes considerable metabolic stress on exposed fishes.

From an ecological perspective, continuous contamination of freshwater bodies with Glufosinate Ammonium through agricultural runoff may compromise fish health by reducing available energy reserves required for growth, swimming performance, predator avoidance and successful reproduction. Such metabolic disturbances may ultimately influence fish population dynamics and freshwater biodiversity.

Therefore, tissue glycogen can be considered a reliable biochemical biomarker for monitoring herbicide contamination in freshwater ecosystems. Future investigations integrating glycogen estimation with oxidative stress biomarkers, enzyme assays, histopathology, molecular biomarkers and gene expression analysis will provide a comprehensive understanding of the ecotoxicological effects of Glufosinate Ammonium in aquatic organisms.

CONCLUSION

The present study demonstrated that acute exposure to the herbicide Sweep Power (Glufosinate Ammonium) significantly reduced the total glycogen content in the liver, muscle, brain, and gill tissues of *Labeo rohita*. The depletion of glycogen was observed in both LC_0 (0.01 ppm) and LC_{50} (0.05 ppm) exposure groups, with a more pronounced reduction at the LC_{50} concentration, indicating a concentration-dependent toxic effect. The marked decrease in tissue glycogen suggests enhanced glycogenolysis and increased utilization of carbohydrate reserves to meet the elevated energy demand under herbicide-induced stress. Among the tissues examined, the gills exhibited the greatest depletion of glycogen, indicating their high susceptibility to waterborne herbicide exposure.

The findings of the present investigation indicate that tissue glycogen is a sensitive biochemical biomarker for assessing herbicide-induced metabolic stress in freshwater fishes. Continuous discharge of Glufosinate Ammonium into aquatic ecosystems through agricultural runoff may adversely affect fish health and disturb

normal energy metabolism. Further investigations involving oxidative stress biomarkers, enzyme assays, histopathological alterations, and molecular studies are recommended to elucidate the precise mechanisms of Glufosinate Ammonium toxicity and its long-term ecological consequences.

REFERENCES

1. Barton, B. A. (2002). Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology, 42(3), 517–525. https://doi.org/10.1093/icb/42.3.517
2. De Zwaan, A., & Zandee, D. I. (1972). Body distribution and seasonal changes in the glycogen content of the common sea mussel, Mytilus edulis. Comparative Biochemistry and Physiology Part A: Physiology, 43(1), 53–58.
3. Ferrari, S., Mettifogo, O. S., Cunha, M. L., Cordeiro, L. F., Polycarpo, G. V., et al. (2021). Effects of low doses of glufosinate-ammonium on upland rice agronomic traits. Gesunde Pflanzen, 73, 1–10.
4. Geng, Y., Jiang, L., Zhang, D., Liu, B., Zhang, J., et al. (2021). Occurrence and environmental risk assessment of glyphosate, aminomethylphosphonic acid, and glufosinate-ammonium in agricultural groundwater and surface water. Science of the Total Environment, 769, 144396. https://doi.org/10.1016/j.scitotenv.2020.144396
5. Kang, G. R., Song, H. Y., & Kim, D. S. (2014). Toxicity and effects of glufosinate-ammonium on the marine medaka (Oryzias dancena). Journal of Fish Pathology, 27(1), 35–43.
6. Lekeshmanaswamy, M. (2018). Studies on the impact of malathion insecticide on certain biochemical constituents of freshwater fish Labeo rohita. Kongunadu Research Journal, 5(1), 93–96.
7. Pazhanisamy, K., & Indra, N. (2007). Toxic effects of arsenic on glycogen content in the freshwater fish Labeo rohita. Nature Environment and Pollution Technology, 6(1), 113–116.
8. Prakash, S. A., & Verma, A. K. (2020). Impact of arsenic on carbohydrate metabolism in freshwater fish Mystus vittatus. Uttar Pradesh Journal of Zoology, 41(5), 16–19.
9. Qian, H., Chen, W., Sheng, G. D., Xu, X., Liu, W., Fu, Z., & others. (2008). Effects of glufosinate on antioxidant enzymes, subcellular structure, and gene expression in Chlorella vulgaris. Aquatic Toxicology, 88(4), 301–307. https://doi.org/10.1016/j.aquatox.2008.05.009
10. Somaiah, K., Satish, P. V., Sunita, K., Nagaraju, B., & Oyebola, O. O. (2014). Toxic impact of phenthoate on protein and glycogen levels in the Indian major carp Labeo rohita. IOSR Journal of Environmental Science, Toxicology and Food Technology, 8(9), 65–73.
11. Takano, H. K., & Dayan, F. E. (2020). Glufosinate-ammonium: A review of the current state of knowledge. Pest Management Science, 76(12), 3911–3925. https://doi.org/10.1002/ps.5965
12. Veeraiah, K., Padmavathi, P., Rao, S. T., & Vivek, C. H. (2014). Pesticide-induced biochemical alterations in freshwater fish. International Journal of Bioassays, 4(4), 3632–3638.
13. Watanabe, T., & Iwase, T. (1996). Developmental and dysmorphogenic effects of glufosinate-ammonium on mouse embryos in culture. Teratology, Carcinogenesis, and Mutagenesis, 16(6), 287–299.
14. Xiong, G., Deng, Y., Li, J., Cao, Z., Liao, X., Liu, Y., Lu, H., & colleagues. (2019). Immunotoxicity and transcriptome analysis of zebrafish embryos in response to glufosinate-ammonium exposure. Chemosphere, 236, 124423.