Chapter

Toxicological Interaction Effects of Herbicides and the Environmental Pollutants on Aquatic Organisms

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Abstract

Although herbicides are designed to remove or control weeds, pollution of water ecosystems with herbicides could have adverse effects on aquatic animals such as fish. The effect of herbicides on nontarget organisms may be different than expected, as herbicides may interact with another environmental contaminant. Since there are different contaminants in the water, fish may live in the cocktail of xenobiotics, including herbicides. Therefore, herbicides alone and in combination with other pollutants could affect fish physiology. Thus, the interaction of environmental contaminants with pesticides may create a situation in which a chemical affects the activity of a pesticide; that is, its effects increase or decrease or produce a new effect that neither of them creates on its own. These interactions may occur due to accidental misuse or lack of knowledge about the active ingredients in the relevant materials. This study aimed to review the effects of herbicides alone and in combination with other xenobiotics on various aspects of fish biology. In this study, different biomarkers were reviewed in fish exposed to herbicides.

Keywords: biomarkers, herbicides, aquatic ecosystems, xenobiotic, aquatic animals

1. Introduction

The agricultural revolution is the starting point for using various types of pesticides and synthetic and chemical fertilizers to increase agriculture crops' volume and maintenance [1–3]. Thus, the development agriculture industry has caused an increase in the pollution of aquatic ecosystems with agrochemicals. Pesticides, including herbicides, are pollutants that can be found in the water around agriculture fields. Herbicides are usually used to control weeds and unwanted plants in agriculture farms, fruit gardens, aquaculture ponds, and urban green spaces [3, 4]. Herbicides may enter water ecosystems when used or after being applied. Penetrating herbicides into surface and groundwaters may occur through the drainage of agriculture farms during spraying or after that [5]. Although herbicides may enter water bodies through the drainage of agricultural fields, they can also be used to control weeds in pools or lagoons. Therefore, they can affect water ecosystems directly or indirectly [4].

Studies showed that herbicides could be detected in the drinking water. For example, concentrations of glyphosate in drinking water in the United States and Australia were 700 μg L^{-1} and 1000 μg L^{-1}, respectively [6].

Tracing some herbicides, such as atrazine, acetochlor, and 2,4-D, in groundwater [7], streams [8], river [9], lake [10], marine ecosystems [11], and estuaries [12] indicates that herbicides are highly mobile. Toxicological data showed that more than 99% of pesticides never affect target organisms. In other words, a significant part of pesticides is released into the environment and influences nontarget organisms [13]. Therefore, the different concentrations of herbicides can impact aquatic organisms' health. Similar reports indicate that even humans and pets are exposed to herbicides.

Although herbicides' chemical structure is designed to affect weeds, they could have toxicity effects on aquatic animals. Herbicides are lipophilic compounds that can easily cross biological barriers and penetrate animals' bodies. The physiological and behavioral changes in aquatic animals exposed to herbicides indicate that herbicides have a potentially toxic effect on nontarget animals. We could observe toxicity effects after aquatic organisms' exposure to herbicides.

Herbicides may be absorbed via gills, skin, or intestinal epithelium. Next, they may enter the blood and distribute it in the various tissues by circulating blood. Although herbicides may be repelled in the urine and feces, they may be reached into the liver via the blood circulation system and metabolized in the hepatocytes by detoxification enzymes. A significant part of herbicides may conjugate with a nonenzyme antioxidant such as glutathione and excrete quickly. Other part of metabolites may be repelled through renal and digestive systems; however, reactive oxygen species (ROS) and some metabolites produced during detoxification remain in animals' bodies. These metabolites and ROS may be conjugated with nonenzyme antioxidants and removed or may be neutralized by antioxidant enzymes. Reactive oxygen species production in the detoxification process of herbicides can induce oxidative stress in aquatic organisms. This phenomenon would occur if detoxification mechanisms in the liver work very well or animals are exposed to a sublethal dose of herbicides. Otherwise, various toxicity effects would be detected in organisms challenged by herbicides.

This chapter aims to illustrate toxicology herbicides to fill gaps in information about the toxicity effects of herbicides on aquatic animals. In this chapter, we try to provide documentation on the effects of herbicides on various aspect of aquatic animals' biology. In addition, we will discuss the interaction of other xenobiotics with herbicides.

2. Interaction of herbicides with other xenobiotics

The natural aquatic ecosystems usually contain various xenobiotics that can affect fish [14, 15]. In other words, fish may live in the cocktails of different pollutants [16, 17]. Thus, fish must be able to survive and resist a range of environmental pollutants [18].

Furthermore, various contaminants may interact with each other [19, 20]. Interaction between pollutants includes additive effects and synergic or antagonistic effects. In the additive and synergistic effects, toxicity and bioavailability of xenobiotics are increased. In contrast, in the antagonistic situation, one or more pollutants reduce toxicity and bioavailability of other xenobiotics [21, 22].

Tabche, et al. [23] studied the combined effects of paraquat and lead (Pb) on the liver of *Oreochromis hornorum*. They found that paraquat and lead had synergistic effects on fish. A synergic effect of microplastic on paraquat toxicity was shown in common carp (*Cyprinus carpio*) by Nematdoost Haghi and Banaee [22]. Also, Xu, et al. [24] displayed that exposure of goldfish (*Carassius auratus*) to paraquat and Pb caused activation of detoxification enzymes in the hepatocytes. The effect of iron oxide nanoparticles (γ-Fe2O3) and glyphosate on the liver of *Poecilia reticulata* was assayed by de Lima Faria, et al. [25]. Changes in the biochemical parameters were detected in the crayfish (*Astacus leptodactylus*) exposed to glyphosate and chlorpyrifos [26, 27]. Bonifacio, Zambrano and Hued [28] displayed that co-exposure to glyphosate and chlorpyrifos changed blood biochemical parameters in *Cnesterodon decemmaculatus*.

3. Biological response of aquatic organisms to herbicides

Therefore, to understand the herbicide effects on aquatic life, herbicide's anecdote is told since primarily its entered aquatic ecosystems, in this chapter. Then, it is said about herbicide's fate in animal's body to its excretion.

After draining herbicides in water ecosystems, they could penetrate the cellular membrane and cytoplasm. These chemical toxicants may influence cell permeability, ion transport, electron transport, and enzyme activities associated membrane. Next, herbicides could disrupt the cellular organelles' functions, which may lead to induce apoptosis, cell necrosis, or activation of the tumorigenesis in cells. Thus, herbicides could affect different functions of the biological membrane.

But the question that may be on readers' minds is whether animal cells are defenseless against herbicides? No!

4. Detoxification and metabolism of herbicides

In two phases, herbicides may be converted into excretable metabolites in hepatocytes of aquatic animals. Maternal compounds combine with oxygen and oxidize in the primary phase (Phase I), known as the biotransformation step. Then, oxidized metabolites are conjugated with water-soluble polar biomolecules in the cell (Phase II). Next, herbicides' metabolites may be excreted through urine or bile [29].

Active compounds as reactive oxygen species are often produced during detoxification that could cause the oxidation of macromolecules. However, a cellular antioxidant defense system could neutralize reactive oxygen species (ROS) and inhibit peroxidation reactions. There is a balance between ROS and cellular antioxidant defense capacity in normal conditions. If this balance is collapsed and ROS levels are more than cellular antioxidant defense potential, oxidative stress would occur. ROS attacks macromolecules in this situation, leading to severe histopathological damage to vital tissues.

The disruption in the detoxification enzymes' function may occur in the fish exposed to herbicides. Therefore, defects in the function of the detoxification system can make fish vulnerable to the toxicity of herbicides. A significant decrease in mitochondrial cytochrome content was reported in *Oreochromis niloticus* exposed to pendimethalin [30]. Zhang et al. [31] assayed mitochondria-immune responses in zebrafish, *Danio rerio* following challenge with dinoseb. They reported a significant

decrease in the expression of genes involved in mitochondrial respiration and cellular detoxification [31].

We know very well that exposure of fish to xenobiotics such as herbicides could cause an imbalance between ROS contents and cellular antioxidant defense capacity [32]. Therefore, exposure of fish to herbicides could lead to oxidative stress. Damage to membrane phospholipids decreases the cellular chance of survival and increases apoptosis and necrosis rates. Disruption in the cellular membrane's physiological function also affects metabolism, biochemical hemostasis, gene expression, and DNA replication in the cells [15]. In the following, we want to explain the effects of herbicides on aquatic animals in more detail.

Involvement of cellular detoxification and biotransformation systems to remove xenobiotics may reduce its ability to detoxify herbicides. Therefore, the toxic effects of herbicides on fish would be increased if the detoxification mechanism was collapsed.

5. Oxidative stress

The oxidative stress in fish exposed to herbicides can be attributed to ROS. Furthermore, ROS production during the detoxification of other xenobiotics may further contribute to oxidative stress due to herbicide exposure.

Like other vertebrates, the antioxidant defense system of fish includes antioxidant enzymes and nonenzyme antioxidants. Therefore, change in the antioxidant enzyme activities and nonenzyme antioxidant contents are biomarkers that show activation of the antioxidant defense system against ROS. Pereira, Fernandes and Martinez [33] showed that hepatic antioxidant enzymes activated after exposure of *Prochilodus lineatus* to clomazone. Oxidative damage was seen in the hepatocytes of *O. niloticus* and *Geophagus brasiliensis* after treatment with mesotrione herbicide [34].

Changes in the antioxidant enzyme activities indicated oxidative stress in the gills and liver of tetra fish (*Astyanax altiparanae*) exposed to atrazine [35]. Moraes, et al. [36] found that oxidative stress occurred in the teleost fish (*Leporinus obtusidens*) after exposure to clomazone and propanil.

Otherwise, interaction of ROS with vital macromolecules such as DNA, lipids, proteins, etc., can lead to their peroxidation. Thus, these macromolecules may be lost their biological functions, and their metabolites may disrupt the cellular hemostasis.

In the assessment of oxidative damages, a measure of malondialdehyde, protein carbonyl, oxidized thiol groups, and 7,8-dihydro-8-oxoguanine (8-oxo-dG) is routine.

Malondialdehyde is a more critical metabolite produced during lipid peroxidation. Therefore, a significant increase in malondialdehyde contents in the target cells indicates oxidative stress. Moreover, an increase in the malondialdehyde expedites cascading reactions of lipid peroxidation. Protein carbonyl is known as a metabolite of protein oxidation. Furthermore, increasing the peroxidation rate of thiol groups can be a physiological response to ROS increase at the cellular level. A significant decrease in the total antioxidant and increase in the protein carbonyls and malondialdehyde contents were reported in the liver and brain of hybrid surubim (*Pseudoplatystoma* sp) exposed to glyphosate and roundup [37].

Also, a significant increase in 7,8-dihydro-8-oxoguanine (8-oxo-dG) contents is a biomarker of nucleic acid oxidation and gene damage.

However, other biomarkers can be used to detect oxidative stress indirectly. We will describe each of them in the following sections.

6. Neurotoxicity

Studies showed that xenobiotics could often influence nerve systems. Therefore, this is a problem in distinguishing the primary neurotoxicity agent in fish when exposed to herbicides combined with other pollutants. Thus, if we observed neurotoxicity response in fish, evaluation of the additive or synergistic effects of xenobiotics on herbicides' toxicity should be a priority.

Peroxidation of phospholipids that cover nerves can disrupt transport of neural signals or information processing in neural centers. Also, herbicides can change neurotransmitters' biochemical structure or disable enzymes involved in biosynthesis or biodegradation of neurotransmitters.

Moraes, et al. [36] found that exposure of teleost fish (*L. obtusidens*) to clomazone and quinclorac decreased acetylcholinesterase (AChE) activity in the brain, while AChE activity increased in muscle tissue after exposure to clomazone, propanil, and metsulfuron-methyl. Similarly, the inhibition of AChE activity was reported in the brain of teleost fish (*L. obtusidens*) exposed to herbicides clomazone and propanil [36]. Thanomsit et al. [38] could design a monoclonal antibody-ACHE that is used to detect acetylcholinesterase activity in the brain of fish exposed to herbicides. Thus, they could measure AChE activity in the brain of hybrid catfish, Nile tilapia, and climbing perch [38].

One of the consequences of neurotoxicity is the occurrence of behavioral changes in aquatic animals exposed to herbicides.

7. Behavioral response

Changes in the behavior of animals may be related to disrupting nerve systems or muscle spasms. Previous research showed that exposure to aquatic animals to herbicides could alter the behavior and rate of their response to environmental stimuli. Herbicides can affect the relationship between hunters and prey. Also, exposure to animals to herbicides may change animals' romantic, reproductive, and parenting behaviors. Thus, changes in feeding behavior can decrease the growth performance of organisms exposed to herbicides [39].

Faria et al. [25] documented that changes in the behavior of fish exposed to herbicides had a significant relationship with changes in the monoaminergic neurotransmitters in the brain. They found that a significant increase in dopamine (DA), serotonin (5-HT), and a decrease in norepinephrine (NE) could change the exploratory and social behaviors of zebrafish following exposure to glyphosate.

Butyrylcholinesterase (BChE) is known as pseudocholinesterase. Fluctuations in the BChE activity may change the behavior of aquatic animals. A significant change in the BChE activity was observed in freshwater fish *Labeo rohita* exposed to Roundup® [40]. Geetha [40] found that increased BChE activity could relieve the Roundup® induced stress in fish.

8. Genotoxicity and gene damage

The genotoxicity effects of herbicides may be due to the interaction of ROS with DNA [41]. Exposure to herbicides and their metabolites may degrade DNA or adduct to DNA structure. The DNA damage to erythrocytes, liver, and gills was detected by comet

assay in the *O. niloticus* and *G. brasiliensis* exposed to Mesotrione [34]. DNA damage was reported in the European eel (*Anguilla anguilla*) exposed to Roundup® (glyphosate-based) and Garlon® (triclopyr-based) [42]. Ruiz de Arcaute, Soloneski and Larramendy [41] observed that exposure of *C. decemmaculatus* to dicamba could cause micronuclei and DNA single-strand breaks in circulating blood cells. Similar results were observed in the *P. lineatus* [43], *C. auratus* [44], and *C. decemmaculatus* [45] exposed to Roundup, atrazine, and glyphosate, respectively. DNA damage and genotoxicity were detected in the egg of silver catfish (*Rhamdia quelen*) exposed to 2,4-D and glyphosate [46].

Enhancement or depression in the mRNA expression of enzymes involved in detoxification and biotransformation of xenobiotics was reported in fish exposed to herbicides. For example, Velki, et al. [47] reported a significant increase in *Ces2* gene expression in the zebrafish embryos following the exposure to 2.15 μM diuron for 96 h. Exposure to Roundup and other glyphosate changed gene expression patterns in the reproductive tissue of Japanese medaka fish (*Oryzias latipes*) [48].

Increased genetic defects and neoplasia in fish embryos and larvae can be caused by exposure to xenobiotics [49], including herbicides. Also, mutation due to exposure of fish to herbicides may lead to tumor generation.

9. Blood biochemical parameters

Moreover, the rapture of cellular membranes may cause the release of cytoplasmic contents or organelles into intercellular fluid such as serum. Hence, assessing biochemical parameters in serum can indicate the stability of cellular membranes after exposure to herbicides [32]. Geetha [40] demonstrated that exposure to Roundup® could affect the balance of plasma electrolytes and transaminase activity in *L. rohita* [40]. The disruption in biochemical hemostasis was reported in the crayfish exposed to glyphosate and chlorpyrifos [26, 27].

The increase in the serum enzyme activities and changes in the blood biochemical parameters were observed in *C. carpio* exposed to paraquat [22]. Similar results were detected in *C. carpio* following glyphosate [50]. A significant change in glucose, cholesterol, and triglyceride levels in the blood may be due to elevated energy needs to alleviate the cytotoxic effects of herbicides.

10. Suppression of the immune system

Exposure to xenobiotics can suppress immune system functions by increasing corticosteroid hormones. A significant increase in corticosteroid hormones can affect cytokine gene expression. Thus, an increase in inflammation response can depress immune system power.

Maddalon, et al. [51] showed that glyphosate herbicide could induce immunotoxicity by interfering with the hormonal pathway and biosynthesis of cytokines and neuropeptides. Also, Acar, et al. [52] displayed that changes in the immune-related genes could mitigate immune functions in Nile tilapia (*O. niloticus*) exposed to glyphosate.

11. Reproductive disorders

Some herbicides can disrupt reproduction physiology. Herbicides may act as endocrine disruptors. They can block hormone receptors or induce changes in enzyme

function involved in hormones' biosynthesis. Furthermore, some herbicides may act as analogs of natural hormones. Reproduction products may be denatured after animals' exposure to herbicides. Therefore, the rate of fecundity, fertility, and survival of embryos may be collapsed. This phenomenon can also affect the hatchling rate and percentage of larvae survival. Decreased adaptability of larvae to environmental conditions may be the reason for the reduced survival rate after exposure to herbicides [53].

Yusof, Ismail and Alias [54] found that exposure of Java medaka (*Oryzias javanicus*) to glyphosate reduced fertility, hatching eggs, and larval survival. Furthermore, Zebral, et al. [53] discovered that Roundup exposure changes the diapausing pattern of *Austrolebias nigrofasciatus* embryos. Thus, Roundup could affect the survival of *A. nigrofasciatus* embryos. Decreased fecundity rates were also observed in *A. nigrofasciatus* breeders exposed to Roundup. Also, Dehnert, Karasov and Wolman [55] displayed that 2,4-D exposure could reduce zebrafish and perch survival rates during larval stages. They explained that a decrease in the survival rate of larvae could be due to the toxicity effect of 2,4-D on the development and function of neural circuits underlying the vision of larval fish. Moreover, Dehnert et al. [56] revealed that the application of 2,4-D to control Eurasian watermilfoil (*Myriophyllum spicatum*) in aquatic ecosystems could threaten fish survival.

12. Growth dysfunction

Previous studies showed that herbicides could decrease growth performance in aquatic animals. A significant weight reduction may be related to disruption in nutrient absorption in digestive systems. Deficiency in the assimilation of vital macromolecules can alter energy budgeting. As a result, animals have to consume energy storage in the liver and muscles to supply their needs. Therefore, weight loss and general weakness, anorexia, were often reported in the aquatic animals exposed to herbicides [39].

13. Hemotoxicity

Herbicides could change white blood cell (WBC), red blood cell (RBC) counts, and hematological indexes such as hemoglobin and hematocrit contents in fish. These phenomena can be related to hematopoietic tissue damage. Moreover, disruption in the blood circulation systems may occur in fish exposed to herbicides. Hemolysis of erythrocytes, a decline in erythropoietin levels, and histopathological damage to hematopoietic organs can reduce blood cell counts in animals exposed to herbicides. Pereira, Fernandes and Martinez [33] declared that changes in the hematological parameters could be due to the toxicity effects of clomazone on the hematopoietic tissue of fish *P. lineatus*. Exposure of *P. lineatus* to clomazone changed hematological parameters after 96 h. Moreover, Merola, et al. [57] showed that exposure of zebrafish to pendimethalin could cause blood congestion, impair blood flow, and reduce heartbeat.

14. Histopathological damage

Histopathological injuries could be related to oxidative damage to the cellular membrane of fish exposed to herbicides. Furthermore, apoptosis and cellular necrosis

may intensify histopathological damages in various tissues of fish exposed to herbicides. Destro, et al. [35] found that atrazine exposure could damage the liver tissue of tetra fish (*A. altiparanae*). They showed that the histopathological damage in the liver was due to an increase in lipid peroxidation. Moreover, Nassar, Abdel-Halim and Abbassy [30] reported histopathological damage in the gills and liver of fish exposed to the herbicide pendimethalin.

15. Bioaccumulation of herbicides

The bioaccumulation of xenobiotics is directly related to their bioavailability. Therefore, environmental pollutants that may increase the bioavailability of herbicides can significantly impact their bioaccumulation capacity in aquatic animals.

Furthermore, the half-life of herbicides in water ecosystems can also affect their bioaccumulation capacity. The half-life of herbicides in the various environments is different. Herbicides in environmental conditions can be quickly degraded into various metabolites. Some herbicides are durable in the environment. The breakdown rate of herbicides depends on their chemical structures and environmental conditions [20].

Therefore, the probability of their bioaccumulation in the body of aquatic animals is also high. Various authors reported the bioaccumulation of herbicides in aquatic animals. Tyohemba et al. [10] measured the bioaccumulation of various herbicides in African mud catfish (*Clarias gariepinus*), and Mozambique tilapia (*Oreochromis mossambicus*) inhabited Lake St. Lucia, South Africa. They detected phenoxy-acid herbicides, acetochlor, atrazine, and terbuthylazine in the muscle tissues of fish [10]. The analysis of fresh fish tissues collected from four markets in Nanning City, Guangxi Province, China, showed that the bioaccumulation of atrazine, acetochlor, metolachlor, and their metabolites could be worrying [58]. Furthermore, herbicides have also been found in fish and seafood [59, 60]. Therefore, the bioaccumulation of herbicides could threaten consumers' health.

16. Conclusion

We tried to present an overview of herbicides' toxicity in this chapter. However, we must update our information because newborn pollutants could be found in water ecosystems that can affect herbicides' half-life, toxicity, and bioavailability. Overall, if we want to discuss the effects of herbicides alone or in combination with other xenobiotics, we should be well known of their toxicity mechanisms and pathways and how they can affect the physiology of aquatic animals. Therefore, if we find the source of herbicide pollution, we can prevent their destructive effects on fish before penetrating aquatic ecosystems. Also, if we cognize about biotransformation and detoxification of herbicides, we can better manage the adverse effects of herbicides on fish. Therefore, studies on toxicity, bioavailability, and interaction of herbicides with other pollutants can be useful in recognizing the physiological response of fish exposed to herbicides.

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