Chapter

Ecotoxicology of Glyphosate-Based Herbicides on Aquatic Environment

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Abstract

Glyphosate-based herbicides (GBHs) are chemicals developed to control unwanted plants such as weeds or algae. These chemicals act on EPSPS enzyme that blocks the production of tyrosine, phenylalanine, and tryptophan amino acids causing plant death. This biochemical pathway exists only in plant organisms. Despite the target use, GBHs have been related to toxic effects on nonplant organisms, such as invertebrates, fishes, amphibians, reptiles, birds, and mammals, including humans. This chapter is focused on ecotoxicological effects of GBHs on aquatic environment, showing a perspective of studies since this kind of product was developed until nowadays, an analysis of how many studies for each taxonomic group. Furthermore, we analyzed specifically the toxic effect of GBHs on each taxon, and finally, we discuss future perspectives and suggestions for a better regulation and application for this chemical.

Keywords: ecotoxicology, water quality, weed control, Roundup®, Monsanto

1. Introduction

Herbicides are chemical compounds used mostly to control weed (i.e., uncultivated) plants in agriculture and forestry and also for algae control [1, 2]. Herbicide formulations are designed to affect mainly plants, affecting specific plant biochemical pathways. However, it is common that this kind of pesticides affects nontarget organisms such animals, including aquatic organisms [3, 4].

The most used herbicide worldwide is glyphosate-based herbicide (GBH), such as Roundup® from Monsanto, and its usage has been increased [5] mainly due to the development of transgenic glyphosate-resistant crops [6]. Glyphosate (N-(phosphonomethyl) glycine (CAS no. 1071-83-6)) is a weak organic acid with a molecular weight of 169.09 M and has a half-life of 7–142 days in water and 76–240 in soil [6, 7]. Glyphosate has high solubility in water (10,000–15,700 mg L^{-1} at 25°C), and it readily dissolves and disperses in an aquatic environment.

Glyphosate affects a specific plant biochemical pathway, inhibiting the action of the enzyme 3-enolpyruvylshikimic acid 5-phosphate synthase (EPSPS) that is necessary for biosynthesis of amino acids such as phenylalanine, tyrosine, and tryptophan [8] (**Figure 1**). Animals do not have this biochemical pathway, and hypothetically, they would be safe from glyphosate. However, the use of glyphosate requires that some other compounds as surfactants are added to the commercial formulation to increase adhesion to the leaf surface and absorbance by plants, trespassing the waxy cuticle [6]. There are a variety of surfactants, but the most common used on glyphosate-based formulations has been polyethoxylated amine (POEA). This surfactant is known to be more toxic to animals then glyphosate itself [6, 9].

As mentioned above, glyphosate *per se* has low toxicity when compared to its commercial formulation containing surfactants. However, those formulations are toxic to a large number of organisms due mainly to products added to the formulae. Many studies have reported tissue damages, DNA damages, enzyme inhibition such as acetylcholinesterase (AChE) and aromatase, endocrine disruption, development disruption causing malformations, and carcinogenesis caused by GBH in animals as fish, amphibians, and mammals, including humans [6, 10–17].

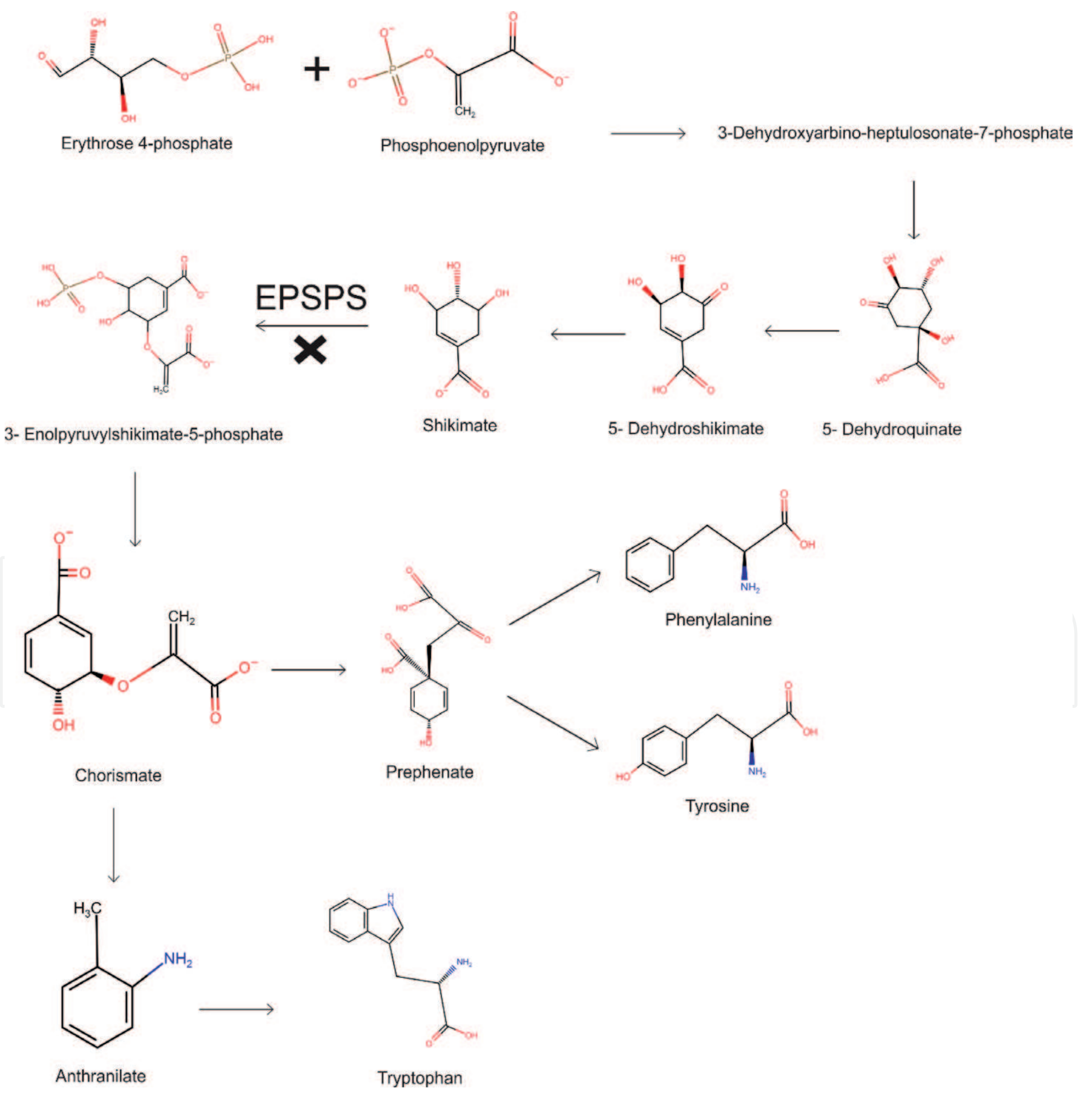

Figure 1.
Glyphosate action on the biochemical pathway of plants inhibiting 3-enolpyruvylshikimic acid 5-phosphate synthase (EPSPS) enzyme and production of essential amino acids as phenylalanine, tyrosine, and tryptophan, causing plant death.

In terrestrial animals, glyphosate reaches these organisms through direct application and contaminated food consumption. However, application of GBH in an aquatic environment is not so common when compared to terrestrial environments. Despite this, GBH can reach the aquatic environment through many ways. It can be applied directly on water bodies for algae control, although the opposite effect can be found, with proliferation of some species of algae due to the increase of phosphorus levels [18]. GBH can also reach the aquatic environment through leaching, run-off, and contaminated food source [6].

As mentioned, glyphosate has high solubility in an aquatic environment. Some studies say that 50% of glyphosate in natural waters dissipates by water flow and decomposition in a few days to 2 weeks [19–21]. Despite that, glyphosate binds to soil particles and solid surfaces [22], which makes its dissipation difficult. The by-products of glyphosate decomposition are sarcosine and aminomethylphosphonic acid (AMPA). The first one is known to be nontoxic [23] and the second one less or equally toxic for aquatic organisms than glyphosate [24, 25]. This substance has also a great solubility and dissipates in water in 7–14 days. POEA in natural environments degrades by microbial decomposition in 14 weeks and its half-life is estimated in 21–42 days [24].

Considering that glyphosate *per se* and the commercial formulations are widely used around the world, being the most popular herbicide, this chapter summarizes the available data from the literature on the ecotoxicity of glyphosate and its formulation compounds, as well as its degraded products, to aquatic organisms (aquatic plants, invertebrates, fish, reptiles, amphibians, and birds) and analyzes the worldwide politics about glyphosate use and environment safety.

2. Studies about glyphosate-based herbicides on the aquatic environment

One of the first studies that evaluated the effects of glyphosate and GBH in aquatic environments was performed by Folmar et al. [26]. According to Thomson's ISI WoS (Institute for Scientific Information, Web of Science) database, using keywords as "glyphosate," and "aquatic environment," since 1979 to the present day, 233 papers have been published that evaluated the toxicological effects of glyphosate in aquatic environments (**Figure 2**). These papers addressed the toxic effects of glyphosate on various types of organisms. The invertebrate group was the most studied, with 52 published articles (21.3%), followed by fish with 51 (20.9%), amphibians 40 (16.4%), plant 31 (12.7%), and aquatic environment 30 (12.3%).

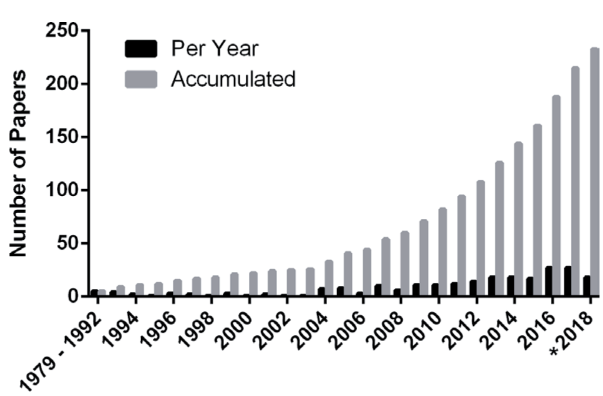

Figure 2.
Number of papers published per year. Black bars represent the number of papers published in each year. Grey bars represent the number of papers accumulated per year. (*) Papers published until August 2018.

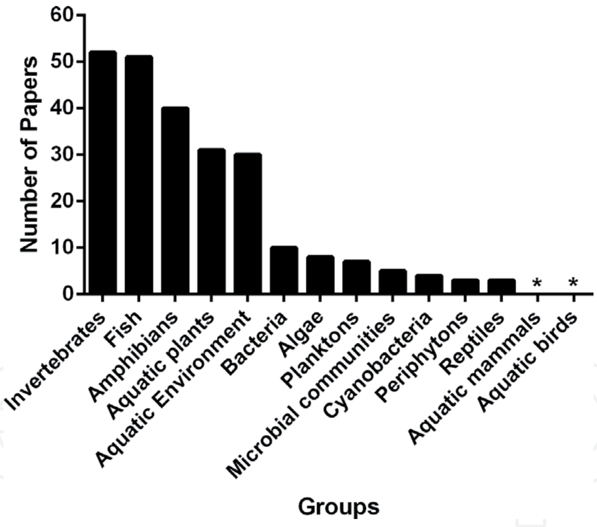

Figure 3.
Number of papers per organism group. Black bars represent the number of papers on toxicological effects of glyphosate published for each aquatic organism groups. Asterisk indicates lack of studies evaluating the toxicological effects of glyphosate in aquatic mammals and birds.

The other groups were present in 40 published articles (16.4%) (**Figure 3**). For the investigated period and database, there were no papers which have evaluated the toxicological effects of glyphosate in aquatic mammals and birds. This scarcity of studies demonstrates the lack of knowledge on the risk of exposure of these groups in aquatic environments contaminated by glyphosate.

2.1 Aquatic plants

Glyphosate in the aquatic environment causes the death of the macrophyte community, which serves as a microhabitat for zooplanktonic, phytoplanktonic, and periphytic communities, and this leads to top-down control of planktonic organisms, affecting refuge and feeding to fish [27], triggering a chain effect. Studies have evaluated the effects of glyphosate on aquatic lentils (*Lemna gibba*) [28] (**Table 1**), showing that larval mortality of tadpoles was caused by predation without their micro-habitats in the absence of macrophytes, due to contamination of water body by glyphosate.

Dörr [18] studied the effect of glyphosate on the growth and production of secondary metabolites by toxigenic strains of the cyanobacteria *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii*. The author assessed the influence of different concentrations of glyphosate on the growth and production of these cyanobacteria and observed that toxin production and growth increased at 15 mg L^{-1}. When exposed to 20 mg L^{-1}, their growths and toxin production increased as well, while concentration above 20 mg L^{-1} prevented their growth. The species *C. raciborskii* was more resistant to GBH, and this species uses the metabolite AMPA as a source of nitrogen for its growth. Considering that microalgae and cyanobacteria are the principal primary producers in aquatic ecosystems, use of the herbicide can stimulate the growth and production of toxins of certain groups. This affects water quality and modifies the functionality of the ecosystem of interest.

The effects of herbicides on nontarget aquatic plants are emerging as a major conservation issue in aquatic biodiversity [29]. *Ludwigia peploides*, an aquatic macrophyte, showed that glyphosate bioaccumulates in water surface and can, therefore, be used as a biomonitoring organism to evaluate glyphosate levels in freshwater. This is because it increases the concentration of the herbicide in the leaf, facilitating its detection in the biological matrix instead of the water. In the study,

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Species	Group	Chemical	Glyphosate concentration ($\mu g\ L^{-1}$)	Effect	Reference
Amphora veneta	Catenulaceae	Roundup®	8456	Increases mortality	[36]
Anabaena sp.	Nostocaceae	Gly. (acid)	0.1–8.8 mM	Increases growth	[28]
Arthrospira fusiformis	Phormidiaceae	Gly. (acid)	0.005–0.048 mM	Increases growth	[2]
Chlorella vulgaris	Chlorellaceae	Gly. (acid)	293,000	Chlorophyll fluorescence/decreases PP	[35]
Gomphonema parvulum	Naviculaceae	Roundup®	1000–10,000	Increases mortality	[30]
Halophila ovalis	Hydrocharitaceae	DCMU Gly. (acid)	11,600	Decreases chlorophyll fluorescence	[31]
		Roundup®			[30]
Lemna gibba	Lemnaceae	Roundup®	2800	Increases growth	[2]
		Gly. (acid) Roundup®	46,900	Increases growth	[29]
Leptolyngbya boryana	Leptolyngbyaceae	Gly. (acid)	0.003–0.02 mM	Increases growth	[2]
Ludwigia peploides	Onagraceae	Gly. (acid)	4000 and 108,000	Bioaccumulation	[2]
Microcystis aeruginosa	Microcystaceae	Gly. (acid)	3–37	Increases growth and toxin production	[28]
					[18]
		Gly. (acid)	15,000	Increases growth and toxin production	[2]
Myriophyllum aquaticum	Haloragaceae	Gly. (acid) Roundup®	840	Decreases root	[30]
		Gly. (n.c.)	220	Chlorophyll fluorescence	[33]
Myriophyllum spicatum	Haloragaceae	Rodeo®	1000	Increases growth	[34]
Nostoc punctiforme	Nostocaceae	Gly. (acid)	>50 mM	Increases growth	[2]
Scenedesmus quadricauda	Chlorophyceae	Gly. (acid)	200,000	Chlorophyll fluorescence/decreases primary productivity (PP)	[2]
Spirulina platensis	Nostocaceae	Gly. (acid)	0.005–0.02 mM	Increases growth	[2]

Table 1.
Ecotoxicity of glyphosate-based herbicide (GBH) to aquatic plants worldwide.

surface water and sediment samples were collected at the same time to measure glyphosate and calculate both the bioconcentration factors (BCFs) and biota-sediment accumulation factors (BSAFs). Glyphosate was detected in 94.11% in the leaves, presenting concentrations between 4 and 108 mg kg^{-1}. In surface waters and sediments, it was detected in 75 and 100% of the samples at concentrations ranging from 0 to 1.7 mg L^{-1} and 5 and 10.50 mg kg^{-1} of dry weight, respectively. The mean BCF and BSAFs were 88.10 and 7.61 L kg^{-1}, respectively. These results indicate that *L. peploides* bioaccumulates glyphosate that is mainly bioavailable in surface waters. Thus, since the plant accumulates the herbicide, the high concentrations in the organisms are evidence of the trophic levels that will feed or interact with the plant [28]. The researchers also observed that only 0.5 mg L^{-1} glyphosate was sufficient to inhibit the growth of *Lemna gibba*, change its shape, and lower chlorophyll content, decreasing its photosynthetic rate and consequently its metabolism.

Another important community in aquatic ecosystems that is also affected by the use of glyphosate is the periphyton. In terms of primary production, the periphyton has a photosynthetic contribution 77% higher than that of phytoplankton [30]. Among the most common and potentially toxic outcrossing cyanobacteria, *M. aeruginosa* uses glyphosate as a source of phosphorus, growing uncontrollably and causing eutrophication of the aquatic ecosystem that modifies ecological conditions. As shown by Forlani and collaborators [31], there is a tolerance to glyphosate by cyanobacteria *Spirulina platensis*, *Nostoc punctiforme*, *Arthrospira fusiformis*, *Anabaena* sp., and *Leptolyngbya boryana*, and four of them were able to use phosphorus as the only source. *Anabaena* sp. presented the highest toxicity (C = 50 mg L^{-1}). Vera and collaborators [32] observed that the interaction of the periphyton with other communities and also with the abiotic environment was low when the mesocosms were treated with glyphosate, presenting an imbalance in the trophic webs of the ecosystem.

The exposure to GBH reduced 78% of the primary productivity of phytoplankton when used at low concentrations (0.125 mg L^{-1}) [33] and at high concentrations (3.8 mg L^{-1}) [34], causing a disturbance in the trophic levels. In freshwater systems, glyphosate at high levels stimulated eutrophication by increasing total phosphorus and favoring the growth of cyanobacteria on the periphyton, which altered the typology of the study ecosystem that was a mesocosm [32].

Species-based differences in sensitivity to GBH exposure may lead to decreased richness and abundance of ecosystem species [34]. Even though herbicides are thought to kill terrestrial plants, it can have an even more devastating effect in water, due to the imbalance that causes mortality of algae and aquatic plants. This causes an increase in decomposing organic matter in the water, which will reduce the concentrations of dissolved oxygen in the system and increase the stress of aquatic communities [35]. Thus, algae and aquatic plants are considered as nontarget organisms that are sensitive to the effects of glyphosate, and the damage to the balance of the aquatic environment is of concern. The damage of glyphosate on the aquatic plant community ranges from the death of the plant itself to the reduction of environmental heterogeneity promoted by the local plants. Consequently, this leads to the death of other aquatic species, causing an imbalance in the ecosystem.

2.2 Aquatic invertebrates

One of the pioneer studies of the effects of GBH on invertebrate organisms was carried out by Tsui and Chu [9] that studied the effects of this chemical on *Ceriodaphnia dubia* and *Acartia tonsa*, both crustaceans, in addition to other organisms such as algae, bacteria, and protozoans. They found the toxicity of this pesticide to these organisms and the most sensible was *A. tonsa* with a LC_{50} of 1.77 mg L^{-1}. There is a high variability of sensibility of invertebrate organisms to GBHs (**Table 2**).

Species	Chemical	Exposure time (h)	LC_{50} (μg L^{-1})	Reference
Acartia tonsa	Roundup®	48	1770 (1330–2340)	[38]
Burnupia stenochorias	Roundup®	96	4304 (2121–7902)	[44]
Caridina nilotica	Roundup®	96	2842 (2524–3190)	[44]
Ceriodaphnia dubia	Roundup®	48	5390 (4810–6050)	[38]
	Eskoba®, Panzer Gold®, Roundup Ultramax®, Sulfosato Touchdown®	48	250–16,770	[45]
Chironomus plumosus	Roundup®, POEAE, Glyphosate acid	96	18,000 (9400–32,000)	[46]
Chironomus riparius	Rodeo®, X-77 Spreader®, ChemTrol®	48	1,216,000 (996,000–1,566,000)	[47]
Daphnia magna	Eskoba®, Panzer Gold®, Roundup Ultramax®, Sulfosato Touchdown®	48	2670–15,430	[45]
	Roundup®, POEAE, Glyphosate acid	48	3000 (2600–3400)	[46]
	Eskoba®, Sulfosato Touchdown®	48	1620–31,410	[48]
	Rodeo®, X-77 Spreader®, ChemTrol®	48	218,000 (150,000–287,000)	[47]
Daphnia pulex	Roundup®	96	657 (472–914)	[44]
Gammarus pseudolimnaeus	Roundup®, POEAE, Glyphosate acid	48	62,000 (40,000–98,000)	[46]
	Roundup®, POEAE, Glyphosate acid	96	43,000 (28,000–66,000)	[46]
	Roundup®	96	340,000	[49]
Hyalella azteca	Rodeo®, X-77 Spreader®, ChemTrol®	96	720,000 (399,000–1,076,000)	[47]
Laeonereis acuta	Roundup®	96	8199 (6690–9580)	[50]
Nephelopsis obscura	Rodeo®, X-77 Spreader®, ChemTrol®	96	1,177,000 (941,000–1,415,000)	[47]
Notodiaptomus conifer	Eskoba®, Sulfosato Touchdown®	48	1220–1,282,000	[48]
Ruditapes decussatus	Roundup®	1440	2200	[51]
Tanytarsus flumineus	Roundup®	96	12,240 (9454–22,360	[44]
Utterbackia imbecillis	Roundup®	24	18.3 ± 12.9	[52]

Table 2.
Ecotoxicity of glyphosate-based herbicide (GBH) to aquatic invertebrates, exposure time, LC_{50} value (lower-upper values), and reference.

Specifically about microinvertebrates (<35 μm), these organisms persist within resting eggs (or egg banks) in lake sediments [36]. They represent a major source of regenerative potential in lake ecosystems near agricultural areas, and play a key role in influencing the active population and community dynamics, seasonal succession, biogeographic patterns, and the evolution of populations [36, 37]. Despite the widely accepted importance of resting egg banks in the ecology of aquatic micro-invertebrates' communities, recently, experimental studies have demonstrated that the extensive and inappropriate use of commercial GBH, associated with agricultural activities, may impair the hatching of resting eggs in the sediment of lakes [38, 39]. Gutierrez and collaborators [38] indicated that the GBHs (Sulfosato Touchdown®) affect the hatching dynamics of micro-invertebrates, and selectively alter the species richness and abundance of community hatched from lake sediment. Portinho and associates [39] extended these findings and indicated that commercial herbicides as Roundup® (a.i. glyphosate) separate or in combination with 2,4-dichlorophenoxyacetic acid (2,4-D) have the potential to suppress emergences of micro-invertebrates from resting egg banks from lake sediments.

The environmental implication of this scenario suggests that changes in micro-invertebrates' structure and composition induced by herbicides will occur, causing not only negative impacts on the process of recolonization from resting egg banks but also shifts in community composition. Recent attempts to develop guidelines for protecting aquatic organisms have focused on emergence from resting egg banks within the context of an ecological community [40], with potential implications for studies related to environmental risk to, and integrity assessment of, aquatic ecosystems.

2.3 Fish

Fish species are particularly vulnerable to GBH and their susceptibility depends on the commercial formulation, fish species, fish developmental stages, and exposure conditions, such as concentrations, exposure time, and route of exposure. Furthermore, gender-specific response of fish to GBH has been indicated in guppy *P. reticulata* exposed to glyphosate (50–73.2 mg L^{-1}) and their metabolite AMPA (86.8–180 mg L^{-1}) for 96 h [25], indicating the need for further studies about the molecular mechanisms of gender-specific effects.

In general, the surfactant and the commercial formulation showed higher toxicity to fish when compared to active ingredient (glyphosate pure) and their metabolite (AMPA). The 50% lethal concentration (i.e., LC_{50}) of GBHs for fish has high variability, ranging from 1000 to 9750 μg L^{-1} [6, 41]. Chandrasekera and Weeratunga [42] found a LC_{50} of 976 μg L^{-1} for 48 h of exposure in fries of *P. reticulata*, while Sadeghi and Hedayati [43] found a LC_{50} = 12,640 μg L^{-1} in adults for a 41% commercial formulation and Souza-Filho and collaborators [44] found 4212 μg L^{-1} for 48 h.

Glyphosate and formulation compounds can be taken by fish via gills and digestive tract through ingestion of contaminated food or water [6, 45]. Once inside the organisms, glyphosate is absorbed and distributed to the whole body through blood circuit, reaching several tissues. GBHs can affect fishes in different ways, affecting many organs and as well molecular levels. In liver, vacuolization process was reported in hepatocytes and nuclear pyknoses; in kidney, studies report Bowman capsule dilatation and accumulation of hyaline drops in tubular cells; and in gills, glyphosate causes hyperplasia, lamellar fusion and aneurism [46–50]. Besides that, Langiano and Martinez [49] showed activation of the stress axis, with increased blood glucose levels. Souza-Filho and collaborators [44] also showed genotoxic effects in fish cells. Concerning to enzymes, Sandrini and collaborators [17] showed that glyphosate impairs acetylcholinesterase activity in synapses, preventing detaching of acetylcholine from receptors, impairing electric transmission by neurons. This can impair muscle contraction and information transmittance. GBH in sub-lethal levels can also impair fish feeding behavior as shown by Giaquinto and collaborators [51]. Also, a recent *in vitro* study [52] showed that low concentrations of GBH, even those allowed by the USA, Canadian, and Brazilian laws (50 μg L^{-1}) kill yellowtail tetra fish (*Astyanax lacustris*) sperm cells, compromising fish reproduction and natural population persistence.

OMIC technologies, such as proteomics, transcriptomics, and metabolomics, have been applied to investigate the molecular mechanisms and toxicity of GBHs on fish. For example, proteomics-based methods (two-dimensional gel electrophoresis associated with mass spectrometry and bioinformatics) were used to complement the knowledge about the ecotoxicity of GBH on *P. reticulata* [53, 54]. The female guppy exposed to GBH (1.82 mg L^{-1}) for 24 h changed different cell processes in the gills (energy metabolism, regulation and maintenance of cytoskeleton, nucleic acid metabolism, and stress response) [53] and liver (cellular structure, motility and transport, energy metabolism, and apoptosis) [54], confirming tissue-specific responses at molecular levels.

2.4 Herpetofauna

The herpetofauna is composed of reptiles and amphibians, and due to the low mobility, physiological requirements, and habitat specificity, this group has become ideal models for environmental conservation studies [55]. Amphibians are sensitive to exposure to contaminants and are considered good bioindicators in monitoring water quality [56]. Characteristics such as permeable skin, reproduction, and larval stages dependent on the aquatic environment make anuran amphibians highly vulnerable to pesticide contamination [57]. Evidence suggests that anuran species decline is related to the intensive use of pesticides [58–60].

The decline of amphibian populations is related to the increase of environmental pollutants, the influence of climate change, habitat fragmentation, exposure to ultraviolet radiation, and human-induced environmental changes [61, 62]. Contamination of water bodies next to agricultural areas generally increases during the rainy season, that is, widely used to breed by most species of amphibians, and many species use temporary ponds and small streams adjacent to agricultural areas as part of their life cycle, harming the reproductive period and larval development [57, 58, 63]. During the rainy season, the agrochemical present in the soil are susceptible to be transported down the soil profiles and/or surfaces/underground water bodies and consequently affect the amphibian population [58] and other environmental (a) biotic elements [6, 64].

Herbicides may delay or inhibit the metamorphosis of amphibians directly impacting their reproduction [57]. According to Walker and collaborators [65], the main routes of herbicide absorption in anuran amphibians are through contaminated food ingestion and skin absorption of pollutants dissolved or suspended in water. After absorption, the substance is transported to different compartments of the body through blood. The effect of herbicides on tadpoles is less known when compared to adult amphibians, since the larvae of the anurans are less visible, and unlike adults, they do not have vocalization. Tadpoles of various species have not yet been described, which makes it even more difficult to study these organisms in depth [66].

The reduction in larval survival due to exposure to glyphosate was observed by Simioni and collaborators [67], Figueiredo and Rodrigues [68], and Costa and collaborators [69] in larvae of *Physalaemus albonotatus*, *Physalaemus centralis*, and *Physalaemus cuvieri* [70]. Rissoli and collaborators [71] also observed that the exposure of bullfrog tadpoles to Roundup Original® causes damage to the epithelium causing hypoxia in these animals. In the last 30 years, populations of amphibians have been suffering a great decline or even being extinct; almost half of the species are experiencing some population decline. On the basis of toxicity studies, sensitivity to glyphosate differs among species; however, there are several variations in experimental conditions and pesticide formula (different commercial formulations of glyphosate, different exposure times, different surfactant substances, number of replicates, abiotic conditions in the experiment, and stage of development) which make it difficult to compare and define which groups or species are more tolerant to contamination [67, 72, 73]. The LC_{50} values for the herpetofauna species are shown in **Table 3**.

Reptiles are extremely sensitive to herbicide formulations and may exhibit changes in their behavior after exposure of these xenobiotics [74]. This group is fairly uniform and exposure to GBHs may affect its energy storage process [75, 76]. Schaumburg and collaborators [77] found that exposure to sublethal concentrations of glyphosate during the embryonic phase of *Salvator merianae* may cause an increase in genetic damage. Therefore, it is assumed that glyphosate is capable of causing DNA damage, promoting chromatin fragmentation of epidermal cells, impairing cell division. Exposure to glyphosate does not alter the thermoregulatory behavior of lizards of the species *Oligosoma polychroma* [78]. Sub-lethal concentrations of the commercial glyphosate formulation (Roundup®) cause genotoxic damage and chromosome breaks in *Anguilla anguilla*. The increase in the damage index in this species can cause reproductive damage and adverse effects in the long term [79].

Currently in the Neotropical region, about 40 studies relate the indiscriminate use of herbicides based on glyphosate with the risk to biodiversity of herpetofauna. Schiesari and collaborators [80] reported that some species of amphibians, including tadpoles and adults and some reptiles are sensitive to exposure to formulations based on glyphosate. Exposure to sublethal concentrations of glyphosate is

Species	Chemical	Exposure time (h)	LC_{50} mg a.i./L	Reference
Anaxyrus americanus	Roundup®	384	0.55–2.52	[31]
	Roundup®	96	0.8–2.0	[80]
Anaxyrus boreas	Roundup®	96	0.8–2.0	[31]
Crinia insignifera	Roundup®	48	2.9–11.6	[31]
Dendropsophus minutus	Roundup®	96	0.28	[85]
Heleioporus eyrei	Roundup®	48	2.9–11.6	[31]
Hyla versicolor	Roundup®	384	0.55–2.52	[31]
	Roundup®	96	0.8–2.0	[31]
Litoria moorei	Roundup®	48	2.9–11.6	[31]
Lithobates sylvaticus	Roundup®	384	0.55–2.52	[31]
	Roundup®	96	0.8–2.0	[31]
Lithobates pipiens	Roundup®	384	0.55–2.52	[31]
	Roundup®	96	0.8–2.0	[31]
Lithobates clamitans	Roundup®	384	0.55–2.52	[31]
	Roundup®	96	0.8–2.0	[31]
Lithobates catesbeianus	Roundup®	384	0.55–2.52	[31]
	Roundup®	96	0.8–2.0	[31]
Limnodynastes dorsalis	Roundup®	48	2.9–11.6	[31]
Pseudacris crucifer	Roundup®	96	0.8–2.0	[31]
Rana cascadae	Roundup®	96	0.8–2.0	[31]
Rhinella arenarum	Roundup®	48	2.42	[83]
Scinax nasicus	Roundup®	48	1.74	[82]

Table 3.
Ecotoxicity of glyphosate-based herbicide (GBH) to herpetofauna, exposure time, and LC50 value.

sufficient to cause irreversible damage to the DNA of amphibians and reptiles, so the use of GBH should be controlled in arable areas avoiding the decline of species that make up the herpetofauna group.

2.5 Aquatic birds

Glyphosate when used in recommended rates is considered not bioaccumulative and of low toxicity in birds [81]. However, the present acquaintance is not enough to make affirmation about low toxicity risk and low exposure of birds to herbicide considering the possible complex process behind the movement and accumulation of glyphosate, additives, and waste in the environment. Moreover, even the few available studies [82–96] have found direct and indirect effects of glyphosate on bird species (**Figure 4**). Among those, only five studies along years 1994 and 2017 on Google Scholar database have analyzed effects on aquatic bird species. Direct effects have been analyzed on male ducks (*Anas platyrhynchos*) that receive two different concentrations of Roundup dissolved in distilled water according to the body weight (5 and 100 mg kg^{-1}). There was a decrease in testosterone level in blood plasma of about 90%. Moreover, anatomical and histological changes in seminiferous tubes and anatomical changes in the epididymis region have also been found [82].

Indirect effects have been found in wetlands where the glyphosate is used to control the increase of *Typha* spp. population [83–85]. Species of blackbirds and wren can be affected by habitat changes in target and nontarget plant communities that decrease available places to sheltering, nesting, and feeding. The lacks of those places lead birds to starvation, strong competition for resources, or leave the environment [84]. Part of control in coastal dunes of invasive species *Chrysanthemoides monilifera* ssp. *rotundata* is due to glyphosate. An 8-year study has found that a typical bird from coastal region, *Myzomela sanguinolenta,* was the rarest in places that receive the handling herbicide [86]. Environmental heterogeneity (e.g., microclimate and flora) and specific vegetation that is dead by glyphosate can be very important for conservation of some bird populations in the environment [85]. Sometimes, there is the increase of some bird populations after the

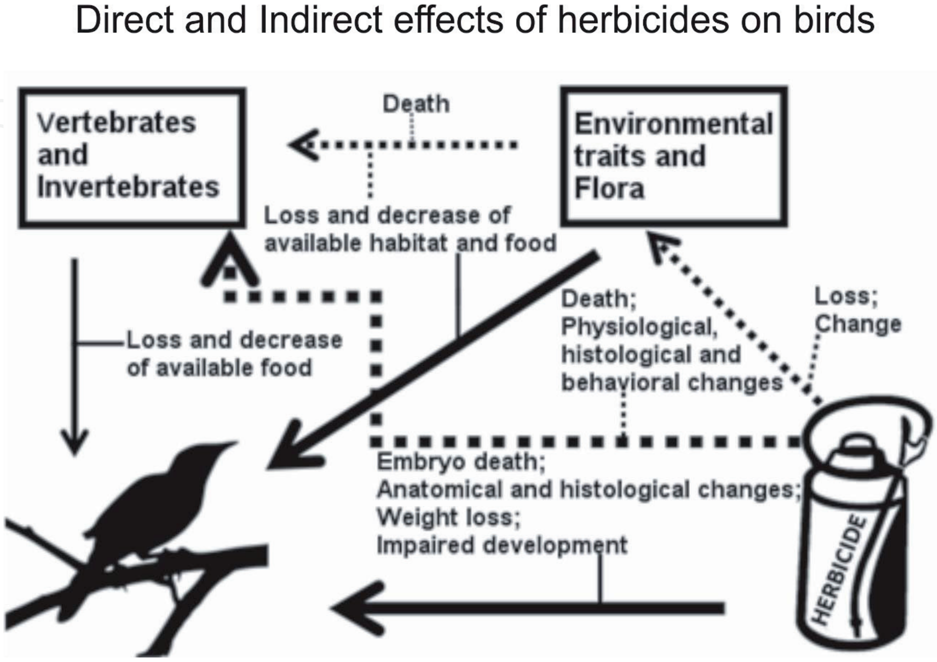

Figure 4.
Ecotoxicity of glyphosate-based herbicide (GBH) to aquatic birds. Direct (continuous arrows) and indirect (dashed arrows) effects of GBH on birds.

glyphosate application. However, it can be related with an immediate advantage due the removal of abundant plant species and other changes in the environment and in available food. Under those circumstances, other population traits, like reproductive success, could have been affected but not detected [83].

The direct effect of glyphosate on aquatic plants and macroalgae [87] can also affect aquatic birds once they make up the varied and plentiful diet of many of those birds. Changes in physiological, histological, and behavioral levels and lethal cases have been documented in fishes due to use of glyphosate [87, 88]. In this way, piscivorous birds can also be suffering indirect effects. In fact, all aquatic birds' food chain can be affected by glyphosate once effects on invertebrates [81, 87, 88], amphibians [89], and reptiles [90] have already been confirmed.

Birds are very similar in their physiology and anatomy. Then, studies that have tested direct and indirect effects of glyphosate on nonaquatic birds can be also considered here. In Japanese quails (*Coturnix japonica*), the low food consumption due to reduced palatability and the low absorption of nutrients in the digestive tract are responsible for body weight loss. Moreover, those birds have been fed with high glyphosate doses (250 and 500 mg kg^{-1} of food) and have exposed clinical symptoms of behavioral changes, malformed feathers, and slow development [91]. A total of 57.5% of dead embryos from chicken eggs have received glyphosate solution (0.1 ml with 2% Glialka Star) inside shell [92]. Herbicides can also act in synergy with other agrochemicals turning these toxic effects more complex. In this way, the combined effect between glyphosate and other chemicals on birds has been analyzed and all studies have demonstrated the increase of potential toxicological: 97.5% of dead embryos (0.1% of lead acetate plus 2% of glyphosate) [92] and decrease of hemoglobin and leucocytes (indoxacarb, an insecticide, plus glyphosate) [91]. Indirect effects on nonaquatic birds due the low vegetation complexity have also been reported: habitat loss replacing shrub by trees, for example, [93]; imbalance in the population structure (i.e., sex ratio) eliminating only habitats of one bird group [94, 95]; and changes in richness of the communities benefiting only birds related to sparse vegetation [96].

Therefore, the controlled and scaled use of glyphosate in large areas is necessary to contribute to conservation of environmental heterogeneity and biological diversity avoiding the plausible effects on bird communities [83–85, 94]. To know what plants are important to bird diet and to promote techniques that do not eliminate all of those plants from the place are important activities before glyphosate application [91]. More studies that aim to analyze the bird contamination by herbicides are also necessary [97]. Long-term studies that encourage collaborative work between ecologist, toxicologist, and chemist are more pertinent [98].

2.6 Aquatic mammals

For the best of our knowledge, GBH or glyphosate only was not tested in aquatic mammals. Searching on Web of Science website for the terms "Glyphosate AND mammal AND aquatic," there is no study reported to date. Despite that, mammals in general are considered less sensible to GBH damages than other groups due to reduced contact with the environment of mammals when compared to other groups as fishes, amphibians, or aquatic invertebrates [99]. The main way that GBH or the active ingredient glyphosate reaches mammals' bodies is through the digestive tract. However, it seems to be poorly absorbed and is excreted essentially nonmetabolized [100]. Essentially, mammals that were tested were rats, mice, and dogs [101], tested through injection or ingestion. Some studies report glyphosate in humans in medical case studies. Reported direct effects of GBH on mammals are described as a "wide range of clinical manifestations" such as skin and throat irritation, hypotension, or death [102] and include heart arrhythmias and atrioventricular block, cardiac electrophysiological changes and conduction blocks [103], pregnancy problems [104], disrupt transcriptional expression of the steroidogenic acute regulatory protein in testicle [105] and aromatase activity, alter mRNA levels, and interact with enzymes [106]. Indirect effects on mammals can be due to reduction of vegetation and animals that are a source of food such as invertebrates [101] and fishes. Although these mentioned studies were conducted in nonaquatic mammals, it is expected that aquatic mammals have similar or even more accentuated effect, since they have intense contact with water, and if it is contaminated, the exposure will be higher.

3. Regulations and perspectives

Despite the fact that GBHs were developed to control weeds, acting specifically in a restrict plan biochemical pathway, several studies demonstrated that there are many side effects on nontarget organisms in all great groups as reported extensively here. Looking to control these side effects, governments for many countries around the world established limits for usage and concentrations in water bodies. The USA, for example, allows 700 μg L^{-1} in water bodies, while Canada allows 280 μg L^{-1} in drink water. The Brazilian law is a little more restrictive, allowing 65 μg L^{-1} in water bodies class 2 that is used for crop and recreation of first degree (direct contact) [107]. However, we could check here that these maximum concentrations allowed are not safe for biodiversity conservation. Considering the Brazilian law, the more restrictive in American countries, populations of yellowtail tetra fish (*A. lacustris*) are not safe since sperm cells of this species are dead in lower concentrations than 65 μg L^{-1} [52]. In this way, European regulations are more plausible, because it is more restrictive (0.1 μg L^{-1}) [108] and can be more precise on conservation of aquatic biodiversity.

However, even with all those regulations, it is not being obeyed, since there is a large range of glyphosate and its metabolite (e.g., AMPA) concentrations in hydroresources [6, 64]. Therefore, another way of action for environment safety is preserving marginal forests of rivers, surveillance, and environment education. Another sustainable way to achieve this goal is changing the crop production matrix from large scale, that is, conventional-based production model to a smaller integrative-/organic-based production system, with controlled or restrictive usage of pesticides and other agrochemicals.

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Conflict of interest

The authors declare that there is no conflict of interest.

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