

Review

Overview of Environmental and Health Effects Related to Glyphosate Usage

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Abstract: Since the introduction of glyphosate (*N*-(phosphomethyl) glycine) in 1974, it has been the most used nonselective and broad-spectrum herbicide around the world. The widespread use of glyphosate and glyphosate-based herbicides is due to their low-cost efficiency in killing weeds, their rapid absorption by plants, and the general mistaken perception of their low toxicity to the environment and living organisms. As a consequence of the intensive use and accumulation of glyphosate and its derivatives on environmental sources, major concerns about the harmful side effects of glyphosate and its metabolites on human, plant, and animal health, and for water and soil quality, are emerging. Glyphosate can reach water bodies by soil leaching, runoff, and sometimes by the direct application of some approved formulations. Moreover, glyphosate can reach nontarget plants by different mechanisms, such as spray application, release through the tissue of treated plants, and dead tissue from weeds. As a consequence of this nontarget exposure, glyphosate residues are being detected in the food chains of diverse products, such as bread, cereal products, wheat, vegetable oil, fruit juice, beer, wine, honey, eggs, and others. The World Health Organization reclassified glyphosate as probably carcinogenic to humans in 2015 by the IARC. Thus, many review articles concerning different glyphosate-related aspects have been published recently. The risks, disagreements, and concerns regarding glyphosate usage have led to a general controversy about whether glyphosate should be banned, restricted, or promoted. Thus, this review article makes an overview of the basis for scientists, regulatory agencies, and the public in general, with consideration to the facts on and recommendations for the future of glyphosate usage.

Keywords: aminomethyl phosphoric acid; environment; herbicide; Roundup[®]; Rangerpro[®]; toxicity



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1. Introduction

Since its introduction in 1974, glyphosate (*N*-(phosphonomethyl) glycine)—the active ingredient in the commercial Roundup[®] and RangerPro[®] products—has been the most used nonselective and broad-spectrum herbicide around the world [1]. Chemically, it is formulated as ammonium, di-ammonium, dimethyl ammonium, potassium, and isopropylamine salts [2]. The glyphosate-based herbicides (GBHs) are also formulated with adjuvants (such as the polyoxyethylene amine (POEA), alkyl polyglycolide, polyethylene alkyl ether phosphates, and quaternary ammonium compounds) as surfactants to promote the uptake and translocation of the active ingredient in plants [3,4]. It is an organophosphate molecule that contains $-PO_3H_2$, $-COOH$, and $-NH_2$ as the functional groups [5].

Glyphosate acts by inhibiting the enzyme 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) (EC. 2.5.1.19) with the interruption of aromatic amino acid biosynthesis in the shikimate pathway [6]. These inhibited amino acids are essential for protein and

secondary-metabolite biosynthesis, such as that of flavonoids, lignin, and phytoalexins [7]. Moreover, the shikimate-pathway interruption affects the carbon flow and fixation to produce energy, and the whole metabolism function [8]. Because the shikimate pathway is not present in mammals, it could be a desirable herbicide. Unfortunately, this pathway is also present in some fungi and bacteria that are present in diverse microbiota (i.e., gut, soil, and plant-surface microbiota) [9,10].

GBHs are traditionally applied at high concentrations (6.7–8.9 kg ha⁻¹) and low concentrations (0.53–1.0 kg ha⁻¹), respectively, before and after the establishment of conventional crops [11]. They have also been used to control invasive vegetation in forestry [12], algae proliferation in aquaculture [13], and invading weeds around perennial trees [14]. They have been used too for weed control in home gardens, parks, and across urban areas [15]. The global use of glyphosate and GBHs rose from 56, 296 tons in 1994 to 825, 804 tons in 2014, with an estimation of 740–920 thousand tons in 2025 [16].

The widespread use of glyphosate and GBHs in agricultural fields and home gardens is due to their low-cost efficiency in killing weeds, their rapid absorption by plants, and the general erroneous perception of their low toxicity and slow generation of herbicide resistance [6]. The commercialization of the first glyphosate-resistant soybean (*Glycine max*) variety (Roundup Ready) in 1996, and subsequent resistant-tolerant varieties of maize (*Zea mays*), canola (*Brassica napus*), and cotton (*Gossypium hirsutum*), have resulted in the increased commercialization and dose administration of GBHs [17]. Currently, the use of glyphosate and GBHs is widespread around the world in developed and developing countries [18]. Next-generation resistant varieties to glyphosate are encoded to produce a glyphosate oxidase enzyme to convert glyphosate into aminomethyl phosphonic acid (AMPA) and glyoxylate [19]. Despite this, the residue persistence of glyphosate and AMPA are determined by factors such as the soil properties and environmental conditions [20]. Moreover, after 46 years of glyphosate-based product application, approximately 38 different glyphosate-resistant (GR) weed species have been reported [21]. Several studies show that the half-lives of glyphosate and AMPA range between 0.8 and 151 and 10 and 98 days, respectively [1].

As a consequence of the intensive use and accumulation of glyphosate and GBHs on environmental sources and food [22], major concerns about the harmful side effects of glyphosate and AMPA on human, plant, and animal health, and on water and soil quality, are emerging [6]. Moreover, the glyphosate residues in the effluents are too difficult to purify, and, thus, they have a long-term life in water and soils [23]. Exposure to glyphosate and AMPA has been shown to induce antibiotic resistance in *Salmonella* spp. and *Escherichia coli*, and in soil bacteria in general [24]. Toxicity to honeybees, birds, amphibians, fishes, and others, has also been documented [25–28]. Moreover, reports indicate that exposure to GBHs, even at below the indicated concentrations, causes tumorigenic, carcinogenic, teratogenic, hepatorenal, and endocrine disruption effects, in addition to oxidative stress [29].

Since the World Health Organization reclassified glyphosate as probably carcinogenic (Group 2A) to humans in 2015 by the IARC [30–32], many review articles concerning different glyphosate-related aspects and its controversy have been published [16,33–36]. Recently, the use of GBHs has been restricted or banned in many countries, including Germany, Italy, France, the Netherlands, Belgium, the Czech Republic, Denmark, the United Arab Emirates, Bermuda, Qatar, Costa Rica, and Mexico [34,37,38]. By contrast, regulatory authorities, such as the European Commission, the United States Environmental Protection Agency (U.S. EPA), and the Canadian Pest Management Regulatory Agency, reviewed the matter and concluded that glyphosate and GBHs are safe and do not pose adverse effects to human health [38,39]. These disparities have led to a general controversy and different regulatory laws around the world, ranging from complete bans to unrestricted policies [40].

The risks, disagreements, and concerns regarding glyphosate usage have generated controversy about whether glyphosate, the most used nonselective and broad-spectrum

herbicide around the world, should be banned, restricted, or promoted. There is a need for an overview with a risk assessment for scientists, regulatory agencies, and the public in general that considers the facts and recommendations about the future of glyphosate usage. Therefore, in this review article, two topics are summarized: (1) the environmental impact of glyphosate; and (2) the health effects of glyphosate.

2. Environmental Impact of Glyphosate

2.1. Behavior and Fate of Glyphosate

After its administration, glyphosate can biotransform in soil by mineralization, immobilization, or leaching; however, it cannot be significantly volatilized because of its high vapor pressure (Figure 1) [38]. Mineralization is the principal mechanism of degradation, involving biotic and abiotic pathways, with AMPA as the major metabolite, as well as other products, such as methyl phosphonic acid [CH₃P(OH)₂], sarcosine (C₃H₇NO₂), glycine, phosphate (PO₄³⁻), carbon dioxide (CO₂), and ammonia (NH₃) [41,42]. The microbial activity that promotes glyphosate mineralization depends on factors such as the soil physicochemical characteristics, temperature, pH, and organic-matter content [43]. Thus, high levels of organic C and organic matter tend to be beneficial to the environment by delaying leaching and promoting their slow degradation and release in soil [44]. Nonetheless, excessive glyphosate depositions could saturate the soil capacity to delay leaching and its gradual mineralization [45]. Some metals in the soil, such as manganese oxide (MnO₂), promote the abiotic degradation of glyphosate [46].

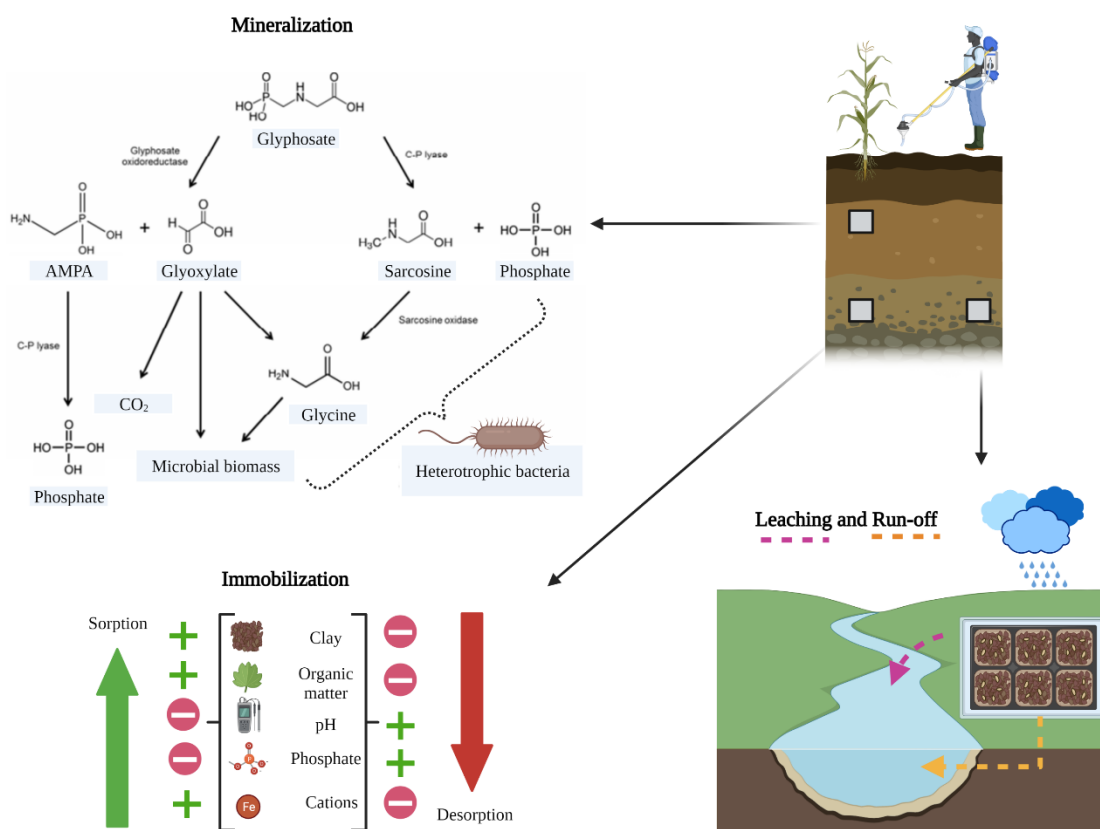


Figure 1. Behavior and fate of glyphosate in the environment.

Glyphosate and AMPA mineralization are related to many soil physicochemical factors, and they too can be variable in short periods under certain specific circumstances [47]. After revision, Bai and Ogbourne [1] concluded that the half-lives of glyphosate and AMPA residues vary between 0.8 and 151 and 10 and 98 days, respectively. The degradation of glyphosate through mineralization is directly affected by the physicochemical properties of

soil (organic-matter content, pH, and texture), climatic conditions (temperature and humidity), and biological properties (microbial diversity and activity) [20,48]. Thus, glyphosate and AMPA exhibit from low to very high persistence in soil (Table 1).

Table 1. Glyphosate and AMPA persistence in soil.

Glyphosate Dose (kg ha ⁻¹)	Half-Life (d)	Soil Properties	Location	Reference
1.54	98 (Gly), 51 (AMPA)	Clay and sandy	Sweden	[20]
5	42 (Gly)	Loamy	China	[43]
1	613 (Gly and AMPA)	Boreal sandy	Finland	[49]
2.1	60 (Gly and AMPA)	Silty loam	Argentina	[50]
0.25	9 (Gly) and 32 (AMPA)	Sandy	Denmark	[51]
1.8	18 (Gly) and 250 (AMPA)	Sandy	Italy	[52]
8	31 (Gly and AMPA)	Sandy loam	Spain	[53]

As mentioned before, glyphosate may biotransform in soil by immobilization and leaching. Glyphosate immobilization occurs naturally rapidly, and it is affected by the organic matter, mineral availability, clay, and phosphate concentration [54]. Thus, glyphosate will accumulate for a long time in soils with high organic matter, phosphate, clay content, Al and Fe concentrations, and low pH, and it is easily leached under the opposite conditions [55]. The phosphonic acid structure of glyphosate binds with the cations contained in clay structures and organic matter in soil [54]. Soil minerals such as Al and Fe in the oxide state have strong chemical affinities with the phosphonate, amino, and carboxyl groups of glyphosate, while inorganic phosphorus binds competitively to its sorption sites [56]. Glyphosate acts as a polyprotic acid that binds anions and cations at 4–8 pH in soils [57]. The immobilization in the soil is not a permanent process, and it decreases after a certain period [58].

Despite glyphosate being immobilized by high soil affinity, factors such as the concentration, prevalence, and mineralization rate are determinants for its leaching [55,59]. Rainfall and the soil structure are determinants for leaching too [60]. Glyphosate can reach the water sediment or water surface either in dissolved or particle form [38]. Leaching is a growing concern because it contaminates the water [16]. Glyphosate is also introduced to water bodies by runoff, but rarely by its direct application (i.e., unwanted seaweed control) [61]. Because AMPA is more mobile, it is found in higher concentrations by leaching [62]. AMPA is also a degradation product of the sweetener acesulfame; nonetheless, the leached AMPA in water bodies is related to glyphosate residues [63].

2.2. Glyphosate Residuality

Glyphosate and GBH application has been rising considerably since the late 1970s because of the false belief in their low toxicity and mobility in the environment, and after the introduction of genetically modified corn, soybean, and cotton [64]. Glyphosate is the most used (>100 crops) and sealed (>130 countries) herbicide around the world [16]. A consequence of the intensive use of glyphosate and AMPA is being detected in the residuality of soil, water, and nontarget plants [1].

Glyphosate and AMPA can appear residually at the cropping site and around it [22]. The principal effects of their residuality are the toxicity to soil microbial communities and the reduction in nutrient availability [65]. Microbial communities have essential functions, such as improving the soil structure and making nutrients available to plants [66]. Nonetheless, their activity is regulated by nematodes that feed them [67]. Because of such importance, microbial communities and nematodes are proposed as indicators of soil quality and health [68]. In a coffee plantation with 22 years of glyphosate application, the soil nematode population was lower than that in a plantation with 7 years of no-glyphosate application [69]. However, glyphosate has not yet been conclusively implicated in the repercussions to nematodes per the current extent of the research [70–72]. The residuality

of glyphosate antagonizes some enzymes in the soil, such as acid phosphomonoesterase (EC. 3.1.3.2), urease (EC. 3.5.1.5), β -glucosidase (EC. 3.2.1.21), and alcohol-dehydrogenase (EC. 1.1.1.1) [73].

As mentioned before, glyphosate and AMPA can reach water bodies by soil leaching, runoff, and sometimes by the direct application of some approved formulations [74]. AMPA could be present in water as the degradation product of detergents; nonetheless, AMPA detection by detergents always corresponds to specific sites, such as plant treatment effluents and stormwater discharge [75]. Glyphosate has been detected in many water bodies, ranging from 2 to 430 $\mu\text{g L}^{-1}$ [22]. In sediment samples from the United States, the glyphosate concentrations ranged from 397 to 476 $\mu\text{g L}^{-1}$ [76]. According to Wang et al. [77], the microbial degradation of glyphosate in water sediments is slower than in soil environments. Some aquatic species, such as fish and amphibians, could be affected by glyphosate concentrations over 400 $\mu\text{g L}^{-1}$ [78]. The free fish species could be exposed via gills and dietary routes [79]. Amphibians are susceptible to glyphosate residues because of their dual life cycle (aquatic/terrestrial) [74]. Despite the studies that argue that glyphosate is not toxic to aquatic species [80,81], the growing evidence suggests the potential impact on aquatic environment species and human health [82–84]. It is important to consider that this can be mixture effects in the soil and water that are not usually taken into account by single-substance dosage studies.

Glyphosate can reach nontarget plants by different mechanisms [36]. Spray application is the primary route, with more than a 10% application rate to nontarget crops after application in crops such as soybean and cotton [85,86]. This spray drift caused distorted fruit in tomatoes, even at minor doses of lethal concentrations [36]. Another mechanism is the release of glyphosate through the tissues of treated plants, such as weeds [87]. Decaying plant matter from weeds is decomposed and absorbed by the soil, and, thus, some traces of glyphosate, available for both target and nontarget plants, are reincorporated by root absorption [36]. Moreover, glyphosate inactivates the EPSPS enzyme, which plays a key role in the synthesis of phenolic compounds that have a function in the plant defense mechanisms [88]. The pathogen's colonization rate was increased in wheat and barley roots when glyphosate was administrated before planting [89]. Nontarget plants are affected indirectly by the alterations in the soil characteristics and their microbial communities, which affect nutrient availability and thus alter the plant defense physiology [36]. Other potential side effects on nontarget plants are root disruption and increasing fruit drop [90]. As a consequence of this nontarget exposure, glyphosate and AMPA residues are being detected in the food chains of diverse products, such as bread, cereal products, wheat, vegetable oil, fruit juice, beer, wine, honey, eggs, and others, at concentrations that range between 2.948 and 0.0005 mg kg^{-1} (Table 2) [91].

Table 2. Residual concentrations of Glyphosate and AMPA from different food sources.

Food Source	Glyphosate (mg kg^{-1})	AMPA (mg kg^{-1})
Beer	<0.0005	<0.001
Wine	0.0031	<0.0007
Mineral water	<0.0006	<0.0005
Milk	<0.0006	<0.0025
Fruit juice	0.0016	<0.0006
Baby food	<0.001	<0.0025
Potatoes and vegetables	<0.001	<0.0025
Honey	0.0030	<0.0025
Eggs	<0.001	<0.0025
Meat and fish	<0.001	<0.0025
Pulses	0.0012	<0.0025
Oilseeds and vegetable oil	<0.001	<0.0025
Pseudo cereals	<0.001	<0.0025
Breakfast cereals	0.0036	<0.0025
Durum wheat	0.139	0.0107

Table 2. Cont.

Food Source	Glyphosate (mg kg ⁻¹)	AMPA (mg kg ⁻¹)
Pastry and snacks	<0.001	<0.0025
Bread	0.0019	<0.0025
Flour and baking mixtures	<0.001	<0.0025
Other cereal products	<0.001	<0.0025

Source: Zoller et al. [91].

3. Health Effects of Glyphosate

After the EPSP inhibition by glyphosate application, the target plants suffer alterations in their physiology and die after 7–21 days [92]. Since the shikimic acid pathway is present in plants, fungi, and some microorganisms, but absent in animals such as mammals, this is the parameter that states that glyphosate is not toxic for animals, even after evidence of exposition and toxicology effects [38]. In mammals, glyphosate and AMPA are considered nontoxic because of their limited tissue and gastrointestinal absorption [39]. Nonetheless, GBHs have demonstrated their toxic effects on nontarget aquatic and terrestrial organisms [26,93,94]. Moreover, a considerable portion of the toxicity of GBHs is attributed to the surfactant POEA [4,93,95].

3.1. Human Health Effects

The increasing global use of GBHs has led to a concern about their residuality on water sources, nontarget plants such as food, and the environment. This human exposure promotes the absorption of residues through ingestion, inhalation, and dermal contact [96]. Residues of glyphosate and AMPA were detected in the urine of the general public from the United States (60–80% of sampled) and Europe (44% of sampled), with 2–3 and <1 µg L⁻¹ means, respectively, and 233 and 5 µg L⁻¹ maximum concentrations, respectively [97,98]. The human health effects of glyphosate and GBHs have been studied and documented (Figure 2) [29,99,100]. Nonetheless, there is a general lack of accord as to glyphosate and GBH health effects.

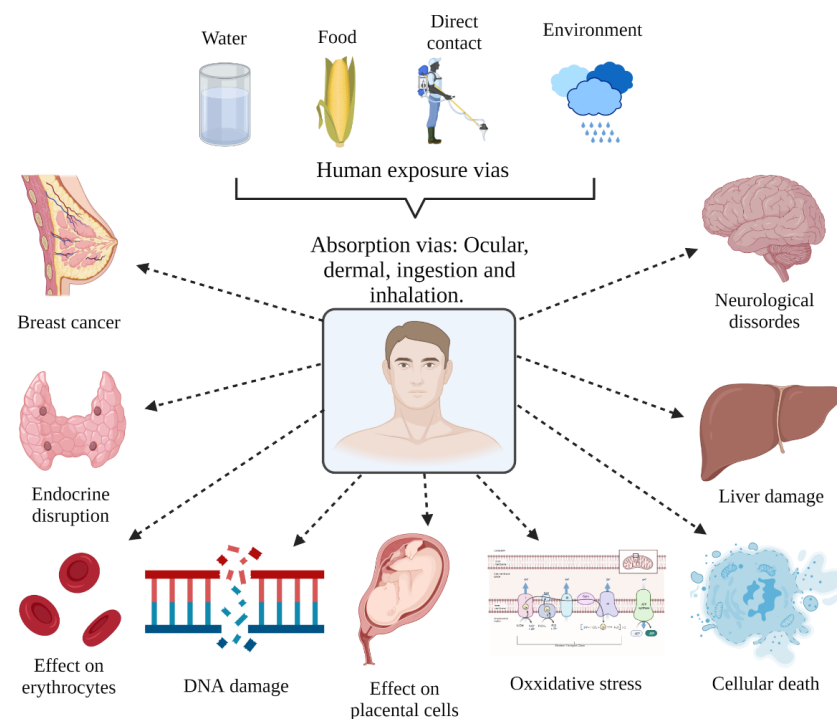


Figure 2. Human health effects after water, food, direct contact, and environmental exposure, and, consequently, absorption.

There are many laboratory-based studies that report the negative effects of glyphosate and GBHs on human cells. Generally, the variation in the results relies on many variables, such as the methodology, dose and exposure time, GBH formulation, and cell type (Table 3) [29]. Despite the inconclusive and sometimes contradictory results on human health, the summarized results are a base statement for future decisions about glyphosate and GBH toxicological effects.

Table 3. Effects of glyphosate and GBHs on human in vitro cell cultures.

Human Cell Type	Dose of Glyphosate ($\mu\text{g mL}^{-1}$)	Exposure Time (h)	Evaluated Effects	References
Blood	0.500	52	Mutagenicity, Cytotoxicity, DNA damage, Hemolysis, Acetyl cholinesterase activity	[101–109]
Epithelial	0.300	18	Oxidative stress, Cell damage, Genotoxicity	[110–112]
Embryonic	0.450	24	Cell damage, Toxicity, Endocrine disruption	[113–115]
Pluripotent stem	0.100	48	Blood–brain barrier	[116]
Renal	0.600	24	Cell viability, Apoptosis, Cell viability	[117]
Hepatic	0.540	24	Transcriptomic changes, Genotoxicity, Oxidative stress, DNA damage,	[112,118–120]
Breast	0.100	48	Endocrine disruption, Toxicity, DNA damage,	[121,122]
Ovarian	0.500	72	Abnormal growth,	[123]
Pulmonary	0.540	24	Cell viability	[119]
Neuronal	0.540	24	Toxicity, DNA damage	[119]
Sperm	0.36	1	Cell viability, DNA fragmentation	[124]

Despite the recognized residuality of glyphosate and AMPA (0.8–151 and 10–98 days, respectively) in the environment [125], it is difficult to predict the significance and the impact of these residues when there are not enough long-term and independent data related to their safety, health, and toxicity. Nonetheless, some epidemiological studies with concluding correlations between glyphosate and/or GBH exposure and health problems, such as cancer, respiratory disease, neurological and congenital effects, and others, have been reported.

In terms of epidemiological cancer studies, Leon et al. [126] conclude that there exists a moderate correlation between glyphosate exposure and β -cell cancer lymphoma. Besides direct exposition and ingestion, glyphosate could be inhaled from the air environment [1]. It is important to mention that the air exposure that was evaluated by Leon et al. [126] was about five times less than the acceptable daily intake proposed by the EFSA [39]. The results by Hoppin et al. [127] show a connection between glyphosate exposure and allergic and nonallergic wheeze in male farmers. Recently, some studies have evaluated the neurological effects of glyphosate exposure [128,129]. Caballero et al. [128] found a 33% higher risk of Parkinson's disease mortality after glyphosate exposure. Von Ehrenstein et al. [129] conclude that there is a correlation between glyphosate prenatal exposure and autism spectrum disorder. During pregnancy, the fetus could be exposed indirectly to glyphosate; thus, Parvez et al. [130] used urine samples and concluded that >90% had glyphosate-detectable levels that were correlated with shortened pregnancy.

The toxicity of glyphosate and GBHs to different human cells has been demonstrated. However, most epidemiological studies lack glyphosate-administrated doses to directly

confirm its effect. On the basis of the in vitro and epidemiological results, it is difficult to directly infer that glyphosate and GBHs pose a risk to human health and safety.

3.2. Health Effects on Other Organisms

Glyphosate and GBHs are perceived as a group of chemicals that are well regulated in their environmental risks and health effects on nontarget organisms [131]. Moreover, some research states that glyphosate is nontoxic or slightly toxic to different organisms [132–135]. However, numerous studies have demonstrated the toxicological effects of glyphosate and GBHs on a wide range of nontarget organisms. Glyphosate showed an adverse effect on unicellular organisms, such as *Euglenia gracilis*, where glyphosate at 3×10^{-3} M reduced the chlorophyll, photosynthesis, and respiration [136]. In rhizobium bacteria, glyphosate applied to glyphosate-resistant crops, such as soybean and corn, decreased the proliferation of Acidobacteria, which are implicated in biogeochemical processes related to nutrient acquisition [137]. In poultry microbiota, exposure to glyphosate affects the availability of some beneficial bacteria, such as *Lactobacillus* spp., *Enterococcus faecium*, *Bifidobacterium adolescentis*, and *Bacillus badius* [10].

Glyphosate also demonstrated negative effects on multicellular organisms found in soil and water. The population and radial growth of some mycorrhiza fungi, such as *Cenococcum geophilum* and *Hebeloma longicaudum*, were reduced after glyphosate exposure at concentrations of >5000 ppm [138]. Kittle and McDermid [139] conclude that glyphosate decreased the macroalgae chlorophyll content. As mentioned, nematodes are important for maintaining a healthy ecosystem in the soil. Dominguez et al. [135] demonstrated that AMPA decreased the bodyweight of juvenile nematodes. Zaller et al. [131] analyzed the effect of GBHs on the correlation between *Lumbricus terrestris* and mycorrhizal fungi. Arthropods are 90% of the animal kingdom, and they have an important function in the ecological balance and in human nutrition [140]. In the research, *Daphnia magna* and *D. spinulata* were treated with glyphosate and, after 48 h at 150 mg L^{-1} , the organisms were immobilized [141]. The effect of glyphosate and POEA on crayfish (*Cherax quadricarinatus*) was evaluated after 50 days of exposure, and the results show a reduction in somatic-cell growth and a reduction in the muscle glycogen and lipid reserves [142].

Insects are invertebrates from the Arthropoda phylum, and they have also been affected by the use of glyphosate and GBHs. Three different concentrations of glyphosate (2.5, 5.0, and 10 mg L^{-1}) were mixed in a sucrose solution, and the results show that glyphosate damaged the cognitive functions of bees (*Apis mellifera*) [143]. GBH was harmful to the larvae eggs of *Trichogramma pretiosum* [144]. The negative effects of glyphosate and GBHs have also been demonstrated in molluscs, such as aquatic snails (*Pseudosuccinea columella*) [145] and terrestrial snails (*Helix aspersa*) [146]. Glyphosate was also toxic to fish [147], amphibians [148], and birds [149].

4. Conclusions

Glyphosate is the most used nonselective and broad-spectrum herbicide around the world. Glyphosate and GBH application has been increasing considerably since the late 1970s because of the false belief in their low toxicity and mobility in the environment. As a consequence of their overuse, glyphosate and AMPA are being detected residually on soil, water, and nontarget plants, causing considerable negative side effects to the environment and to the health of humans and other organisms. This review article presents the state of the art on the environmental and health effects of GBHs, glyphosate, and its principal residue, AMPA. It can be concluded that the indiscriminate use of glyphosate and GBHs has led to documented effects on nontarget organisms. As a consequence of all the recent controversy, glyphosate and GBHs have been either restricted or banned. Nonetheless, further studies are needed on the side effects of glyphosate and GBHs on the environment, human health, and nontarget organisms to fill in the gaps in the knowledge. This general overview provides a risk assessment for scientists, regulatory agencies, and the public in general, with consideration to the facts on the glyphosate-usage risks.

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