Effects of Glyphosate and Its Metabolite AMPA on Aquatic Organisms

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Abstract: Glyphosate (N-(phosphonomethyl)glycine) was developed in the early 1970s and at present is used as a herbicide to kill broadleaf weeds and grass. The widely occurring degradation product aminomethylphosphonic acid (AMPA) is a result of glyphosate and amino-polyphosphonate degradation. The massive use of the parent compound leads to the ubiquity of AMPA in the environment, and particularly in water. Considering this, it can be assumed that glyphosate and its major metabolites could pose a potential risk to aquatic organisms. This review summarizes current knowledge about residual glyphosate and its major metabolite AMPA in the aquatic environment, including its status and toxic effects in aquatic organisms, mainly fish. Based on the above, we identify major gaps in the current knowledge and some directions for future research knowledge about the effects of worldwide use of herbicide glyphosate and its major metabolite AMPA. The toxic effect of glyphosate and its major metabolite AMPA has mainly influenced growth, early development, oxidative stress biomarkers, antioxidant enzymes, haematological, and biochemical plasma indices and also caused histopathological changes in aquatic organisms.

Keywords: toxicity; effect; fish; invertebrate; mussels

1. Introduction

Over the last few years, the importance of knowledge about pesticide’s persistence, mobility, and ecotoxicity has increased. Using pesticides and other agrochemicals is the most cost-effective way to maintain economic viability in the increasing human population [1,2]. On the other hand, the intensive application and repeated use of pesticides in fields in order to increase the crop yield lead to long-term risk for humans, fauna, flora, and the whole ecosystem (soil, air, and water) [1–3]. The extensive use of pesticides is not only a problem in agricultural areas but also in urban settings where pesticides are applied for horticultural purposes. Therefore, it is challenging to control the source of diffuse chemical pollution and its consequences [4]. In particular, the presence of pesticides and their metabolites occurring in residual concentrations in drinking, ground, and surface waters poses a global problem [1,3].

Before World War II, natural and organic pesticides were used. However, after the war, it was necessary to increase crop production to prevent starvation and malnutrition, which was an opportunity for industrial companies to produce new synthetic agrochemicals and disseminate worldwide use of them [5]. A considerable amount of pesticide-based chemicals with different uses and modes of action have been successfully brought to market thanks to the fact that the chemical structures, the way, or period of modes of action had the desired effect on the target organisms (according to the United States Environmental Protection Agency 40% herbicides, following insecticides and fungicides) [6]. Later after World War II, in 1970, John Franz discovered the glyphosate-based herbicide effect working in Monsanto (USA). The herbicide was registered in 1974 under the trade name...
“Roundup”. [7,8]. Due to initial toxicity tests, which showed relatively low risks to nontarget organisms, including mammals, the exposure limits of glyphosate were set relatively high worldwide. In a short time, use of this popular herbicide increased dramatically due to genetically modified crops (soybean, canola, alfalfa, maize, cotton, and corn) which proved to be glyphosate-tolerant. This high frequency of use in agronomy and urban areas caused the general public to perceive this herbicide as having low toxicity and not being very mobile in the environment [9–11]. However, ecotoxicology and epidemiology studies published in the last decade indicate the need for further intensive glyphosate toxicity testing. [11]. Furthermore, the World Health Organization’s International Agency for Research on Cancer concluded recently that glyphosate is “probably carcinogenic to humans” [12–14].

Generally, the mobility and concentration of glyphosate and aminomethylphosphonic acid (AMPA) are mainly influenced by their bioavailability, bioaccumulation, persistence, ecotoxicity, and transfer into the aquatic environment (Figure 1) [1,8,11].

![Figure 1. Distribution and transport of glyphosate and its major metabolite AMPA into the aquatic environment.](image-url)

Directly after spraying herbicide in agriculture or in urban areas, glyphosate is absorbed by crops or weeds and penetrates the soil simultaneously. The glyphosate degradation pathway in bacterial strains is the cleavage of the C-N bond and conversion to AMPA, which is either further decomposed or excreted into the environment [15,16]. AMPA is a primary product of the degradation process of glyphosate and the following nontoxic products are sarcosine and glycine. Unlike AMPA, which is 3–6-fold times more toxic and persistent than glyphosate [17], sarcosine is barely detected in the natural environment [18], except under experimental conditions in a laboratory [16]. On the one hand, the soil has functioned as storage; on the other hand, these contaminants leach below the root zone into groundwater. Glyphosate is also transported by runoff into surface water and consequently accumulated in sediment where glyphosate can be highly mobile [10,17]. The residual concentrations of glyphosate and AMPA in waters contaminate aquatic organisms via the food web (Figure 1) [11,15].
Indeed, pesticides have the ability to dissolve themselves to some extent in the environment. However, there is also the potential risk of residues from the biodegradation process [4,19]. As a result of the extensive use of pesticides, residual concentrations of pesticides and their metabolites are commonly found ubiquitously through different environmental constituents ranging from 1 ng/L to 1 mg/L or higher concentrations [3,4]. There is also a potential risk of banned pesticides. They were excluded because of their long-term persistence and toxicity in the ecosystem. For example, organochlorine insecticides were still detectable in water after 20 years [20] and Acetochlor ESA, the major metabolite of prohibited Acetochlor in the European Union in 2012 no. 1372/2011 [21], was found in the waters of the Czech Republic in recent years [22,23]. Although these pesticides are usually detected only in low concentrations in the environment, they may be present as complex mixtures. The metabolites may be as toxic as their parental compound or even moreso. Therefore, the presence of these substances is of great concern to ecotoxicologists, e.g., [24–26].

Due to repeated application of pesticides, the physical and chemical changes in water properties rise considerably, which is reflected in the modification of the cellular and biochemical biology of aquatic communities, leading to significant changes in their tissues, physiology, and behaviour [27,28]. Therefore, it may affect the daily or seasonal rhythm of aquatic organisms and also their reproduction ability. The environmental stress from xenobiotics may cause loss of habitats and consequently loss of freshwater biodiversity [29,30], which implies that the use of pesticides, despite their advantage in controlling pests, diseases, fungi, etc., has adversely impacted their ubiquity in the environment, e.g., [16,17,31,32].

As far as is known, several studies and reports about the occurrence and toxic effects of different types of pesticides are available in the literature; nevertheless, their global extent and spatial extent of exposure remain largely unknown [2,33]. Considering this information, we decided to write a review to summarise the toxic effects of the often used herbicide glyphosate and its metabolite AMPA on aquatic organisms.

2. Glyphosate (N-(phosphonomethyl)glycine)

Glyphosate (GLY) belongs to the phosphonoamino acid class of pesticides. Glyphosate is an acid that can be associated with different counter cations to form salts [15]. This herbicide is a crop desiccant, broad-spectrum, nonselective, postemergency herbicide that affects all annual and multiannual plants and aquatic weed control in ponds, lakes, canals, etc. [34,35].

Unlike GLY, whose small molecule consists of a linear chain with weak bonds, the molecules of other herbicides are usually arranged in aromatic circular structures. This difference reduces the persistence of glyphosate in the environment [36]. For higher water solubility, GLY is formulated as potassium salts or isopropylamine salts and a surfactant, poly-oxyethylene amine (POEA), is added to enhance the efficacy of the herbicide. Another formulation, Rodeo, contains the isopropylamine salt (IPA) of GLY without the surfactant and is primarily used for controlling aquatic weeds [35,37] or Roundup Transorb, which contains a mix of 15% POEA and additional surfactants [38]. Roundup includes 48% of active agent IPA [34] or potassium salts in the range 167–480 g L$^{-1}$. The exact amount depends on the type of area where the Roundup is applied [39].

2.1. Environmental Fate

Although a strong bond to the soil amount of GLY leaching up or runoff into surface or ground water is low [40], the aerial applications of glyphosate spray drifts from the ground and may enter into aquatic ecosystems (Figure 1) [41]. Height application rates, rainfall, and a flow route that does not include transportation of GLY through the soil from watersheds pose the highest risk to offsite transport of GLY [9]. For example, the United States Environmental Protection Agency [15] reports predicted GLY concentration from direct applications into a standard pond in 103.8–221.5 µg/L for daily peak,
101.8–217.5 µg/L for 21-day average, and 98.4–210 µg/L for 60-day average. In water bodies, the glyphosate-based herbicide is usually detectable as glyphosate acid equivalent at the range level from 0.01 mg/L to 0.7 mg/L and has the worst impact on surface waters with the value of 1.7 mg/L [42–44]. Coupe et al. [9] reported concentration of GLY for Mississippi, Iowa, and France ranged from 0.03 to 73 µg/L, 0.02 to 1.6 µg/L, and 1.9 to 4.7 µg/L, approximately.

This herbicide is unique for its ability to transform itself to the major metabolite AMPA due to microbial degradation [16,40], and its physiochemical properties: water solubility 11.6 g/L at 25 °C, low lipophilicity LogP <−3.2 at 20 °C, dissociation constant of 2.3 at 25 °C [40]. Under aerobic conditions, the halflife of GLY ranges from 1.8 to 109 days in soil and 14–518 days in water-sediment systems; however, in anaerobic water-sediment systems it ranges from 199 to 208 days [15]. Nevertheless, according to the published data the halflife of GLY ranges from 7 to 14 days [40].

GLY contamination has emerged as a pressing issue their high-water solubility and extensive usage in the environment (especially in shallow water systems). Therefore, the exposure of nontarget aquatic organisms to these herbicides is a concern of ecotoxicologists [16,37]. Many objects of ecotoxicologists studies are the toxicity of GLY to different species of fauna and flora and as the final food chain link human. For example, the recent review by Matozzo [45] summarizes only the impact on marine invertebrates but compares glyphosate and its commercial formulations. On the other hand, we report published data about adverse effects of GLY on more aquatic species as a summarisation of harmful impact on aquatic biota.

2.2. Acute Toxicity

It has been already mentioned that the initial testing of GLY did not fully demonstrate its toxic effects, and therefore the amount for use was not strictly regulated. U.S. The EPA divided the toxicity of GLY into slight toxicity with concentrations ranging from 10 to 100 mg/L and almost nontoxicity with concentration higher than 100 mg/L to fish species with acute LC50 values from >10 to >1000 mg/L [15]. Lethal concentrations are various for 24, 48, and 96 h ranging from 0.295 to 645 mg/L for fish species (Table 1); from 6.5 to 115 mg/L for amphibian’s species (Table 2); and from 35 to 461.54 mg/L for invertebrate species (Table 3).

### Table 1. Acute toxicity values (LC50) of glyphosate and its commercial products on fish.

| Species                          | Formulation | Exposure (Hours) | Concentration (mg/L) | References |
|----------------------------------|-------------|-----------------|----------------------|------------|
| Rainbow trout (Oncorhynchus mykiss) | GLY         | 96              | 140                  | [41]       |
|                                  | Roundup     | 96              | 52–55                | [46]       |
| Common carp (Cyprinus carpio)    | GLY         | 48              | 645                  | [47]       |
|                                  |            | 96              | 620                  |            |
|                                  | Roundup     | 96              | 22.19                | [48]       |
|                                  | GLY         | 48              | 602.61               | [49]       |
|                                  |            | 96              | 520.77               |            |
| Blackhead minnow (Pimephales promelas) | GLY         | 96              | 97                   | [41]       |
| Channel catfish (Ictalurus punctatus) | GLY         | 96              | 130                  |            |
| Bluegills (Lepomis macrochirus)   | GLY         | 24              | 150                  | [41]       |
|                                  |            | 96              | 140                  |            |
| Guppy (Poecilia reticulata)      | GLY         | 96              | 69.83                | [50]       |
Table 1. Cont.

| Species                                      | Formulation | Exposure (Hours) | Concentration (mg/L) | References |
|----------------------------------------------|-------------|------------------|-----------------------|------------|
| *Rhamdia quelen*                             | GLY         | 96               | 7.30                  | [51]       |
| North African catfish (*Clarias gariepinus*) | GLY         | 96               | 0.295                 | [52]       |
| *Zebrfish (Danio rerio)*                     | Atnor 48    | 96               | 76.50                 | [53]       |
| Ten spotted live-bearer (*Cnesterodon decemmaculatus*) | Glyfoglex 3 | 96               | 41.40                 | [54]       |

1 Roundup (active substance glyphosate, 41%), 2 Atnor 48 (active substance glyphosate, 48%), 3 Glyfoglex (active substance glyphosate, 48%).

Table 2. Acute toxicity values (LC50) of glyphosate and its commercial products on amphibians.

| Species                                      | Formulation | Exposure (Hours) | Concentration (mg/L) | References |
|----------------------------------------------|-------------|------------------|-----------------------|------------|
| *Boana pardalis*                             | GLY         | 96               | 106                   | [55]       |
| *Physalaemus cuvier*                         | GLY         | 96               | 115                   |            |
| Green frog (*Lithobates clamitans*)          | Roundup 1   | 24               | 6.6                   | [56]       |
| Northern leopard frog (*Lithobates pipiens*) | Roundup 1   | 24               | 11.9                  |            |
| Wood frog (*Lithobates sylvaticus*)          | Roundup 1   | 24               | 18.1                  |            |
| Dwarf American toad (*Anaxyrus americanus*)  | Roundup 1   | 96               | <12.9                 |            |
| *Rhinella arenarum*                          | Roundup Ultra-Max 2 | 48             | 2.42                  | 77.52      | [57]       |

1 Roundup (active substance glyphosate, 41%), 2 Roundup Ultra-Max (active substance glyphosate, 36%).

Table 3. Acute toxicity values (LC50) of glyphosate and its commercial products on invertebrate species.

| Species                                      | Formulation | Exposure (Hours) | Concentration (mg/L) | References |
|----------------------------------------------|-------------|------------------|-----------------------|------------|
| Midge larvae (*Chironomus plumosus*)         | GLY         | 48               | 55                    | [41]       |
| *Ceriodaphnia dubia*                         | Roundup 1   | 48               | 147                   | [37]       |
| *Acartia tonsa*                              | Roundup 1   | 48               | 35                    |            |
| Chinese mitten crab (*Eriocheir sinensis*)   | GLY         | 24               | 461.54                | [58]       |

1 Roundup (active substance glyphosate, 41%).

2.3. Toxic Effects

2.3.1. Fish

In recent years, GLY toxicity has been studied on various kinds of aquatic organisms. The exposure to GLY may cause several changes in fish (Table 4), such as haematologic and biochemical processes in tissues [38], genotoxicity [53,59], histopathological damage, immunotoxicity [50,60], or cardiotoxicity [61].
### Table 4. Toxic effects of glyphosate and its commercial products on fish.

| Species                          | Concentration          | Exposure | Effects                                                                 | References |
|----------------------------------|------------------------|----------|-------------------------------------------------------------------------|------------|
| **Common carp**                  | 2.5, 5, 10 mg/L (GLY)  | 96 h     | ↑ ALP in liver, heart, GOT in liver and kidney, GPT in kidney; Subepithelial edema and epithelial hyperplasia in gills, focal fibrosis in liver | [47]       |
| **Roundup 1**                    | 3.5, 7, 14 mg/L (GLY)  | 16 days  | ↓ MCV, MCH; ↑ AChE in muscle, brain and liver, HB, HCT, RBC, WBC, AST, ALT, LDH | [48]       |
|                                  | 52.08, 104.15 mg/L (GLY)| 7 days   | Vacuolization of the renal parenchyma and intumescence of the renal tubule in kidney, immunotoxicity | [49]       |
| **European eel**                 | 58, 116 µg/L (Roundup 1)| 1, 3 days | ↑ TBARS, LPO, GDI, ENA | [42]       |
| **Curimbata**                    | 10 mg/L (Roundup 1)    | 24 h     | ↑ GDI, damaged nucleoids, EndoIII | [59]       |
| **Spotted snakehead**            | 32.54 mg/L (Roundup 1)| 1, 7, 14, 21, 28, 35 days | ↑ TBARS, DNA damage, LPO, ROS; ↓ CAT, SOD, GR in gill and blood | [62]       |
| **Ten spotted live-bearer**      | 1, 1.75, 35 mg/L (GLY) | 96 h     | ↓ AChE | [63]       |
| **Megaleporinus obtusidens**     | 3, 6, 10, 20 mg/L (Roundup 1)| 96 h | ↑ hepatic GL, GLU, NH₃ in liver and muscle, PCV, HB, RBC, WBC, P; ↓ AChE in brain, LACT, P in liver, muscle GL, GLU | [64]       |
|                                  | 5 mg/L (Roundup 1)     | 90 days  | ↑ LACT in liver and muscle, P in liver; ↓ AChE, GL in liver and muscle, PCV, HB, RBC, WBC | [65]       |
| **Rhamdia quelen**               | 0.2, 0.4 mg/L (Roundup 1)| 96 h | ↑ immature circulating cells; ↓ RBC, THR, WBC, phagocytic activity, agglutination activity, lysozyme activity | [67]       |
| **Rhamdia quelen**               | 0.730 mg/L (GLY)       | 24, 96 h, 10 days | ↑ TP, THR, WBC, phagocytic activity, agglutination activity, lysozyme activity | [67]       |
| **Goldfish**                     | 18, 36, 72 µg/L (GLY)  | 7 days   | ↑ TP in liver, ↑ GL in muscle; ↓ TP, GL, TL in gills, liver, and kidney | [68]       |
|                                  | 2.5–20 mg/L (Roundup 1)| 2 months | ↓ GR in kidney, liver, and brain, G6PDH in kidney, liver and brain, SOD in kidney, liver and brain | [69]       |
| **North African catfish**        | 0.2 mmol/L (Nongteshi 2)| 90 days  | Hyaline cast in kidney, ↑ CRE, BUN, ALT, AST, LDH, MDA, ↑ 3-hydroxybutyrate, LACT, alanine, acetamide, glutamate, glycine, histidine, inosine, GLU↓ SOD, GSH-Px, GR, lysisne, NAA, citrate, choline, phosphocholine, myo-inosine, nicotinamide, | [71]       |
|                                  | 0.22, 0.44, 0.88 mmol/L (GLY) | 96 h | Behaviour abnormalities (observed depression, erratic swimming, partial loss of equilibrium), liver tissue damage (cellular swelling, inflammatory cell infiltration, hydropic degeneration, loose cytoplasm, ↑ brown particles), kidney tissue damage (edema in the epithelial cells of renal tubules, ↑ cell volume, loose cytoplasm, slight staining), changes in plasma (↑ CK, UN, ↓ LDH) | [70]       |
| **Goldfish**                     | 0.2 mmol/L (Nongteshi 2)| 90 days  | Cellular infiltration in gills; fatty degeneration, fat vacuolation, diffuse hepatic necrosis, infiltration of leukocytes in liver; hematopoietic necrosis, pyknotic nuclei in kidney; mononuclear infiltration, neuronal degeneration, spongiosis in brain; respiratory stress, erratic swimming | [52]       |
Table 4. Cont.

| Species | Concentration | Exposure | Effects | References |
|---------|---------------|----------|---------|------------|
| Hybrid fish jundiara \((Leiarius marmoratus \times Psedoplatystoma reticulatum)\) | 1.357 mg/L (Roundup 1) | 6, 24, 48, 96 h | ↑ LACT in liver, P level in liver, ALT, AST, CHOL, TAG in plasma; ↓ GL in liver and muscle, plasma GLU, Hb, PCV, RBC, WBC | [28] |
| | 50 µg/mL (GLY) | 24 h | ↓ gene expression in eye, fore, and midbrain delineated brain ventricles and cephalic regions | [72] |
| Zebrafish \((Danio rerio)\) | 32.5, 65, 130 µg/L (Transorb 3) | 48 h | ↓ integrity of plasma membrane of hepatocytes, viability of cells, mitochondrial activity in the cell, lysosomal integrity, inhibition in ABC transporter activity | [73] |
| Climbing bass \((Anabas testudineus)\) | 17.20 mg/L (Excel Mera 71 4) | 30 days | ↑ AChE, LPO, CAT; ↓ TP, GST | [74] |

1 Roundup (active substance glyphosate, 41%); 2 Nongteshi (active substance glyphosate, 30%); 3 Transorb (active substance glyphosate, 48%); 4 Excel Mera 71 (active substance glyphosate, 71%).

There are just a few data about the chronic effects of glyphosate on nontarget organisms. For example, Le Du-Carrée [75] have studied chronic exposure to glyphosate with a concentration of 1 µg/L on rainbow trout for 10 months. No significant changes in reproduction, metabolism, nor even oxidative response were observed. However, some occasional impacts on immune response have occurred. Other chronic effects have been studied with different concentrations of glyphosate (0.2, 0.8, 4 and 16 mg/L) in Oreochromis niloticus for 80 days [76]. It was found that glyphosate exposure reduces antioxidative ability, disturbs liver metabolism, promote inflammation, and suppresses immunity.

2.3.2. Invertebrate Species

The exposure to GLY may cause several changes in invertebrate species (Table 5), such as biochemical processes in tissues, development, or behaviour; changes in haemolymph [77], changes in the reproduction system, and 50% inhibition of cholinesterase activity [78] in mussels.

Table 5. Toxic effects of glyphosate and its commercial products on invertebrate species.

| Species | Concentration | Exposure | Effects | References |
|---------|---------------|----------|---------|------------|
| Mediterranean mussel \((Mytilus galloprovincialis)\) | 100 µg/L (GLY) | 7, 14, 21 days | ↑ THC, haemocyte proliferation; ↓ Haemocyte diameter, AChE in gills | [77] |
| | 14 days | ↑ AChE in gills, CAT in digestive gland; ↓ CAT in gills | |
| | 21 days | ↑ CAT in gills; ↓ THC, haemocyte diameter, haemocyte volume, HL, AChE in gills | |
| | 10, 100, 1000 µg/L (GLY) | 7, 14, 21 days | ↑ cell volume of haemocyte, haemolymph pH; ↓ HL, haemolymph acid phosphatase activity; AChE in gills; SOD in digestive gland, THC; | [79] |
| Limnoperna fortunei | 1, 3, 6 mg/L (GLY) | 26 days | ↑ TBARS, GST, ALP; ↓ CES, SOD | [80] |
| | 10, 20, 40 mg/L (GLY) | 3 weeks | ↓ presence of large mussel by 40%, presence empty shell by 25% | [81] |
| Pacific oyster \((Crassostrea gigas)\) | 0.1, 1, 100 µg/L (Roundup Expres 1) | 35 days | ↑ GST; ↓ growth; LPO, MDA | [82] |
| California blackworm \((Lumbricus variegatus)\) | 0.05–5 mg/L (GLY) | 4 days | ↑ SOD; ↓ GST, membrane bound GST | [83] |
| Chinese mitten crab \((Eriocheir sinensis)\) | 4.4, 9.8, 44, 98 mg/L (GLY) | 96 h | ↑ % DNA in tail, SOD, POD, β-GD; ↓ THC, granulocytes, phagocytic activity, ACP, AKP | [58] |
Table 5. Cont.

| Species | Concentration | Exposure | Effects | References |
|---------|---------------|----------|---------|------------|
| American bullfrog (*Lithobates catesbeianus*) | 1 mg/L (Roundup 2) | 48 h | ↑ swimming activity, CPM; SOD, CAT and LPO in liver; ↓ LPO in muscle; ↓ SOD, CAT in muscle, TtHR | [84] |
| Rhinella arenarum | 1.85, 2.75, 7.5, 15, 30, 60, 120, 240 mg/L (Roundup Ultra-Max 2) | 48 h | ↓ AChE, BChE, CbE, GST | [57] |
| Northern leopard frog (*Rana pipiens*) | 0.6, 1.8 mg/L (Roundup 2) | 166 days | ↑ TRβ mRNA; Late metamorphic climax, developmental delay, abnormal gonads, necrosis of the tail tip, fin damage, abnormal growth on the tail tip, blistering on the tail fin | [56] |
| Snail (*Biomphalaria alexandrina*) | 3.15 mg/L (Roundup 2) | 6 weeks | ↑ mortality, stopped egg lying, abnormal laid eggs, ↑ GLU, LACT, FAC; ↓ egg hatchability, GL, TP, pyruvate, nucleic acids levels | [85] |
| | 10 mg/L (Roundup 2) | 7 days | ↑ in vitro phagocytic activity, DNA damage in haemocytes | [86] |

1 Roundup Expres (active substance glyphosate, 15%); 2 Roundup (active substance glyphosate, 41%); 3 Roundup Ultra-Max (active substance glyphosate, 36%).

3. AMPA (Aminomethylphosphonic Acid)

AMPA belongs to the aminomethylene phosphonates chemical group. It is the primary metabolite of the GLY degradation process (Figure 1) with a significant measured concentration in the environment. Additional sources of AMPA originate from organic phosphonates used in water treatment [87], from the degradation of phosphonic acids used in Europe in detergent and industrial boilers, and cooling (EDTMA, DTMP, ATMP, and HDTMP) [15,87]. Due to phosphonate and amine functional groups, AMPA will form metal complexes with Ca$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, and Zn$^{2+}$. AMPA is adsorbed firmly to soil [88].

3.1. Environmental Fate

AMPA has a lower water solubility and longer soil half-life than glyphosate. The presence of AMPA in freshwater, sediment, and suspended particulate is commonly measured in significant quantities [10,89], and even more frequently (67.5%) than glyphosate (17.5%) [15,90,91]. The Water Framework Directive [92] provides a procedure to set Environmental Quality Standards for AMPA at level 450 mg/L. Coupe et al. [9] reported concentrations of AMPA in freshwater environments for Mississippi and Iowa were 2.6 µg/L and 0.02–5.7 µg/L. In France, AMPA was detected with the highest concentration at a level of 44 µg/L.

A concentration of AMPA in soil was found from 299 to 2256 µg kg$^{-1}$ by Aparicio et al. [93]. This study pointed out the difficulty of establishing specific conditions for the presence of AMPA in soils. It depends on complex and multifactorial processes, including agronomic conditions, local agrometeorological conditions, mineralogy, and soil conditions. Moreover, AMPA was detected in soil with no exposure time to glyphosate. It could be caused by surface runoff. This movement of soil particles can end up in surface water, and next, the substance can be desorbed, biodegraded, and accumulated in the bottom sediment.

AMPA, like glyphosate, also degrades in water and soil but significantly slower. Because its adsorption to particulates is possibly stronger, its penetrability of cell membranes is lower. The concentration of AMPA in the sediment can fluctuate depending on its degradation rate relative to GLY (Figure 1) [95].

3.2. Acute Toxicity

AMPA toxicity has been already studied in recent years on various kinds of organisms. Although de Brito Rodrigues et al. [53] observed no acute toxic effect of AMPA on fish species, other studies showed acute toxicity values ranging from 27 to 452 mg/L (Table 6).
Table 6. Toxicity values of AMPA for aquatic organisms.

| Species                        | Value          | Concentration (mg/L) | References |
|--------------------------------|----------------|----------------------|------------|
| **Fish**                       |                |                      |            |
| Guppy (*Poecilia reticulata*)  | 96hLC50        | 180 for male         | [50]       |
|                                |                | 164.32 for female    |            |
| **Invertebrate**               |                |                      |            |
| Pacific oyster (*Crassostrea gigas*) | 36hEC10 | 38.55               |            |
|                                | 36hEC20        | 42.68                |            |
|                                | 36hEC50        | 50.78                | [94]       |
|                                | 24hEC10        | 27.08                |            |
|                                | 24hEC20        | 39.80                |            |
|                                | 24hEC50        | 76.90                |            |
| Daphnia magna                  | 48hEC10        | >100<sup>5</sup>     |            |
|                                | 48hEC20        |                      |            |
|                                | 48hEC50        |                      |            |
| **Algae**                      |                |                      |            |
| *Pseudokirchneriella subcapitata* | 72hEC10 | 85.05               | [94]       |
|                                | 72hEC20        | >100                 |            |
|                                | 72hEC50        |                      |            |
| *Desmodesmus subspicatus*      | 72hIC50        | 117.8                | [95]       |
|                                | 72hEC50        | 89.8<sup>1</sup>     | [96]       |
|                                |                | 452<sup>2</sup>      |            |

<sup>1</sup> Biomass test, <sup>2</sup> Algal growth inhibition tests.

3.3. Toxic Effects

Although AMPA has been studied less than glyphosate, Reddy et al. [97] pointed to affecting chlorophyll biosynthesis which leads to plant growth reduction. That means that AMPA can also be translocated to diverse plant tissue. AMPA is also known as a phytotoxin, which can amplify the indirect effects of glyphosate on physiological processes. On the other hand, due to its chemical similarity, AMPA can compete with glycine in biological sites and pathways, affecting chlorophyll biosynthesis and therefore the photosynthetic process as well [98]. Plants treated with AMPA showed a decreased glycine, serine, and glutamate [99]. According to published data, AMPA seems to be highly toxic on aquatic organisms (Table 7).

Table 7. Toxic effects of AMPA on aquatic organisms.

| Species                        | Concentration | Exposure | Effects                                      | References |
|--------------------------------|---------------|----------|----------------------------------------------|------------|
| European eel (*Anguilla Anguilla*) | 11.8, 23.6 µg/L | 1, 3 days | ↑ GDI, FPG, EndoIII | [100]      |
| Zebrafish (*Danio rerio*)      | 1.7, 5, 10, 23, 50, 100 mg/L | 24, 48, 72, 96 h | Genotoxicity with LOEC 1.7 mg/L, induce primary DNA lesions, | [53]       |
| Guppy (*Poecilia reticulata*)  | 82 mg/L       | 96 h     | Proliferation of the interlamellar epithelium, fusion of secondary lamellae in gill, steatosis, pyknotic nuclei in liver, degeneration of hepatocytes | [50]       |
Table 7. Cont.

| Species                        | Concentration | Exposure | Effects                                                                 | References |
|--------------------------------|---------------|----------|------------------------------------------------------------------------|------------|
| Mediterranean mussel (Mytilus galloprovincialis) | 100 µg/L      | 7 days   | ↑ haemocyte diameter, haemocyte volume, haemocyte proliferation, LDH in haemolymph, HL; ↓ THC, AChE in gills | [77]       |
| Mediterranean mussel (Mytilus galloprovincialis) | 14 days       | 100 µg/L | ↑ THC, haemocyte diameter, haemocyte volume, haemocyte proliferation, AChE in gills, CAT in digestive gland; ↓ HL |            |
| Mediterranean mussel (Mytilus galloprovincialis) | 21 days       |          | ↑ haemocyte volume, LDH in haemolymph; ↓ THC, haemocyte proliferation, HL, AChE in gills |            |
| Mediterranean mussel (Mytilus galloprovincialis) | 7 days        |          | ↓ THC                                                                  |            |
| Mediterranean mussel (Mytilus galloprovincialis) | 14 days       | 1, 10, 100 µg/L | ↑ THC, haemocyte diameter and volume, lysosome activity, acid phosphatase; ↓ haemocyte proliferation, SOD in gill and digestive gland | [101]     |
| Mediterranean mussel (Mytilus galloprovincialis) | 21 days       |          | ↑ haemocyte proliferation, lysosome activity, acid phosphatase, LDH; ↓ THC, haemocyte diameter and volume |            |
| Bufo spinosus                    | 0.07, 0.32, 3.57 µg/L | 16 days  | ↓ embryonic survival, development delay, short tail length              | [102]     |

4. Conclusions

There is a large amount of information about the benefits, environmental fate, effects, and risks of using glyphosate throughout the scientific literature. Nevertheless, evaluating chronic exposures of nontarget aquatic organisms is missing. The results of the studies that have been summarized in this review indicate that GLY, as an individual compound or as a component of commercial products used in agriculture, and its main metabolite AMPA may have adverse effects on freshwater and marine organisms at different levels of biological organization. GLY mainly caused oxidative stress, and affected antioxidant enzymes, blood parameters, and caused several histopathologic changes in the gills, liver, and kidneys, and not least genotoxicity, immunotoxicity, and cardiotoxicity in fish and oxidative stress, antioxidant enzymes, and haemocyte parameters in mussels. In comparison to AMPA, there are many gaps in the scientific literature regarding the knowledge of its toxicity on aquatic organisms. AMPA may cause genotoxicity and immunotoxicity in fish, adverse changes in haemolymph parameters, effects on mussels’ antioxidant enzymes, and developmental delay and survival of tadpoles.

There are also concerns about potential bioconcentration effects and breeding in organisms of these compounds. Considering the increasing consumption of herbicides and their repeated application worldwide, the European Commission implemented a regulation (EU 2017/2324 [103]) that GLY can be used as an active substance until 15 December 2022 on the condition that national authorities have to authorize each product. Therefore, we assume that the presence of GLY and AMPA in the aquatic environment requires a stricter control and further studies of the potentially toxic effects of these substances on nontarget organisms. As the lethal concentrations indicate glyphosate, its commercial products, and AMPA at very high levels impact long-term exposure at real environmental concentrations, more detailed information about the ecotoxicity needs to be evaluated.

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Abbreviations

ABC transporter activity: adenosine triphosphate-binding cassette transporters constitute; AChE: acetylcholinesterase; ACP: acid phosphatase; AKP/ALP: alkaline phosphatase; ALT: alanine aminotransferase; AMPA: aminomethylphosphonic acid; AST: aspartate aminotransferase; ATMP: amino tris(methyleneephosphonate); β-GD: β-glucuronidase; BChE: butyrylcholinesterase; BCF: bioaccumulation factor; BUN: blood urea nitrogen; C-N bond: carbon–nitrogen bond; Ca\(^{2+}\): calcium ion; Cacanal1C: L-type calcium channel; CAT: catalase; CB: carboxylesterase, CES: carboxylesterases; ChOL: cholesterol; CK: creatinine; CPM: cardiac pumping capacity; CRE: creatine; DNA: deoxyribonucleic acid; DTPMP: diethylenetriamine penta(methyleneephosphonate); EC10: equivalent to the No observed effect concentration; EC20: equivalent to the Low observed effect concentration; EC50: effective concentration that affects 50% of the population; EDTMA: ethylenediamine tetra(methyleneephosphonate); ENA: erytrocytic nuclear abnormalities; EndoIII: endonuclease III; FAC: free amino acid levels; FPG: formamidopyrimidine DNA glycosylase; G6PDH: glucose-6-phosphate dehydrogenase; GDI: total DNA damage; GL: glycogen; GLU: glucose; GLY: glyphosate; GOT: glutamic–oxaloacetic transaminases; GPx: glutathione peroxidase; GPT: glutamic–pyruvic transaminases; GR: glutathione reductase; GSH: glutathione; GSH-Px: glutathione peroxidase; GST: glutation-S-transferase; Hb: hemoglobin; HCT: hematocrit; HDTMP: hexamethylenediamine tetra(methyleneephosphonate); HL: haemocyte lysate; hspb11: heat shock protein; IC: inhibition concentration; IPA: isopropylamine salt; LACT: lactate; LC: lethal concentration; LDH: lactate dehydrogenase; LPO: lipid peroxidation; Na\(^{+}\)/K\(^{+}\)-ATPase: sodium–potassium adenosine triphosphatase; NOEC: no observed effect concentration; MCH: mean cell hemoglobin; MCV: mean cell volume; MDA: methanedicarboxylic aldehyde; Mg\(^{2+}\): magnesium ion; mg ae/L: miligrams active ingredient per liter; Mn\(^{2+}\): manganese ion; NAA: N-Acetyl aspartate; NH3: ammonia; NO: nitric oxide; P: protein; PC: protein carbonyl; PCV: hematocrit; POD: peroxidase; RBC: erythrocytes; ROS: reactive oxygen species; ryr2a: Ryanodine receptor; SOD: superoxide dismutase; T-AOC: total antioxidant activity; TAG: triacylglycerides; TBARS: thiobarbituric acid reactive substances; THC: total hemocyte count; THR: thrombocytes; TL: Total lipids; TP: total protein; TRβ mRNA: TRβ mRNA: Thyroid hormone receptor beta of messenger ribonucleic acid; ThHR: time to half relaxation; UN: urine nitrogen; U.S. EPA: United States Environmental Protection Agency; WBC: leukocytes; Zn\(^{+}\): zinc ion.

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