Review

Effects of Glyphosate and Its Metabolite AMPA on Aquatic Organisms

Nikola Tresnakova , Alzbeta Stara and Josef Velisek

Research Institute of Fish Culture and Hydrobiology, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, The University of South Bohemia in Ceske Budejovice, Zatisi 728/II, 389 25 Vodnany, Czech Republic; stara01@frov.jcu.cz (A.S.); velisek@frov.jcu.cz (J.V.)

* Correspondence: tresnakova@frov.jcu.cz; Tel.: +420-387-774-625

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

Citation: Tresnakova, N.; Stara, A.; Velisek, J. Effects of Glyphosate and Its Metabolite AMPA on Aquatic Organisms. *Appl. Sci.* **2021**, *11*, 9004. <https://doi.org/10.3390/app11199004>

Academic Editors: Panagiotis Berillis and Božidar Rašković

Received: 31 August 2021

Accepted: 24 September 2021

Published: 27 September 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

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.

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, postemergence 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 half-life 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 half-life 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 (<i>Oncorhynchus mykiss</i>)	GLY	96	140	[41]
	Roundup ¹	96	52–55	[46]
Common carp (<i>Cyprinus carpio</i>)	GLY	48	645	[47]
		96	620	
	Roundup ¹	96	22.19	[48]
		96	22.19	
	GLY	48	602.61	[49]
		96	520.77	
Blackhead minnow (<i>Pimephales promelas</i>)	GLY	96	97	[41]
Channel catfish (<i>Ictalurus punctatus</i>)		96	130	
Bluegills (<i>Lepomis macrochirus</i>)	GLY	24	150	[41]
		96	140	
Guppy (<i>Poecilia reticulata</i>)	GLY	96	69.83	[50]

Table 1. Cont.

Species	Formulation	Exposure (Hours)	Concentration (mg/L)	References
<i>Rhamdia quelen</i>	GLY	96	7.30	[51]
North African catfish (<i>Clarias gariepinus</i>)	GLY	96	0.295	[52]
Zebrafish (<i>Danio rerio</i>)	Atnor 48 ²	96	76.50	[53]
Ten spotted live-bearer (<i>Cnesterodon decemmaculatus</i>)	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
<i>Boana pardalis</i>	GLY	96	106	[55]
<i>Physalaemus cuvieri</i>		96	115	
Green frog (<i>Lithobates clamitans</i>)	Roundup ¹	24	6.6	[56]
		96	6.5	
Northern leopard frog (<i>Lithobates pipiens</i>)		24	11.9	
		96	9.2	
Wood frog (<i>Lithobates sylvaticus</i>)		24	18.1	
		96	16.5	
Dwarf American toad (<i>Anaxyrus americanus</i>)		24	13.5	
		96	<12.9	
<i>Rhinella arenarum</i>	Roundup Ultra-Max ²	48	2.42	[57]
			77.52	

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 (<i>Chironomus plumosus</i>)	GLY	48	55	[41]
<i>Ceriodaphnia dubia</i>	Roundup ¹	48	147	[37]
<i>Acartia tonsa</i>		48	35	
Chinese mitten crab (<i>Eriocheir sinensis</i>)	GLY	24	461.54	[58]
		96	97.89	

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 (<i>Cyprinus carpio</i>)	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]
	3.5, 7, 14 mg/L (Roundup ¹)	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]
			↑ AST, ALT, MDA, PC; ↓ GSH, inhibition of Na ⁺ /K ⁺ -ATPase, SOD, CAT, GPx, GR, T-AOC, induce inflammatory response in gills	[60]
European eel (<i>Anguilla Anguilla</i>)	58, 116 µg/L (Roundup ¹)	1, 3 days	↑ TBARS, LPO, GDI, ENA	[42]
			↑ GDI, damaged nucleoids, EndoIII	[59]
Curimbata (<i>Prochilodus lineatus</i>)	10 mg/L (Roundup ¹)	24 h	↑ GSH, GST, LPO; ↓ SOD, GPx, inhibition of AChE in muscle	[38]
		96 h	↑ GST, LPO; inhibition of AChE in muscle in brain and muscle	
Spotted snakehead (<i>Channa punctatus</i>)	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 (<i>Cnesterodon decemmaculatus</i>)	1, 1.75, 35 mg/L (GLY)	96 h	↓ AChE	[63]
<i>Megaleporinus obtusidens</i>	3, 6, 10, 20 mg/L (Roundup ¹)	96 h	↑ hepatic GL, GLU, NH ₃ 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, P in muscle, PCV, Hb, RBC, WBC	[65]
<i>Rhamdia quelen</i>	0.2, 0.4 mg/L (Roundup ¹)	96 h	↑ hepatic GL, LACT in liver and muscle, P in liver and muscle, NH ₃ in liver and muscle, TBARS in muscle; ↓ muscle GL, GLU in liver and muscle, AChE in brain	[66]
	0.730 mg/L (GLY)	24, 96 h, 10 days	↑ immature circulating cells; ↓ RBC, THR, WBC, phagocytic activity, agglutination activity, lysozyme activity	[67]
<i>Rhamdia quelen</i>	18, 36, 72 µg/L (Roundup ¹)	7 days	↑ TP in liver, ↑ GL in muscle; ↓ TP, GL, TL in gills, liver, and kidney	[68]
Goldfish (<i>Carassius auratus</i>)	2.5–20 mg/L (Roundup ¹)	2 months	↑ CAT in liver and kidney; ↓ GR in kidney, liver, and brain, G6PDH in kidney, liver and brain, SOD in kidney, liver and brain	[69]
	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]
	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, lysine, NAA, citrate, choline, phosphocholine, myo-inosine, nicotinamide,	[71]
	0, 19, 42, 94, 207, 455 mg/L (GLY)	96 h	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 (<i>Leiarius marmoratus</i> × <i>Pseudoplatystoma reticulatum</i>)	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]
Zebrafish (<i>Danio rerio</i>)	50 µg/mL (GLY)	24 h	↓ gene expression in eye, fore, and midbrain delineated brain ventricles and cephalic regions	[72]
	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]
	10, 50, 100, 200, 400 µg/L (GLY)	48 h	↓ heartbeat, NO generation, downregulation of Cacana1C and ryr2a genes, upregulation of hspb11	[61]
Climbing bass (<i>Anabas testudineus</i>) <i>Heteropneustes fossilis</i>	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 (<i>Mytilus galloprovincialis</i>)	100 µg/L (GLY)	7 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]
<i>Limnoperna fortunei</i>	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 (<i>Crassostrea gigas</i>)	0.1, 1, 100 µg/L (Roundup Expres ¹)	35 days	↑ GST; ↓ growth; LPO, MDA	[82]
California blackworm (<i>Lumbriculus variegatus</i>)	0.05–5 mg/L (GLY)	4 days	↑ SOD; ↓ GST, membrane bound GST	[83]
Chinese mitten crab (<i>Eriocheir sinensis</i>)	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 (<i>Lithobates catesbeianus</i>)	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]
<i>Rhinella arenarum</i>	1.85, 3.75, 7.5, 15, 30, 60, 120, 240 mg/L (Roundup Ultra-Max ³)	48 h	↓ AChE, BChE, CbE, GST	[57]
Northern leopard frog (<i>Rana pipiens</i>)	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(<i>Biomphalaria alexandrina</i>)	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 aminomethylenephosphonates 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 Ca²⁺, Mg²⁺, Mn²⁺, and Zn²⁺. 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) [93].

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 (<i>Poecilia reticulata</i>)	96hLC50	<div>180 for male</div> <div>164.32 for female</div>	[50]
Invertebrate			
Pacific oyster (<i>Crassostrea gigas</i>)	36hEC10	38.55	[94]
	36hEC20	42.68	
	36hEC50	50.78	
	24hEC10	27.08	
	24hEC20	39.80	
	24hEC50	76.90	
<i>Daphnia magna</i>	48hEC10	>100 ⁵	
	48hEC20		
	48hEC50		
Algae			
<i>Pseudokirchneriella subcapitata</i>	72hEC10	85.05	[94]
	72hEC20	>100	
	72hEC50		
<i>Desmodesmus subspicatus</i>	72hIC50	117.8	[95]
	72hEC50	89.8 ¹	[96]
452 ²			

¹ biomass test, ² 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 (<i>Anguilla Anguilla</i>)	11.8, 23.6 µg/L	1, 3 days	↑ GDI, FPG, EndoIII	[100]
Zebrafish (<i>Danio rerio</i>)	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 (<i>Poecilia reticulata</i>)	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 (<i>Mytilus galloprovincialis</i>)	100 µg/L	7 days	↑ haemocyte diameter, haemocyte volume, haemocyte proliferation, LDH in haemolymph, HL; ↓ THC, AChE in gills	[77]
Mediterranean mussel (<i>Mytilus galloprovincialis</i>)	100 µg/L	14 days	↑ THC, haemocyte diameter, haemocyte volume, haemocyte proliferation, AChE in gills, CAT in digestive gland; ↓ HL	[101]
		21 days	↑ haemocyte volume, LDH in haemolymph; ↓ THC, haemocyte proliferation, HL, AChE in gills	
	1, 10, 100 µg/L	7 days	↓ THC	
		14 days	↑ THC, haemocyte diameter and volume, lysosome activity, acid phosphatase; ↓ haemocyte proliferation, SOD in gill and digestive gland	
		21 days	↑ haemocyte proliferation, lysosome activity, acid phosphatase, LDH; ↓ THC, haemocyte diameter and volume	
<i>Bufo spinosus</i>	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.

Author Contributions: Conceptualization, data curation, writing—original draft preparation, N.T.; writing—review and editing A.S.; supervision J.V. All authors have read and agreed to the published version of the manuscript.

Funding: The research was funded by the Ministry of Agriculture of the Czech Republic—project No. QK1910282.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not report data.

Conflicts of Interest: The authors declare no conflict of interest regarding the publication of this review paper.

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(methylenephosphonate); β -GD: β - glucuronidase; BChE: butyrylcholinesterase; BCF: bioaccumulation factor; BUN: blood urea nitrogen; C-N bond: carbon–nitrogen bond; Ca^{2+} : calcium ion; Cacan1C: L-type calcium channel; CAT: catalase; CbE: carboxylesterase, CES: carboxylesterases; ChOL: cholesterol; CK: creatinine; CPM: cardiac pumping capacity; CRE: creatine; DNA: deoxyribonucleic acid; DTPMP: diethylenetriamine penta(methylenephosphonate); 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(methylenephosphonate); ENA: erythrocytic 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: glutathione-S-transferase; Hb: hemoglobin; HCT: hematocrit; HDTMP: hexamethylenediamine tetra(methylenephosphonate); 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: milligrams active ingredient per liter; Mn^{2+} : manganese ion; NAA: N-Acetyl aspartate; NH_3 : 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; $\text{TR}\beta$ mRNA: $\text{TR}\beta$ mRNA: Thyroid hormone receptor beta of messenger ribonucleic acid; TtHR: time to half relaxation; UN: urine nitrogen; U.S. EPA: United States Environmental Protection Agency; WBC: leukocytes; Zn^{2+} : zinc ion.

References

1. Arias-Estévez, M.; López-Periago, E.; Martínez-Carballo, E.; Simal-Gándara, J.; Mejuto, J.-C.; García-Río, L. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agric. Ecosyst. Environ.* **2008**, *4*, 247–260. [[CrossRef](#)]
2. Bilal, M.; Iqbal, H.M.N.; Barceló, D. Persistence of pesticides-based contaminants in the environment and their effective degradation using laccase-assisted biocatalytic systems. *Sci. Total Environ.* **2019**, *695*, 133896. [[CrossRef](#)]
3. Riahi, B.; Rafatpanah, H.; Mahmoudi, M.; Memar, B.; Brook, A.; Tabasi, N.; Karimia, G. Immunotoxicity of paraquat after subacute exposure to mice. *Food Chem. Toxicol.* **2010**, *48*, 1627–1631. [[CrossRef](#)]
4. Fenner, K.; Canonica, S.; Wackett, L.P.; Elsner, M. Evaluating Pesticide Degradation in the Environment: Blind Spots and Emerging Opportunities. *Science* **2013**, *341*, 752–758. [[CrossRef](#)]
5. Gill, J.P.K.; Sethi, N.; Mohan, A.; Datta, S.; Girdhar, M. Glyphosate toxicity for animals. *Environ. Chem. Lett.* **2018**, *16*, 401–426. [[CrossRef](#)]

6. Grube, A.; Donaldson, D.; Kiely, T.; Wu, L. *Pesticides Industry Sales and Usage, 2006 and 2007 Market Estimates*; U.S. Environmental Protection Agency: Washington, DC, USA, 2011; p. 41.
7. Franz, J. *N-Phosphonomethyl-Glycine Phytotoxicant Compositions*; Monsanto CO, US: St. Louis, MO, USA, 1974; p. 3799758. Available online: <https://www.freepatentsonline.com/3799758.html> (accessed on 12 February 2021).
8. Henderson, A.M.; Gervais, J.A.; Luukinen, B.; Buhl, K.; Stone, D.; Strid, A.; Cross, A.; Jenkins, J. Glyphosate Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services. 2010. Available online: <http://npic.orst.edu/factsheets/archive/glyphotech.html> (accessed on 10 February 2021).
9. Coupe, R.H.; Kalkhoff, S.J.; Capel, P.D.; Gregoire, C. Fate and transport of glyphosate and Aminomethylphosphonic acid in surface waters of agricultural basins. *Pest. Manag. Sci.* **2012**, *68*, 16–30. [\[CrossRef\]](#)
10. Battaglin, W.A.; Myer, M.T.; Kuivila, K.M.; Dietze, J.E. Glyphosate and its gedradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. *J. Am. Water Resour. Assoc.* **2014**, *50*, 275–290. [\[CrossRef\]](#)
11. Myers, J.P.; Antoniou, M.N.; Blumberg, B.; Carroll, L.; Colborn, T.; Everett, L.G.; Hansen, M.; Landrigan, P.J.; Lanphear, B.P.; Mesnage, R.; et al. Concerns over use of glyphosate-based herbicides and risks associated with exposures: A consensus statement. *Environ. Health* **2016**, *15*, 19. [\[CrossRef\]](#)
12. Guyton, K.Z.; Loomis, D.; Grosse, Y.; El Ghissassi, F.; Benbrahim-Tallaa, L.; Guha, N.; Scoccianti, C.; Mattock, H.; Straif, K. International Agency for Research on Cancer Monograph Working Group, IARC, Lyon, France. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *Lancet Oncol.* **2015**, *5*, 490–491. [\[CrossRef\]](#)
13. IARC (International Agency for Research on Cancer). IARC Rejecets False Claims in Reuters Article (“In Glyphosate Review, WHO Cancer Agency Edited Out “Non-Carcinogenic” Findings”). 2017. Available online: https://www.iarc.who.int/wp-content/uploads/2018/07/IARC_Response_Reuters_October2017.pdf (accessed on 14 February 2021).
14. IARC (International Agency for Research on Cancer). IRAC Response to Critisms of the Monographs and the Glyphosate Evaluation. 2018. Available online: https://www.iarc.who.int/wp-content/uploads/2018/07/IARC_response_to_criticisms_of_the_Monographs_and_the_glyphosate_evaluation.pdf (accessed on 14 February 2021).
15. U.S. EPA (United States Environmental Protection Agency). *Preliminary Ecological Risk Assessment in Support of the Registration Review of Glyphosate and Its Salts*; U.S. EPA: Washington, DC, USA, 2015; p. 318.
16. Zhan, H.; Feng, Y.; Fan, X.; Chen, S. Recent advances in glyphosate biodegradation. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 5033–5043. [\[CrossRef\]](#)
17. Sun, M.; Li, H.; Jaisi, D.P. Degradation of glyphosate and bioavailability of phosphorus derived from glyphosate in a soil-water system. *Water Res.* **2019**, *163*, 114840. [\[CrossRef\]](#)
18. Wang, S.; Seiwert, B.; Kästner, M.; Miltner, A.; Schäffer, A.; Reemtsma, T.; Yang, Q.; Nowak, K.M. (Bio)degradation of glyphosate in water-sediment microcosms—A stable isotope co-labeling approach. *Water Res.* **2016**, *99*, 91–100. [\[CrossRef\]](#)
19. Al-Mamun, A. Pesticide Degradations Resides and Environmental Concerns. In *Pesticide Residue in Foods: Sources, Management, and Control*; Khan, M.S., Rahman, M.S., Eds.; Springer International Publishing AG: Cham, Switzerland, 2017; pp. 87–102. [\[CrossRef\]](#)
20. Larson, S.J.; Capel, P.D.; Majewski, M.S. Pesticide in surface waters—Distribution, trends, and governing factors. In *Series of Pesticides in Hydrologic System*; Gilliom, R.J., Ed.; Ann Arbor Press: Chelsea, MI, USA, 1997; Volume 3, p. 392.
21. Commission Implementing Regulation (EU) no 1372/2011. Concerning the Non-Approval of the Active Substance Acetochlor, in Accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council Concerning the Placing of Plant Protection Products on the Market, and Amending Commission Decision 2008/934/EC. OJEU, L 341/45. Available online: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R1372> (accessed on 25 November 2020).
22. Moulisová, A.; Bendakovská, L.; Kožíšek, F.; Vavrouš, A.; Jelígová, H.; Kotal, F. Pesticidy a jejich metabolismy v pitné vodě. Jaký je současný stav v České Republice? *Vodní Hospodářství* **2017**, *68*, 4–10.
23. CHMI (Czech Hydrometeorological Institute). On-Line Water Quality Database. 2020. Available online: <http://hydro.chmi.cz/> (accessed on 10 December 2020).
24. Kolpin, D.W.; Thurman, E.M.; Linhart, S.M. The environmental occurrence of herbicides: The importance of degradates in ground water. *Arch. Environ. Contam. Toxicol.* **1998**, *35*, 385–390. [\[CrossRef\]](#)
25. Schwarzenbach, R.P.; Escher, B.I.; Fenner, K.; Hofstetter, T.B.; Johnson, C.A.; Von Gunten, U.; Wehrli, B. The challenge of micropollutants in aquatic systems. *Science* **2006**, *313*, 1072–1077. [\[CrossRef\]](#)
26. Ceyhun, S.B.; Şentürk, M.; Ekin, D.; Erdoğan, O.; Çiltaş, A.; Kocaman, E.M. Deltamethrin attenuates antioxidant defense system and induces the expression of heat shock protein 70 in rainbow trout. *Comp. Biochem. Physiol. C* **2010**, *152*, 215–222. [\[CrossRef\]](#)
27. Abrantes, N.; Pereira, R.; Gonçalves, F. Occurrence of pesticides in water, sediments, and fish tissues in a Lake Surrounded by agricultural lands: Concerning risks to humans and ecological receptors. *Water Air Pollut.* **2010**, *212*, 77–88. [\[CrossRef\]](#)
28. De Moura, F.R.; da Silva Lima, R.R.; da Cunha, A.P.S.; da Costa Marisco, P.; Aguiar, D.H.; Sugui, M.M.; Sinhorin, A.P.; Sinhorin, V.D.G. Effects of glyphosate-based herbicide on pintado da Amazônia: Hematology, histological aspects, metabolic parameters and genotoxic potential. *Environ. Toxicol. Pharmacol.* **2017**, *56*, 241–248. [\[CrossRef\]](#)
29. Geist, J. Integrative freshwater ecology and biodiversity conservation. *Ecol. Indic.* **2011**, *11*, 1507–1516. [\[CrossRef\]](#)
30. Malaj, E.; Peter, C.; Grote, M.; Kühne, R.; Mondy, C.P.; Usseglio-Polatera, P.; Schäfer, R.B. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Nat. Acad. Sci. USA* **2014**, *111*, 9549–9554. [\[CrossRef\]](#)

31. Qui, Y.W.; Zeng, E.Y.; Qiu, H.; Yu, K.; Cai, S. Bioconcentration of polybrominated diphenyl ethers and organochlorine pesticides in algae is an important contaminant route to higher trophic levels. *Sci. Total. Environ.* **2017**, *579*, 1885–1893. [CrossRef]
32. Eddleston, M. Poisoning by pesticides. *Medicine* **2020**, *48*, 214–217. [CrossRef]
33. Ippolito, A.; Kattwinkel, M.; Rasmussen, J.J.; Schäfer, R.B.; Fornaroli, R.; Liess, M. Modeling global distribution of agricultural insecticides in surface waters. *Environ. Pollut.* **2015**, *198*, 54–60. [CrossRef]
34. WHO (World Health Organization). Glyphosate/Published under the Joint Sponsorship of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. World Health Organization & International Programme on Chemical Safety. 1994. Available online: <https://apps.who.int/iris/handle/10665/40044> (accessed on 3 December 2020).
35. Franz, J.E.; Mao, M.K.; Sikorski, J.A. *Glyphosate: A Unique Global Herbicide*; American Chemical Society: Washington, DC, USA, 1997; p. 615.
36. Wallace, J.; Lingenfelter, D. Glyphosate (Roundup): Understanding Risks to Human Health. PennState Extension: College of Agricultural Sciences. 1–3. 2019. Available online: <https://extension.psu.edu/glyphosate-roundup-understanding-risks-to-human-health#:~:text=Glyphosate%20fate%20in%20environment.%20The%20chemical%20properties%20of,water.%20Glyphosate%20does%20not%20degrade%20quickly%20in%20plants> (accessed on 10 July 2020).
37. Tsui, M.T.K.; Chu, L.M. Aquatic toxicity of glyphosate-based formulations: Comparison between different organisms and the effects of environmental factors. *Chemosphere* **2003**, *52*, 1189–1197. [CrossRef]
38. Modesto, K.A.; Martinez, C.B.R. Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. *Chemosphere* **2010**, *78*, 294–299. [CrossRef] [PubMed]
39. Roundup.cz. RoundupBioaktiv: Okolo Vodních Toků a Nádrží, Fakta. Monsanto ČR s.r.o. Available online: <https://www.roundup.cz/roundup-biaktiv/fakta> (accessed on 12 December 2020).
40. Blake, R.J.; Pallet, K. The environmental fate and ecotoxicity of glyphosate. *Outlooks Pest. Manag.* **2018**, *29*, 266–269. [CrossRef]
41. Folmar, L.C.; Sanders, H.O.; Julin, A.M. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. *Arch. Environ. Contam. Toxicol.* **1979**, *8*, 269–278. [CrossRef] [PubMed]
42. Guilherme, S.; Gaivao, I.; Santos, M.A.; Pacheco, M. European eel (*Anguilla Anguilla*) genotoxic and prooxidant responses following short-term exposure to Roundup®—A glyphosate-based herbicide. *Mutagenesis* **2010**, *25*, 523–530. [CrossRef]
43. Wagner, N.; Reichenbecher, W.; Teichmann, H.; Tappeser, B.; Lotters, S. Questions concerning the potential impact of glyphosate-base herbicides on amphibians. *Environ. Toxicol. Chem.* **2013**, *32*, 1688–1700. [CrossRef]
44. Rodrigues, N.R.; de Souza, A.P.F. Occurrence of glyphosate and AMPA residue in soy-based infant formula sold in Brazil. *Food Addit. Contam. A* **2018**, *35*, 724–731. [CrossRef]
45. Matozzo, V.; Fabrello, J.; Marin, M.G. The effects of glyphosate and its commercial formulations to marine invertebrates: A review. *J. Mar. Sci. Eng.* **2020**, *8*, 399. [CrossRef]
46. Hildebrand, L.D.; Sullivan, D.S.; Sullivan, T.P. Experimental studies of rainbow trout populations exposed to field applications of Roundup®herbicide. *Arch. Environ. Contam. Toxicol.* **1982**, *11*, 93–98. [CrossRef]
47. Neskovic, N.K.; Poleksic, V.; Elezovic, I.; Karan, V.; Budimir, M. Biochemical and histopathological effects of glyphosate on carp, *Cyprinus carpio* L. *Bull. Environ. Contam. Toxicol.* **1996**, *56*, 295–302. [CrossRef] [PubMed]
48. Gholami-Seyedkolaei, S.; Mirvaghefi, A.; Farahmand, H.; Kosari, A.A. Effect of a glyphosate-based herbicide in *Cyprinus carpio*: Assessment of acetylcholinesterase activity, hematological responses and serum biochemical parameters. *Ecotoxicol. Environ. Saf.* **2013**, *98*, 135–141. [CrossRef] [PubMed]
49. Ma, J.; Bu, Y.; Li, X. Immunological and histopathological responses of the kidney of common carp (*Cyprinus carpio* L.) sublethally exposed to glyphosate. *Environ. Toxicol. Pharmacol.* **2015**, *39*, 1–8. [CrossRef] [PubMed]
50. Antunes, A.M.; Rocha, T.L.; Pires, F.S.; de Freitas, M.A.; Leite, V.R.M.C.; Arana, S.; Moreira, P.C.; Sabóia-Morais, S.M.T. Gender-specific histopathological response in guppies *Poecilia reticulata* exposed to glyphosate or its metabolite Aminomethylphosphonic acid. *J. Appl. Toxicol.* **2017**, *37*, 1098–1107. [CrossRef] [PubMed]
51. Kreutz, L.C.; Barcellos, L.J.G.; Silva, T.O.; Anziliero, D.; Martins, D.; Lorensen, M.; Martheninghe, A.; da Silva, L.B. Acute toxicity test of agricultural pesticides on silver catfish (*Rhamdia quelen*). *Cienc. Rural* **2008**, *38*, 1050–1055. [CrossRef]
52. Ayoola, S.O. Histopathological effects of glyphosate on juvenile African catfish (*Clarius gariepinus*). *Am-Euras. J. Agric. Environ. Sci.* **2008**, *4*, 362–367.
53. de Brito Rodrigues, L.; Costa, G.G.; Thá, E.L.; da Silva, L.R.; de Oliveira, R.; Leme, D.M.; Cestari, M.M.; Grisolia, C.K.; Valadares, M.C.; de Oliveira, G.A.R. Impact of the glyphosate-based commercial herbicide, its components and its metabolite AMPA on non-target aquatic organisms. *Mut. Res.-Genet. Toxicol. Environ. Mut.* **2019**, *842*, 94–101. [CrossRef]
54. Brodeur, J.C.; Malpel, S.; Anglesio, A.B.; Cristos, D.; D’Andrea, M.F.; Poliserpi, M.B. Toxicities of glyphosate- and cypermethrin-based pesticides are antagonistic in the tenspotted livebearer fish (*Cnesterodon decemmaculatus*). *Chemosphere* **2016**, *155*, 429–435. [CrossRef]
55. Daam, M.A.; Moutinho, M.F.; Espindola, E.L.G.; Schiesari, L. Lethal toxicity of the herbicides acetochlor, ametryn, glyphosate and metribuzin to tropical frog larvae. *Ecology* **2019**, *28*, 707–712. [CrossRef] [PubMed]
56. Howe, C.M.; Berrill, M.; Pauli, B.D.; Helbing, C.C.; Werry, K.; Veldhoen, N. Toxicity of Glyphosate-based pesticides to four north American frog species. *Environ. Toxicol. Chem.* **2004**, *23*, 1928–1938. [CrossRef] [PubMed]

57. Lajmanovich, R.C.; Attademo, A.M.; Peltzer, P.M.; Junges, C.M.; Cabagna, M.C. Toxicity of four herbicide formulations with glyphosate on *Rhinella arenarum* (Anura: Bufonidae) tadpoles: B-esterases and glutathion S-transferase inhibitors. *Arch. Environ. Contam. Toxicol.* **2011**, *60*, 681–689. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Hong, Y.; Yang, X.; Yan, G.; Huang, Y.; Zuo, F.; Shen, Y.; Ding, Y.; Cheng, Y. Effects of glyphosate on immune responses and haemocyte DNA damage of Chinese mitten crab, *Eriocheir sinensis*. *Fish Shellfish Immunol.* **2017**, *71*, 19–27. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Guilherme, S.; Santos, M.; Barroso, C.; Gaivão, I.; Mário, P. Differential genotoxicity of Roundup® formulation and its constituents in blood cells of fish (*Anguilla anguilla*): Considerations on chemical interactions and DNA damaging mechanisms. *Ecotoxicology* **2012**, *21*, 1381–1390. [\[CrossRef\]](#)
60. Ma, J.; Zhu, J.; Wang, W.; Ruan, P.; Rajeshkumar, S.; Li, X. Biochemical and molecular impacts of glyphosate-based herbicide on the gills of common carp. *Environ. Pollut.* **2019**, *252*, 1288–1300. [\[CrossRef\]](#)
61. Gaur, H.; Bhargava, A. Glyphosate induces toxicity and modulates calcium and NO signaling in zebrafish embryos. *Biochem. Biophys. Res. Comm.* **2019**, *513*, 1070–1075. [\[CrossRef\]](#)
62. Nwani, C.D.; Nagpure, N.S.; Kumar, R.; Kushwaha, B.; Lakra, W.S. DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, *Channa punctatus*. *Environ. Toxicol. Pharmacol.* **2013**, *36*, 539–547. [\[CrossRef\]](#)
63. Menéndez-Helman, R.; Ferreyroa, G.V.; dos Santos Afonso, M.; Salibán, A. Glyphosate as an Acetylcholinesterase inhibitor in *Cnesterodon decemmaculatus*. *Bull. Environ. Contam. Toxicol.* **2012**, *88*, 6–9. [\[CrossRef\]](#)
64. Gluszcak, L.; dos Santos Miron, D.; Crestani, M.; da Fonseca, M.B.; de Araújo Pedron, F.; Duarte, M.F.; Pimentel Vieira, V.L. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens*). *Ecotoxicol. Environ. Saf.* **2006**, *65*, 237–241. [\[CrossRef\]](#)
65. Salbego, J.; Pretto, A.; Gioda, C.R.; de Menezes, C.C.; Lazzari, R.; Neto, J.R.; Baldisserotto, B.; Loro, V.L. Herbicide formulation with glyphosate affects growth, acetylcholinesterase activity, and metabolic and hematological parameters in Piava (*Leporinus obtusidens*). *Arch. Environ. Contam. Toxicol.* **2010**, *58*, 740–745. [\[CrossRef\]](#)
66. Gluszcak, L.; dos Santos Miron, D.; Moares, B.S.; Simoes, R.R.; Chitolina Schetinger, M.R.; Morsch, V.M.; Loro, V.L. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish. *Comp. Biochem. Physiol. C* **2007**, *146*, 519–524. [\[CrossRef\]](#)
67. Kreutz, L.C.; Barcellos, L.J.G.; de Faria Valle, S.; de Oliveira Silva, T.; Anziliero, D.; dos Santos, E.D.; Pivato, M.; Zanatta, R. Altered hematological and immunological parameters in silver catfish fish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate. *Fish Shellfish Immunol.* **2011**, *30*, 51–57. [\[CrossRef\]](#)
68. Persch, T.S.P.; Weimer, R.N.; Freitas, B.S.; Oliveira, G.T. Metabolic parameters and oxidative balance in juvenile (*Rhamdia quelen*) exposed to rice paddy herbicides: Roundup®, Primoleo®, and Facet®. *Chemosphere* **2017**, *174*, 98–109. [\[CrossRef\]](#)
69. Lushchak, O.V.; Kubrak, O.I.; Storey, J.M.; Storey, K.B.; Lushchak, V.I. Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. *Chemosphere* **2009**, *76*, 932–937. [\[CrossRef\]](#) [\[PubMed\]](#)
70. Li, M.H.; Xu, L.D.; Liu, Y.; Chen, T.; Jiang, L.; Fu, Y.H.; Wang, J.S. Multi-tissue metabolic responses of goldfish (*Carassius auratus*) exposed to glyphosate-based herbicide. *Toxicol. Res.* **2016**, *5*, 1039–1052. [\[CrossRef\]](#)
71. Li, M.H.; Ruan, L.Y.; Zhou, J.W.; Fu, Y.H.; Jinag, L.; Zhao, H.; Wang, J.S. Metabolic profiling of goldfish (*Carassius auratus*) after long-term glyphosate-base herbicide exposure. *Aquatic. Toxicol.* **2017**, *188*, 159–169. [\[CrossRef\]](#) [\[PubMed\]](#)
72. Roy, N.M.; Carneiro, B.; Ochs, J. Glyphosate induces neurotoxicity in zebrafish. *Environ. Toxicol. Pharmacol.* **2016**, *42*, 45–54. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Goulart, T.L.S.; Boyle, R.T.; Souza, M.M. Cytotoxicity of the association of pesticides Roundup Transorb® and Furadan 350 SC® on the zebrafish cell line, ZF-L. *Toxicol. Vitro.* **2015**, *29*, 1377–1384. [\[CrossRef\]](#)
74. Samanta, P.; Pal, S.; Mukherjee, A.K.; Gosh, A.R. Biochemical effects of glyphosate based herbicide, Excel Mera 71 on enzyme activities of acetylcholinesterase (AChE), lipid peroxidation (LPO), catalase (CAT), glutathion-S-transferase (GST) and protein content on teleostean fishes. *Ecotoxicol. Environ. Saf.* **2014**, *107*, 120–125. [\[CrossRef\]](#)
75. Le Du-Carrée, J.; Morin, T.; Danion, M. Impact of chronic exposure of rainbow trout, *Oncorhynchus mykiss*, to low doses of glyphosate or glyphosate-based herbicides. *Aquat. Toxicol.* **2021**, *230*, 105687. [\[CrossRef\]](#) [\[PubMed\]](#)
76. Zheng, T.; Jia, R.; Cao, L.; Du, J.; Gu, Z.; He, Q.; Xu, P.; Yin, G. Effects of chronic glyphosate exposure on antioxidative status, metabolism and immune response in tilapia (GIFT, *Oreochromis niloticus*). *Com. Biochem. Physiol. C* **2021**, *239*, 108878. [\[CrossRef\]](#) [\[PubMed\]](#)
77. Matozzo, V.; Munari, M.; Maseiro, L.; Finos, L.; Marin, M.G. Ecotoxicological hazard of a mixture of glyphosate and aminomethylphosphonic acid to the mussel *Mytilus galloprovincialis* (Lamarck 1819). *Sci. Rep.* **2019**, *9*, 14302. [\[CrossRef\]](#)
78. Sandrini, J.Z.; Rola, R.C.; Lopes, F.M.; Buffon, H.F.; Freitas, M.M.; Martins, C.M.; da Rosa, C.E. Effects of glyphosate on cholinesterase activity of the mussel *Perna perna* and the fish *Danio rerio* and *Jenynsia multidentata*: In vitro studies. *Aquat. Toxicol.* **2013**, *130–131*, 171–173. [\[CrossRef\]](#) [\[PubMed\]](#)
79. Matozzo, V.; Fabrello, J.; Masiero, L.; Ferraccioli, F.; Finos, L.; Pastore, P.; Di Gangi, I.M.; Bogianni, S. Ecotoxicological risk assessment for the herbicide glyphosate to non-target aquatic species: A case study with the mussel *Mytilus galloprovincialis*. *Environ. Pollut.* **2018**, *233*, 623–632. [\[CrossRef\]](#)
80. Iummato, M.M.; Di Fiori, E.; Sabatini, S.E.; Cacciatori, L.C.; Cochón, A.C.; del Carmen Ríos de Molina, M.; Juárez, Á.B. Evaluation of biochemical markers in the golden mussel *Limnoperna fortune* exposed to glyphosate acid in outdoor microcosms. *Ecotoxicol. Environ. Saf.* **2013**, *95*, 123–129. [\[CrossRef\]](#) [\[PubMed\]](#)

81. Di Fiori, E.; Pizarro, H.; dos Santos Afonso, M.; Cataldo, D. Impact of the invasive mussels *Limnoperna fortunei* on glyphosate concentration in water. *Ecotoxicol. Environ. Saf.* **2012**, *81*, 106–113. [\[CrossRef\]](#)
82. Séguin, A.; Mottier, A.; Perron, C.; Lebel, J.M.; Serpentine, A.; Costil, K. Sub-lethal effects of glyphosate-based commercial formulation and adjuvants on juvenile oysters (*Crassostrea gigas*) exposed for 35 days. *Mar. Pollut. Bull.* **2017**, *117*, 348–358. [\[CrossRef\]](#) [\[PubMed\]](#)
83. Contardo-Jara, V.; Klingelmann, E.; Wiegand, C. Bioaccumulation of glyphosate and its formulations Roundup Ultra in *Lumbricus variegatus* and its effects on biotransformation and antioxidant enzymes. *Environ. Pollut.* **2009**, *157*, 57–63. [\[CrossRef\]](#)
84. Costa, M.J.; Monteiro, D.A.; Oliveira-Neto, A.L.; Rantin, T.F.; Kalinin, A.L. Oxidative stress biomarkers and heart function in bullfrog tadpoles exposed to Roundup Original. *Ecotoxicology* **2008**, *17*, 153–163. [\[CrossRef\]](#)
85. Barky, F.A.; Abdelsam, H.A.; Mahmoud, M.B.; Hamdi, S.A.H. Influence of Atrazine and Roundup pesticides on biochemical and molecular aspects of *Biomphalaria alexandrina* snails. *Pest. Biochem. Physiol.* **2012**, *104*, 9–18. [\[CrossRef\]](#)
86. Mohamed, A.H. Sublethal toxicity of Roundup to immunological and molecular aspects of *Biomphalaria alexandrina* to *Schistosoma mansoni* infection. *Ecotoxicol. Environ. Saf.* **2011**, *74*, 754–760. [\[CrossRef\]](#)
87. Levine, S.L.; von Mérey, G.; Minderhout, T.; Manson, P.; Sutton, P. Aminomethylphosphonic acid has low chronic toxicity to *Daphnia magna* and *Pimephales promelas*. *Environ. Toxicol. Chem.* **2015**, *34*, 1382–1389. [\[CrossRef\]](#) [\[PubMed\]](#)
88. Poppov, K.; Ronkkomaki, H.; Lajunen, L.H.J. Critical evaluation of stability constants of phosphonic acids. *Pure Appl. Chem.* **2001**, *73*, 1641–1677. [\[CrossRef\]](#)
89. Bonansea, R.I.; Filippi, I.; Wunderlin, D.A.; Marino, D.J.G.; Amé, M.V. The Fate of Glyphosate and AMPA in a Freshwater Endorheic Basin: An Ecotoxicological Risk Assessment. *Toxics* **2018**, *6*, 3. [\[CrossRef\]](#)
90. Battaglin, W.A.; Koplin, D.W.; Scribner, E.A.; Kuivila, K.M.; Sandstorm, M.W. Glyphosate, other herbicides, and transformation products in midwestern streams, 2002. *J. Am. Water Resour. Assoc.* **2005**, *41*, 323–332. [\[CrossRef\]](#)
91. Battaglin, W.A.; Rice, K.C.; Focazio, M.J.; Salmons, S.; Barry, R.X. The occurrence of glyphosate, atrazine, and other pesticides in vernal pool and adjacent streams in Washington, DC, Maryland, Iowa, and Wyoming, 2005–2006. *Environ. Monitor. Assess.* **2009**, *155*, 281–307. [\[CrossRef\]](#)
92. Directive 2000/60/EC of the European Parliament and of the Council. 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. Available online: <https://eur-lex.europa.eu/legal-content/CS/ALL/?uri=CELEX%3A32000L0060> (accessed on 22 December 2020).
93. Aparicio, V.C.; De Gerónimo, E.; Marino, D.; Primost, J.; Carriquiriborde, P.; Costa, J.L. Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins. *Chemosphere* **2013**, *93*, 1866–1873. [\[CrossRef\]](#)
94. Di Poi, C.; Costil, K.; Bouchart, V.; Harm-Lemeille, M.P. Toxicity assessment of five emerging pollutants. Alone and in binary or ternary mixtures, towards three aquatic organisms. *Environ. Sci. Pollut.* **2018**, *25*, 6122–6134. [\[CrossRef\]](#)
95. Tajnaiová, L.; Vurm, R.; Kholomyeva, M.; Kobera, M.; Koci, V. Determination of the ecotoxicity of herbicides Roundup®Classis Pro and Garlon New in aquatic and terrestrial environments. *Plants* **2020**, *9*, 1203. [\[CrossRef\]](#)
96. EFSA (European Food and Safety Authority). Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. *EFSA J.* **2015**, *13*, 4302. [\[CrossRef\]](#)
97. Reddy, K.N.; Rimando, A.M.; Duke, S.O. Aminomethylphosphonic acid, a metabolite of glyphosate, causes injury in glyphosate-treated, glyphosate-resistant soybean. *J. Agric. Food Chem.* **2004**, *52*, 5139–5143. [\[CrossRef\]](#) [\[PubMed\]](#)
98. Gomez, M.P.; Smedbol, E.; Chalifour, A.; Hénault-Ethier, L.; Labrecque, M.; Lepage, L.; Lucotte, M.; Juneau, P. Alteration of plant physiology by glyphosate and its by-product Aminomethylphosphonic acid: An overview. *J. Exp. Bot.* **2014**, *65*, 4691–4703. [\[CrossRef\]](#) [\[PubMed\]](#)
99. Serra, A.A.; Nuttens, A.; Larvor, V.; Renault, D.; Couée, I.; Sulmon, C.; Gouesbet, G. Low environmentally relevant levels of bioactive xenobiotics and associated degradation products cause cryptic perturbations of metabolism and molecular stress responses in *Arabidopsis thaliana*. *J. Exp. Bot.* **2013**, *64*, 2753–2766. [\[CrossRef\]](#)
100. Guilherme, S.; Santos, M.A.; Gaivao, I.; Pacheco, M. DNA and chromosomal damage induced in fish (*Anguilla anguilla* L.) by aminomethylphosphonic acid (AMPA)—The major environmental breakdown product of glyphosate. *Environ. Sci. Pollut. Res.* **2014**, *21*, 8730–8739. [\[CrossRef\]](#) [\[PubMed\]](#)
101. Matozzo, V.; Marin, M.G.; Maseiro, L.; Tremonti, M.; Biamonte, S.; Viale, S.; Finos, L.; Lovato, G.; Pastore, P.; Bogialli, S. Effects of aminomethylphosphonic acid, the main breakdown product of glyphosate, on cellular and biochemical parameters of the mussel *Mytilus galloprovincialis*. *Fish Shellfish Immunol.* **2018**, *83*, 321–329. [\[CrossRef\]](#)
102. Cheron, M.; Brischoux, F. Aminomethylphosphonic acid alters amphibian embryonic development at environmental concentrations. *Environ. Res.* **2020**, *190*, 109944. [\[CrossRef\]](#)
103. Commission Implementing Regulation (EU) 2017/2324. Renewing the Approval of the Active Substance Glyphosate in Accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council Concerning the Placing of Plant Protection Products on the Market, and Amending the Annex to Commission Implementing Regulation (EU) No 540/2011. OJEU, L333/10. 2017. Available online: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32017R2324> (accessed on 19 September 2021).