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Review

Effects of glyphosate and their metabolite AMPA on aquatic organisms

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Abstract: Glyphosate (N-(phosphonomethyl)glycine) is a herbicide used to kill broadleaf weeds and grass, developed in the early 1970s. The widely occurring degradation product aminomethylphosphonic acid (AMPA) is a result of glyphosate and amino-polyphosphonate degradation. The massive use of the parent compounds 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 summarises current knowledge about residual glyphosate and their major metabolite AMPA in the aquatic environment, including status and toxic effects in aquatic organisms, mainly fish, are reviewed. 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 their major metabolite AMPA has mainly influenced growth, early development, oxidative stress biomarkers, antioxidant enzymes, haematological, biochemical plasma indices, caused histopathological changes in the aquatic organism.

Keywords: toxicity, effect, fish, invertebrate, mussels

Abbreviations: ABC transporter activity: adenosine triphosphate-binding cassette transporters constitute; AChE: acetylcholinesterase; ACP: acid phosphatase; AKP: alkaline phosphatase; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AMPA: aminomethylphosphonic acid; AST: aspartate aminotransferase; ATMP: amino tris(methylenephospohonate); 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 chanel; 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 Lo observed effect concentration; EC50: effective concentration that affects 50% of the population; EDTMA: ethylenediamine tetra(methylenephosphonate); 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(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: 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:

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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; TtHR: time to half relaxation; UN: urine nitrogen; U.S. EPA: United States Environmental Protection Agency; WBC: leukocytes; Zn^{+}: zinc ion.

1. Introduction

In recent years, knowledge about pesticide's persistence, mobility and ecotoxicity is valuable. Using pesticides and other agrochemicals is the most cost-effective way to maintain economic viability in an increasing human population [1-2]. On the other hand, the intensive application and repeated use of pesticides in the field for increasing the crop yield lead to long-term risk for humans, fauna, flora, and the whole ecosystem (soil, air, water) [1-3]. Extensive use of pesticides is not a problem only in agricultural areas, but also in urban settings are pesticides applied for horticultural purposes. Therefore, it is challenging to control the source of diffuse chemical pollution and its consequences [4]. Especially presence of pesticides and their metabolites occurring in the residual concentration in drinking, ground- and surface waters comprises a global problem [1,3].

Before World War II, natural and organic pesticides were used. However, after this was necessary to increase the production of crops as a defence against starvation and malnutrition, this was an opportunity for an industrial company to produce new synthetic agrochemicals and worldwide use of them [5]. A considerable amount of pesticide-based chemicals with different uses and modes of action have been brought to market. Despite chemical structures, way or period of action, and target organisms (according to 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 company (United States). Under the trade name, "Roundup" was registered in 1974 [7-8]. Thanks to initial toxicity tests, which posed relatively low risks to non-target organisms, including mammals, relatively high exposure limits of glyphosate have been set worldwide. In a short time, use of this popular herbicide dramatically increased due to genetically modified crops (soybean, canola, alfalfa, maize, cotton, corn) to be tolerant to glyphosate, its utility in agronomy and urban settings, and the perception that it has low toxicity and little mobility in the environment [9-11]. However, ecotoxicology and epidemiology studies published in the last decade point to the need for intensive testing toxicity of glyphosate [11]. Furthermore, the World Health Organization's International Agency for Research on Cancer recently concluded that glyphosate is "probably carcinogenic to humans" [12-14].

The most prevalent glyphosate degradation pathway in bacterial strains is the cleavage of C-N bond and conversion to aminomethylphosphonic acid (AMPA), which is either further decomposed or excreted to the environment [15-16]. AMPA is a primary product of the degradation process of glyphosate, and the following non-toxic 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 the experimental condition in a laboratory [16].

Natural processes in motion in the environment, pesticides can be removed to a certain extent. But also, potential risk of residues from the biodegradation process [4,19]. By implication of widespread use of pesticides, residual concentrations of pesticides and their metabolites are commonly found ubiquitously through different environment constituents ranging from 1 ng/L to 1 mg/L or higher concentration [3-4]. There is also a potential risk of banned pesticides. They were excluded because of their long persistence and toxicity in the ecosystem for many years. For example, organochlorine insecticides were still detectable in waters after 20 years [20] or Acetochlor ESA, the major metabolite of banned Acetochlor in the European Union in 2012 no 1372/2011 [21], was found in waters of the Czech Republic in recent years [22-23]. Although these are usually in low

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concentrations in the environment, they may be present as complex mixtures. The metabolites may be as toxic as their parental compound or even higher. Therefore, the presence of these substances raises significant ecotoxicological concerns [e.g., 24-26].

Due to repeated application of pesticides arise to physical and chemical changes in water properties that are reflected in the modification of the cellular and biochemical biology of aquatic communities, leading to significant changes in tissues, physiology, and behaviour [27-28]. Therefore, it may affect the daily or seasonal rhythm and reproduction ability of aquatic organisms. Environmental stress from xenobiotics may cause losing of habitats and consequently losing freshwater biodiversity [29-30], which imply 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, in the literature is several studies and reports about an occurrence or toxic effects of different type of pesticides and their metabolites; nevertheless, their global extents 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 often using herbicide glyphosate and their metabolite AMPA on aquatic organisms.

2. Glyphosate (N-(phosphonomethyl)glycine)

Glyphosate (GLY) is belonging 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, non-selective, post-emergency 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 contain a mix of 15% POEA and additional surfactants [38]. The Roundup includes 48% of active agent IPA [34] or potassium salts in the range 167-480 $g \cdot l^{-1}$ depends on the type of area where the Roundup is applied [39].

2.1 Environmental fate

Even though solid bond to the soil amount of GLY which leach or runoff to surface- or ground- waters is low [40], spray drift from the ground and aerial applications of glyphosate may enter to aquatic ecosystems [41]. Hight application rates, rainfall, and a flow route that does not include transportation of GLY through the soil from watersheds comprise the most risk for 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 a range level from 0.01 mg/L to 0.7 mg/L and reaching the worst case for surface waters 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, 0.02-1.6 μg/L, and 1.9-4.7 μg/L, approximately.

This herbicide is unique for its high efficiency, transformation on major metabolite AMPA due to microbial degradation [16,40], and 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 ranges from 199 to 208 days [15]. Nevertheless, according to published data half-life of GLY ranges from 7 to 14 days [40].

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Owing to their high-water solubility and extensive usage in the environment (especially in shallow water systems), GLY contamination has emerged as an urgent issue. Therefore the exposure of non-target aquatic organisms to these herbicides is a concern of ecotoxicologists [16,37].

2.2 Acute toxicity

It has been already mentioned that initial testing of GLY did not fully demonstrate its toxic effects, and therefore the amount for use was not over-regulated. U.S. EPA divided toxicity of GLY into slightly toxicity with concentration 10 - 100 mg/L and almost non-toxicity with higher concentration > 100 mg/L to fish species with acute LC50 values from > 10 to > 1000 mg/L [15]. The lethal concentration for fish is in the range 0.295 to 645 mg/L (Table 1), for amphibians is in the range 6.5 to 115 mg/L (Table 2) and for invertebrate species from 35 to 461.54 mg/L (Table 3).

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

Species	Formu-lation	Exposure (hours)	Concentration (mg/L)	Referen-ces
Rainbow trout (*Oncorhynchus mykiss*)	GLY	96	140	[41]
	Roundup[1]	96	52-55	[45]
Common carp (*Cyprinus carpio*)	GLY	48	645	[46]
		96	620	
	Roundup[1]	96	22.19	[47]
	GLY	48	602.61	[48]
		96	520.77	
Black-head minnow (*Pimephales promelas*)	GLY	96	97	[41]
Channel catfish (*Ictalurus punctatus*)		96	130	
Bluegills (*Lepomis macrochirus*)		24	150	
		96	140	
Barbados millions (*Poecilia reticulata*)	GLY	96	69.83	[49]
Rhamdia quelen	GLY	96	7.30	[50]
North African catfish (*Clarias gariepinus*)	GLY	96	0.295	[51]
Leopard Danio (*Danio rerio*)	Atnor 48[2]	96	76.50	[52]
Ten spotted live-bearer (*Cnesterodon decemmaculatus*)	Glyfoglex[3]	96	41.40	[53]

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

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

Species	Formu-lation	Exposure (hours)	Concentration (mg/L)	Referen-ces
Boana pardalis	GLY	96	106	[54]
Physalaemus cuvieri		96	115	
Green frog (*Lithobates clamitans*)	Roundup[1]	24	6.6	[55]
		96	6.5	
Northern leopard frog *(Lithobates pipiens)*		24	11.9	
		96	9.2	
Wood frog (*Lithobates sylvaticus*)		24	18.1	
		96	16.5	
Dwarf American toad (*Anaxyrus americanus*)		24	13.5	
		96	< 12.9	
Rhinella arenarum	Roundup Ultra-Max[2]	48	2.42	[56]
			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 product on invertebrate species.

Species	Formu-lation	Exposure (hours)	Concentration (mg/L)	References
Midge larvae (*Chironomus plumosus*)	GLY	48	55	[41]
Ceriodaphnia dubia	Roundup[1]	48	147	[37]
Acartia tonsa		48	35	
Chinese mitten crab (*Eriocheir sinensis*)	GLY	24	461.54	[57]
		96	97.89	

[1]Roundup (active substance glyphosate, 41%).

2.2 Toxic effects

2.2.1 Fish

GLY toxicity has been studying in recent years on various kinds of aquatic organisms. Exposure to GLY may cause several changes in fish (Table 4), such as haematologic changes, biochemical processes in tissues [38], genotoxicity [52,58], histopathological damage, immunotoxicity [48,59], or cardiotoxicity [60].

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Table 4. Toxic effects of glyphosate and its commercial product on fish.

Species	Concentration	Exposure	Effects	References
Common carp (*Cyprinus carpio*)	2.5, 5, 10 mg/L (GLY)	96 hours	↑ ALP in liver, heart, GOT in liver and kidney, GPT in kidney; Subepithelial edema and epithelial hyperplasia in gills, focal fibrosis in liver	[46]
	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	[47]
	52.08, 104.15 mg/L (GLY)	7 days	Vacuolization of the renal parenchyma and intumescence of the renal tubule in kidney, immunotoxicity,	[48]
			↑ AST, ALT, MDA, PC; ↓ GSH, inhibition of NA^+/K^+ -ATPase, SOD, CAT, GPx, GR, T-AOC, induce inflammatory response in gills	[59]
European eel (*Anguilla Anguilla*)	58, 116 µg/L (Roundup[1])	1, 3 days	↑ TBARS, LPO, GDI, ENA	[42]
			↑ GDI, damage nucleoids, EndoIII	[58]
Curimbata (*Prochilodus lineatus*)	10 mg/L (Roundup[1])	24 hours	↑ GSH, GST, LPO; ↓ SOD, GPx, inhibition AChE in muscle	[38]
		96 hours	↑ GST, LPO; inhibition AChE in muscle in brain and muscle	
Spotted snakehead (*Channa punctatus*)	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	[61]
Ten spotted live-bearer (*Cnesterodon decemmaculatus*)	1, 1.75, 35 mg/L (GLY)	96 hours	↓ AChE	[62]
Megaleporinus obtusidens	3, 6, 10, 20 mg/L (Roundup[1])	96 hours	↑ hepatic GL, GLU, NH_3 in liver and muscle, PCV, Hb, RBC, WBC, P; ↓ AChE in brain, LACT, P in liver, muscle GL, GLU	[63]
	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	[64]
Rhamdia quelen	0.2, 0.4 mg/L (Roundup[1])	96 hours	↑ 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	[65]
	0.730 mg/L (GLY)	24, 96 hours, 10 days	↑ immature circulating cells; ↓ RBC, THR, WBC, phagocytic activity, agglutination activity, lysozyme activity	[66]
	18, 36, 72 µg/L (Roundup[1])	7 days	↑ TP in liver, ↑ GL in muscle; ↓ TP, GL, TL in gills, liver and kidney	[67]
Goldfish (*Carassius auratus*)	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	[68]
	0.22, 0.44, 0.88 mmol/L	96 hours	Behaviour abnormalities (observed depression, erratic swimming, partial loss of equilibrium), liver	[69]

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	(GLY)		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)	
	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,	[70]
North African catfish (*Clarias gariepinus*)	0, 19, 42, 94, 207, 455 mg/L (GLY)	96 hours	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	[51]
Hybrid fish jundiara (*Leiarius marmoratus* x *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]
Leopard danio (*Danio rerio*)	50 µg/mL (GLY)	24 h	↓ gene expression in eye, fore and midbrain delineated brain ventricles and cephalic regions	[71]
	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	[72]
	10, 50, 100, 200, 400 µg/L (GLY)	48 h	↓ heartbeat, NO generation, downregulation of Cacana1C and ryr2a genes, upregulation of hspb11	[60]
Climbing bass (*Anabas testudineus*) *Heteropneustes fossilis*	17.20 mg/L (Excel Mera 71[4])	30 days	↑ AChE, LPO, CAT; ↓ TP, GST	[73]

[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 several data about the chronic effects of glyphosate on non-target organisms. For example, [74] 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, occasional impacts on immune response have occurred. Other chronic effects were studied with different concentrations of glyphosate (0.2, 0.8, 4 and 16 mg/L) in *Oreochromis niloticus* for 80 days [75]. It was evaluated that glyphosate exposure reduced antioxidative ability, disturbed liver metabolism, promoted inflammation and suppressed immunity.

2.2.2 Mussels

Glyphosate caused changes in hemolymph [76], changes in the reproduction system and 50% inhibition of cholinesterase activity [77] in mussels (Table 5).

Table 5. Toxic effects of glyphosate and its commercial product on mussels.

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Species	Concentration	Exposure	Effects	References
Mediterranean mussel (*Mytilus galloprovincialis*)	100 µg/L (GLY)	7 days	↑ THC, haemocyte proliferation; ↓ Haemocyte diameter, AChE in gills	[76]
		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,	[78]
Limnoperna fortunei	1, 3, 6 mg/L (GLY)	26 days	↑ TBARS, GST, ALP; ↓ CES, SOD	[79]
	10, 20, 40 mg/L (GLY)	3 weeks	↓ presence of large mussel by 40%, presence empty shell by 25 %	[80]
Pacific oyster (*Crassostrea gigas*)	0.1, 1, 100 µg/L (Roundup Expres[1])	35 days	↑ GST; ↓ growth; LPO, MDA	[81]

[1]Roundup Expres (active substance glyphosate, 15%).

2.2.3 Invertebrate species

Exposure to GLY may cause several changes in invertebrate species (Table 6), such as biochemical processes in tissues, development, or behaviour.

Table 6. Toxic effects of glyphosate and its commercial product on invertebrate species.

Species	Concentration	Exposure	Effects	References
California blackworm (*Lumbriculus variegatus*)	0.05 - 5 mg/L (GLY)	4 days	↑ SOD; ↓ GST, membrane bound GST	[82]
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	[57]
American bullfrog (*Lithobates catesbeianus*)	1 mg/L (Roundup[1])	48 h	↑ swimming activity, CPM; SOD, CAT and LPO in liver; LPO in muscle; ↓ SOD, CAT in muscle, TtHR	[83]
Rhinella arenarum	1.85, 3.75, 7.5, 15, 30, 60, 120, 240 mg/L (Roundup Ultra-Max[2])	48 h	↓ AChE, BChE, CbE, GST	[56]
Northen leopard frog (*Rana pipiens*)	0.6, 1.8 mg/L (Roundup[1])	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	[55]
Snail (*Biomphalaria alexandrina*)	3.15 mg/L (Roundup[1])	6 weeks	↑ mortality, stopped egg lying, abnormal laid eggs, ↑ GLU, LACT, FAC; ↓ egg hatchability, GL, TP, pyruvate, nucleic acids levels	[84]

	10 mg/L (Roundup[1])	7 days	↑ in vitro phagocytic activity, DNA damage in hemocytes	[85]

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

3. AMPA (aminomethylphosphonic acid)

AMPA belongs to the aminomethylenephosphonates chemical group. It is the primary metabolite of GLY degradation process with a significant measured concentration in the environment. Additional sources of AMPA originate from organic phosphonates using in water treatment [86], from the degradation of phosphonic acids used in Europe in detergent and industrial boilers and cooling (EDTMA, DTMP, ATMP. HDTMP) [15,86]. Because of phosphonate and amine functional groups, AMPA will form metal complexes with Ca^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+}. AMPA is sorb firmly to soil [87].

3.1 Environmental fate

AMPA has a lower water solubility and longer soil half-life than glyphosate. Presence of AMPA in freshwater, sediment, and suspended particulate is commonly measured in significant quantities [10,88], and even more frequently (67.5%) than glyphosate (17.5%) [15,89-90]. The Water Framework Directive [91] provides a procedure to set Environmental Quality Standards for AMPA at level 450 mg/L. Coupe et al. [9] reported concentration of AMPA in freshwater environments for Mississippi and Iowa ranged 2.6 μg/L, 0.02-5.7 μg/L. In France, AMPA was detectable with the highest concentration at level 44 μg/L.

AMPA, like glyphosate, also degrade in water and soil but significantly slower. Because its adsorption to particulates is possibly stronger is lower penetrability to cell membranes. The concentration of AMPA in the sediment can fluctuate depending on its degradation rate relative to GLY [92].

3.2 Acute toxicity

AMPA toxicity has been already studied in recent years on various kinds of organisms. Although [52] observed no acute toxic effect of AMPA on fish species, other studies showed acute toxicity values from 27 to 452 mg/L (Table 7).

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Table 7. Toxicity values of AMPA for aquatic organisms.

Species	Value	Concentration (mg/L)	References
Barbados millions (*Poecilia reticulata*)	96hLC50	180 for male 164.32 for female	[49]
Pacific oyster (*Crassostrea gigas*)	36hEC10	38.55	[93]
	36hEC20	42.68	
	36hEC50	50.78	
	24hEC10	27.08	
	24hEC20	39.80	
	24hEC50	76.90	
Daphnia magna	48hEC10	> 100[5]	
	48hEC20		
	48hEC50		
Pseudokirchneriella subcapitata	72hEC10	85.05	
	72hEC20	> 100	
	72hEC50		
Desmodesmus subspicatus	72hIC50	117.8	[94]
	72hEC50	89.8[1] 452[2]	[95]

[1]biomass test, [2]algal growth inhibition tests.

3.3 Toxic effects

Although AMPA has been studied less than glyphosate, Reddy et al. [96] pointed on 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 thus the photosynthetic process [97]. Plants treated with AMPA showed a decreased glycine, serine, and glutamate [98].

Table 8. Toxic effects of AMPA on aquatic organisms.

Species	Concentration	Exposure	Effects	References
Europaen eel (*Anguilla Anguilla*)	11.8, 23.6 µg/L	1, 3 days	↑ GDI, FPG, EndoIII	[99]
Leopard danio (*Danio rerio*)	1.7, 5, 10, 23, 50, 100 mg/L	24, 48, 72, 96 hours	Genotoxicity with LOEC 1.7 mg/L, induce primary DNA lesions,	[52]
Barbados millions (*Poecilia reticulata*)	82 mg/L	96 hours	Proliferation of the interlamellar epithelium, fusion of secondary lamellae in gill, steatosis, pyknotic nuclei in liver, degenerate of hepatocytes	[49]
Mediterranean mussel (*Mytilus galloprovincialis*)	100 µg/L	7 days	↑ haemocyte diameter, haemocyte volume, haemocyte proliferation, LDH in haemolymp, HL; ↓ THC, AChE in gills	[76]

		14 days	↑ THC, haemocyte diameter, haemocyte volume, haemocyte proliferation, AChE in gills, CAT in digestive gland; ↓ HL	
		21 days	↑ haemocyte volume, LDH in haemolymph; ↓ THC, haemocyte proliferation, HL, AChE in gills	
	1, 10, 100 µg/L	7 days	↓ THC	[100]
		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	
Bufo spinosus	0.07, 0.32, 3.57 µg/L	16 days	↓ embryonic survival, development delay, short tail length	[101]

There is almost no data for chronic effects and exposure to AMPA for aquatic organisms. The chronic toxicity of AMPA to *Pimephales promelas* and *Daphnia magna* was studied by Levine et al. [86]. Evaluating NOEC for *P. promelas* was determined 12 mg/L, and no-observed-effect concentration for *D. magna* was 15 mg/L.

4. Conclusion

GLY and AMPA et environmental relevant concentrations usually do not cause direct lethality. However, glyphosate as a separate compound or as a component of commercial products used in agriculture and its primary metabolite AMPA may have adverse effects on non-target aquatic organisms. GLY mainly affected oxidative stress, antioxidant enzymes, blood parameters and cause several histopathologic changes in gills, liver and kidneys, and not least genotoxicity, immunotoxicity and cardiotoxicity in fish; oxidative stress, antioxidant enzymes, and haemocyte parameters in mussels. In comparison to AMPA, in literature is gaps in knowledge about its toxicity on aquatic organisms. AMPA may cause genotoxicity, immunotoxicity in fish, adverse changes in haemolymph parameters, affected 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, we assume that the presence of GLY and AMPA in the aquatic environment requires stricter control and further studies of the potentially toxic effects of these substances on the non-target organism. Further needs to be found bioindicators for polluted aquatic environments of GLY and AMPA.

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