Glyphosate(N-(phosphonomethyl)glycine)

Subjects: Environmental Sciences

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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.

toxicity effect fish invertebrate glyphosate AMPA

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][2][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 concentratiosn in drinking, ground, and surface waters poses a global problem [1][3].

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 [5][6]. 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 [7], sarcosine is barely detected in the natural environment [8], except under experimental conditions in a laboratory [6]. 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 [9][7]. The residual concentrations of glyphosate and AMPA in waters contaminate aquatic organisms via the food web (**Figure 1**) [10][5].

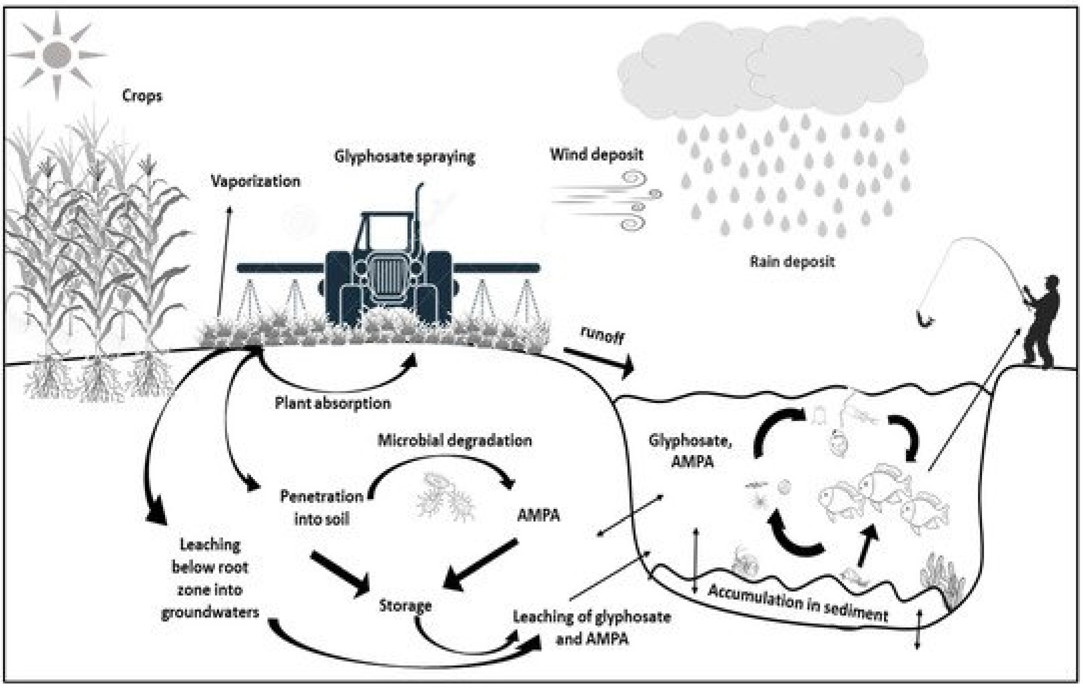

Figure 1. Distribution and transport of glyphosate and its major metabolite AMPA into the aquatic environment.

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 [5]. 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. [11][12].

For higher water solubility, GLY is formulated as potassium salts or isopropylamine salts and a surfactant, polyoxyethylene 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 [12][13] or Roundup Transorb, which contains a mix of 15% POEA and additional surfactants [14]. Roundup includes 48% of active agent IPA [11] 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 [15].

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 [16], the aerial applications of glyphosate spray drifts from the ground and may enter into aquatic ecosystems (**Figure 1**) [17]. 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 [18]. For example, the United States Environmental Protection Agency [5] 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 [19][20][21]. Coupe et al. [18] reported concentration of GLY for Mississippi, Iowa, and France ranged from 0.03 to 73 µg/L, 0.02 to1.6 µg/L, and 1.9 to 4.7 µg/L, approximately.

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 [5]. Nevertheless, according to the published data the halflife of GLY ranges from 7 to 14 days [16].

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 [6][13].

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 [5]. 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	[17]
	Roundup [1]	96	52–55	[22]
Common carp (*Cyprinus carpio*)	GLY	48	645	[23]
		96	620	
	Roundup [1]	96	22.19	[24]
	GLY	48	602.61	[25]
		96	520.77	
Blackhead minnow (*Pimephales promelas*)	GLY	96	97	[17]
Channel catfish		96	130	

Species	Formulation	Exposure (Hours)	Concentration (mg/L)	References
(*Ictalurus punctatus*)				
Bluegills (*Lepomis macrochirus*)	GLY	24	150	[17]
		96	140	
Guppy (*Poecilia reticulata*)	GLY	96	69.83	[26]
Rhamdia quelen	GLY	96	7.30	[27]
North African catfish (*Clarias gariepinus*)	GLY	96	0.295	[28]
Zebrafish (*Danio rerio*)	Atnor 48 [2]	96	76.50	[29]
Ten spotted live-bearer (*Cnesterodon decemmaculatus*)	Glyfoglex [3]	96	41.40	[30]

[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	[31]
Physalaemus cuvieri		96	115	
Green frog (*Lithobates clamitans*)	Roundup [1]	24	6.6	[32]
		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	[33]

Species	Formulation	Exposure (Hours)	Concentration (mg/L)	References
			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 (*Chironomus plumosus*)	GLY	48	55	[17]
Ceriodaphnia dubia	Roundup [1]	48	147	[13]
Acartia tonsa		48	35	
Chinese mitten crab (*Eriocheir sinensis*)	GLY	24	461.54	[34]
		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 [14], genotoxicity [29][35], histopathological damage, immunotoxicity [26][36], or cardiotoxicity [37].

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

Species	Concentration	Exposure	Effects	References
Common carp (*Cyprinus carpio*)	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	[23]
	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	[24]
	52.08, 104.15 mg/L (GLY)	7 days	Vacuolization of the renal parenchyma and intumescence of the renal tubule in kidney, immunotoxicity	[25]

Species	Concentration	Exposure	Effects	References
European eel (*Anguilla Anguilla*)	58, 116 μg/L (Roundup [1])	1, 3 days	↑ AST, ALT, MDA, PC; ↓ GSH, inhibition of NA^{+}/K^{+} -ATPase, SOD, CAT, GPx, GR, T-AOC, induce inflammatory response in gills	[36]
			↑ TBARS, LPO, GDI, ENA	[19]
			↑ GDI, damaged nucleoids, EndoIII	[35]
Curimbata (*Prochilodus lineatus*)	10 mg/L (Roundup [1])	24 h	↑ GSH, GST, LPO; ↓ SOD, GPx, inhibition of AChE in muscle	[14]
		96 h	↑ GST, LPO;inhibition of 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	[38]
Ten spotted live-bearer (*Cnesterodon decemmaculatus*)	1, 1.75, 35 mg/L (GLY)	96 h	↓ AChE	[39]
Megaleporinus obtusidens	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	[40]
	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	[41]
Rhamdia quelen	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	[42]
	0.730 mg/L (GLY)	24, 96 h, 10 days	↑ immature circulating cells; ↓ RBC, THR, WBC, phagocytic activity, agglutination activity, lysozyme activity	[43]
Rhamdia quelen	18, 36, 72 μg/L (Roundup [1])	7 days	↑ TP in liver, ↑ GL in muscle; ↓ TP, GL, TL in gills, liver, and kidney	[44]

Species	Concentration	Exposure	Effects	References
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	[45]
	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)	[46]
	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,	[47]
North African catfish *(Clarias gariepinus*)	0, 19, 42, 94, 207, 455mg/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	[28]
Hybrid fish jundiara (*Leiarius marmoratus × 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	[48]
Zebrafish (*Danio rerio*)	50 µg/mL (GLY)	24 h	↓ gene expression in eye, fore, and midbrain delineated brain ventricles and cephalic regions	[49]
	32.5, 65, 130 µg/L (Transorb [3])	48 h	↓ integrity of plasma membrane of hepatocytes, viability of cells, mitochondrial activity in the cell,	[50]

Species	Concentration	Exposure	Effects	References
			lysosomal integrity, inhibition in ABC transporter activity	
	10, 50, 100, 200, 400 μg/L (GLY)	48 h	↓ heartbeat, NO generation, downregulation of Cacana1C and ryr2a genes, upregulation of hspb11	[37]
Climbing bass (*Anabas testudineus*)	17.20 mg/L (Excel Mera 71 [4])	30 days	↑ AChE, LPO, CAT; ↓ TP, GST	[51]
*Heteropneustes fossi*lis				

[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%).

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 [52], changes in the reproduction system, and 50% inhibition of cholinesterase activity [53] 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 days	↑ THC, haemocyte proliferation; ↓ Haemocyte diameter, AChE in gills	[52]
		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	[54]

Species	Concentration	Exposure	Effects	References
			gills; SOD in digestive gland, THC,	
Limnoperna fortunei	1, 3, 6 mg/L (GLY)	26 days	↑ TBARS, GST, ALP; ↓ CES, SOD	[55]
	10, 20, 40 mg/L (GLY)	3 weeks	↓ presence of large mussel by 40%, presence empty shell by 25%	[56]
Pacific oyster *(Crassostrea gigas*)	0.1, 1, 100 μg/L (Roundup Expres [1])	35 days	↑ GST; ↓ growth; LPO, MDA	[57]
California blackworm (*Lumbriculus variegatus*)	0.05–5 mg/L(GLY)	4 days	↑ SOD; ↓ GST, membrane bound GST	[58]
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	[34]
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	[59]
Rhinella arenarum	1.85, 3.75, 7.5, 15, 30, 60, 120, 240 mg/L (Roundup Ultra-Max [3])	48 h	↓ AChE, BChE, CbE, GST	[33]
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	[32]
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	[60]

Species	Concentration	Exposure	Effects	References
	10 mg/L (Roundup [2])	7 days	↑ in vitro phagocytic activity, DNA damage in haemocytes	[61]

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

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