
ÁREA AMBIENTAL
TOXICITY OF GLYPHOSATE AND ITS
DEGRADATION PRODUCTS IN AQUATIC
ECOSYSTEMS: A REVIEW

TOXICIDAD DEL GLIFOSATO Y SUS PRODUCTOS DE
DEGRADACIÓN EN ECOSISTEMAS ACUÁTICOS:
UNA REVISIÓN



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Abstract: Context: The intensifying utilization of glyphosate has provoked global scientific concern due to the potential large-scale impact on ecosystems. In 2015, the International Agency for Research on Cancer (IARC) reclassified glyphosate and its degradation byproduct, aminomethylphosphonic acid (AMPA), as Category 2A, indicating probable human carcinogenicity.

Knowledge Gap: A review of the existing literature reveals a relative scarcity of information on the aquatic impact of glyphosate and its degradation products.

Purpose: To determine the scope and implications of the impact of glyphosate and its degradation products on aquatic ecosystems, in order to better understand their ecotoxicological effects and provide a foundation for future research and policy decisions in this area.

Methodology: A systematic review of ecotoxicological studies published in Spanish and English over the last 12 years was conducted to assess the effects of glyphosate and its degradation products on aquatic ecosystems and the risks to various species. Approximately 95 documents were reviewed, including data from 69, addressing toxicity, biodegradation, contamination, and international regulations.

Results: Studies have shown that chronic exposure to glyphosate can alter the metabolism in fish and affect the life cycle of organisms such as *Daphnia Magna*. The composition of glyphosate, along with its adjuvants, can increase its toxicity and pose risks to the aquatic ecosystem, making it crucial to understand the specific formulations and their concentrations.

Conclusions: The increased use of glyphosate raised global scientific concerns due to its potential impact on ecosystems, with the IARC classifying it as possibly carcinogenic to humans. Studies showed acute toxicity to aquatic organisms and highlighted the need for risk assessment methodologies for long-term impacts on ecosystems and human.

Keywords: Agriculture, Degradation Product, Environmental Risk, Glyphosate, Herbicide, Organophosphate, Risk assessment, Water Quality, Ecotoxicity.

Resumen: **Contextualización:** la creciente utilización de glifosato ha generado preocupación científica a nivel global debido a su potencial impacto a gran escala en los ecosistemas. En 2015, la Agencia Internacional de Investigación sobre el Cáncer (IARC) reclasificó el glifosato y su producto de degradación, el ácido aminometilfosfónico (AMPA), como Categoría 2A, indicando probable carcinogenicidad en humanos.

Vacío de conocimiento: una revisión de la literatura existente revela una relativa escasez de información sobre el impacto acuático del glifosato y sus productos de degradación.

Propósito: determinar el alcance y las implicaciones del impacto del glifosato y sus productos de degradación en ecosistemas acuáticos, a fin de comprender mejor sus efectos ecotoxicológicos y proporcionar una base para futuras investigaciones y decisiones políticas en esta área.

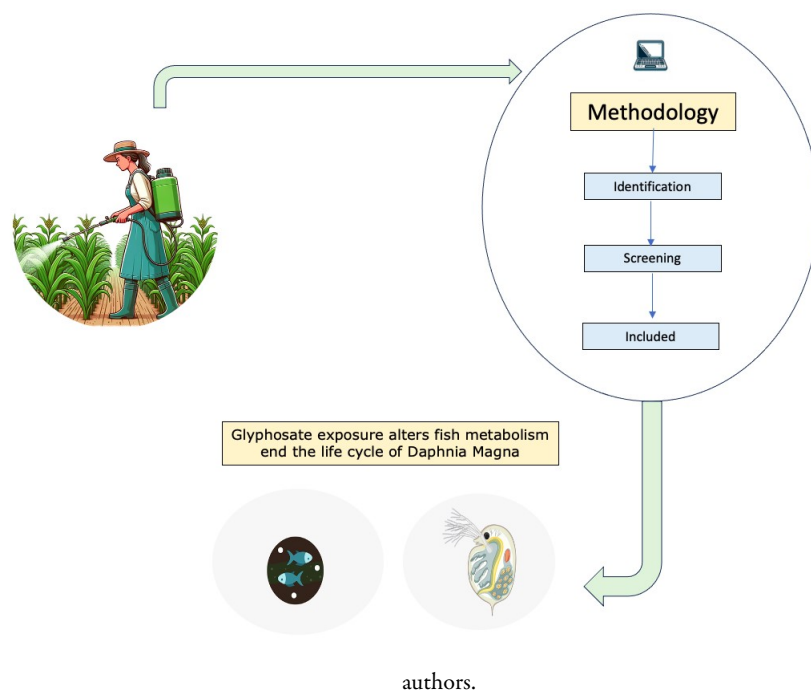
Metodología: se realizó una revisión sistemática de estudios ecotoxicológicos en español e inglés publicados en los últimos 12 años para evaluar los efectos del glifosato y sus productos de degradación en ecosistemas acuáticos y los riesgos para diversas especies. Se revisaron aproximadamente 95 documentos, incluyendo información de 69, abordando toxicidad, biodegradación, contaminación y regulaciones internacionales.

Resultados: estudios han demostrado que la exposición crónica a glifosato puede alterar el metabolismo en peces y afectar el ciclo de vida de organismos como *Daphnia Magna*. La composición de glifosato, junto con sus coadyuvantes, puede incrementar su toxicidad y representar riesgos para el ecosistema acuático, siendo clave entender las formulaciones específicas y sus concentraciones.

Conclusiones: el aumento en el uso del glifosato generó preocupaciones científicas a nivel mundial debido a su potencial impacto en los ecosistemas, con la IARC clasificándolo como posiblemente carcinogénico para los humanos. Los estudios mostraron toxicidad aguda en organismos acuáticos y resaltaron la necesidad de metodologías de evaluación de riesgos para los impactos a largo plazo en los ecosistemas y la exposición humana a estas sustancias.

Palabras clave: agricultura, calidad del agua, ecotoxicidad, evaluación de riesgo, glifosato, herbicida, organofosforado, producto de degradación, riesgo ambiental.

GRAPHIC ABSTRACT



1. INTRODUCTION

Pesticides are substances or mixtures composed of chemical or biological components designed for repelling, destroying, or controlling pests, as well as for plant growth regulation. Globally, the most prevalent pesticides are herbicides (Argüello-Rangel et al., 2015), utilized to manage invasive or undesirable plant species (Torres & Romero-Natale, 2019). They function by disrupting crucial physiological processes at target sites within plants, thereby affecting survival or normal growth (Mantilla, 2020).

In view of the transport of chemical compounds in the atmosphere, in general, is governed by two phenomena: wind erosion and spray drift that later falls toward the earth's surface (Grandcoin et al., 2017); the behavior depends largely on their physical-chemical properties and the type of formulation (Mas et al., 2020), in the case pesticides involve a complex series of events that allow them to be distributed through water, air, soil, or through the food chain (Faillaci, 2017), also impacting non-target organisms. In ecotoxicology, these 'non-target' organisms are those not intended to be affected by a chemical substance, such as a pesticide (Curieses, 2015). Hence, pesticides are substances that lack real selectivity, affecting a greater or lesser degree, both the "target species" and other categories of living beings (Schaaf, 2017).

According to the FAO in the world until 2018 and since 1990, the ten countries that used the most pesticides were China, USA, Brazil, Argentina, USSR, France, Italy, Japan, Colombia, and Canada as shown in Figure 1; in addition to doubling its use in that period (1990-2018), This situation responds to population growth and the improvement of emerging markets (Stagnaro, 2017). In the case of Brazil, this country surpassed its production of cereals, legumes, and oilseeds in 2017 by 29.2% compared to its production in 2016 (Tauhata et al., 2020). In Argentina, agricultural production significantly contributes to exports. As

reported by the Rosario Stock Exchange (2019), the principal export commodities are two soybean by-products - pellets and oil, which represent over 60% of the total export value (Terré & Santa, 2020). This has consequently led to an intensification in the use of herbicides. That is the increase in the use of synthetic inputs per surface unit (Pamela, 2018). In Colombia, glyphosate, a commonly used herbicide, has been extensively applied for over three decades in the cultivation of various crops, including sugarcane, coffee, bananas, rice, cocoa, African palm, and citrus. (Restrepo & Rincón, 2021). Additionally, the Colombian government implemented a program from the Ministry of Justice and Law in 2001 to eradicate illicit crops (*Erythroxylum coca*) through glyphosate aerial spraying (Julio & Ramírez, 2017) which was suspended in 2015 after the International Agency for the Study of Cancer [IARC] ruled on their carcinogenic potential as a preventive measure (Ruano-Ibarra & Carreño, 2020).

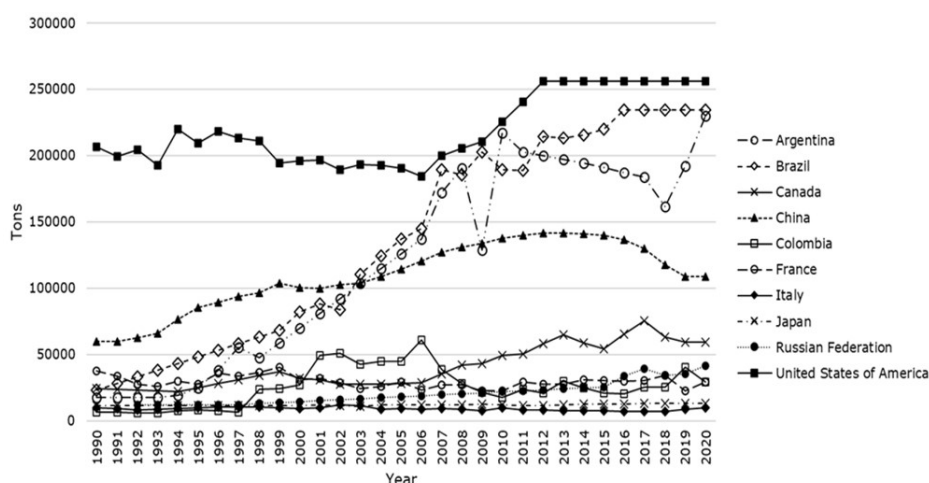


Figure 1.

Highest Pesticide-Consuming Countries from 1990-2020. Note. Chart developed from the FAOSTAT database, The Food and Agriculture Organization of the United Nations (2023)

Recovered from: FAO (2023).

In this scenario of great changes at the agricultural and market level, the rise in glyphosate use can be traced mostly to its widespread use on an extensive range of plants, and the development of genetically modified organisms, which contain a gene that confers resistance to the herbicide molecule, suppressing the ability to generate aromatic amino acids such as tryptophan-tyrosine and phenylalanine in the plants (Argüello-Rangel et al., 2015) In the instance of Monsanto's patented line of Roundup Ready® seeds, which was developed in 1996 (Tubio, 2016). Which includes a wide variety of crops that have been genetically modified to be tolerant to Glyphosate inhibition (Torres & Romero-Natale, 2019); some of the best-known seeds released on the market are some varieties of soybean, canola, cotton, and corn crops (Guijarro, 2019); which, in addition to containing glyphosate as the active ingredient, contain surfactant agents and other additives that improve the absorption of glyphosate in the foliage of vascular plants (Mantilla, 2020). The extended use of herbicides has raised concern worldwide about the direct or indirect effects that can be

caused by large-scale use (Chen et al., 2022; Van Bruggen et al., 2018) due to the increase in the concentrations of these substances in the environment (Benbrook, 2016), moreover because of the adverse effects of its degradation products, and the negative impacts that it can have on the quality of soil, water, plants, animals and human beings (Zirena, et al., 2018).

Ecological Risk Assessments (ERAs) are systematic tools for quantifying the potential impacts of stressors, typically chemicals, on ecosystems. Despite being crucial for guiding conservation decisions, ERAs face challenges such as data scarcity and the difficulty of generalizing across different contexts (Landis et al., 2013).

The primary objective of the literature review is to provide a comprehensive analysis of the global usage patterns of pesticides, particularly glyphosate, considering their physical-chemical properties, distribution mechanisms, impacts on non-target organisms and broader ecosystems, contributing socio-economic and agricultural factors, interplay with genetically modified organisms, and potential ecotoxicological risks to the environment and human health.

2. MATERIALS AND METHODS

To comprehend what are the immediate and long-term effects of chronic and acute exposure to glyphosate and its degradation products on aquatic ecosystems, and how can the risks for the different species present the ecosystems, a systematic review was conducted in accordance with the PRISM methodology suggested by Page et al., (2021), (supplementary material) of ecotoxicological studies published within the past 12 years, in either Spanish or English languages.

Approximately 95 documents were examined, which included academic papers, theses, and information from exhibitions or lectures available on the internet. After applying the process detailed in Figure 2, information from a total of 69 documents was included in this review. We used the keywords: Glyphosate, Organophosphate, Herbicide, Environmental Risk, Degradation Product, Agriculture, Risk assessment, Water Quality in the databases of Scielo, Dialnet, Google Scholar, Elsevier, Springer, Science Of The Total Environment, Environmental Sciences Europe to obtain a comprehensive set of data for this research. To conduct a more detailed analysis, we directed the review towards the methodology employed, the results presented, and the conclusions drawn. The selected studies allowed for the characterization of the behavior of glyphosate, AMPA, and sarcosine in the environment, including their mechanisms of transport and persistence. Studies on the toxicity, biodegradation, persistence, and environmental contamination of glyphosate were considered, as were the effects of glyphosate-based herbicides on the environment, the relationship between the use of glyphosate herbicide and its impact on human health and biodiversity. Studies that evaluated the ecotoxicological risk of these compounds in water and monitored their presence were also included. Additionally, information was gathered on international parameters such as regulations and studies evaluated by international organizations such as the EPA, IARC, and the EU. Studies that were considered outside the scope of the research question were excluded. Additionally, risk assessments conducted on matrices other than water, as well as outdated or incomplete information, duplicates, and studies with unclear methodology, were discarded.

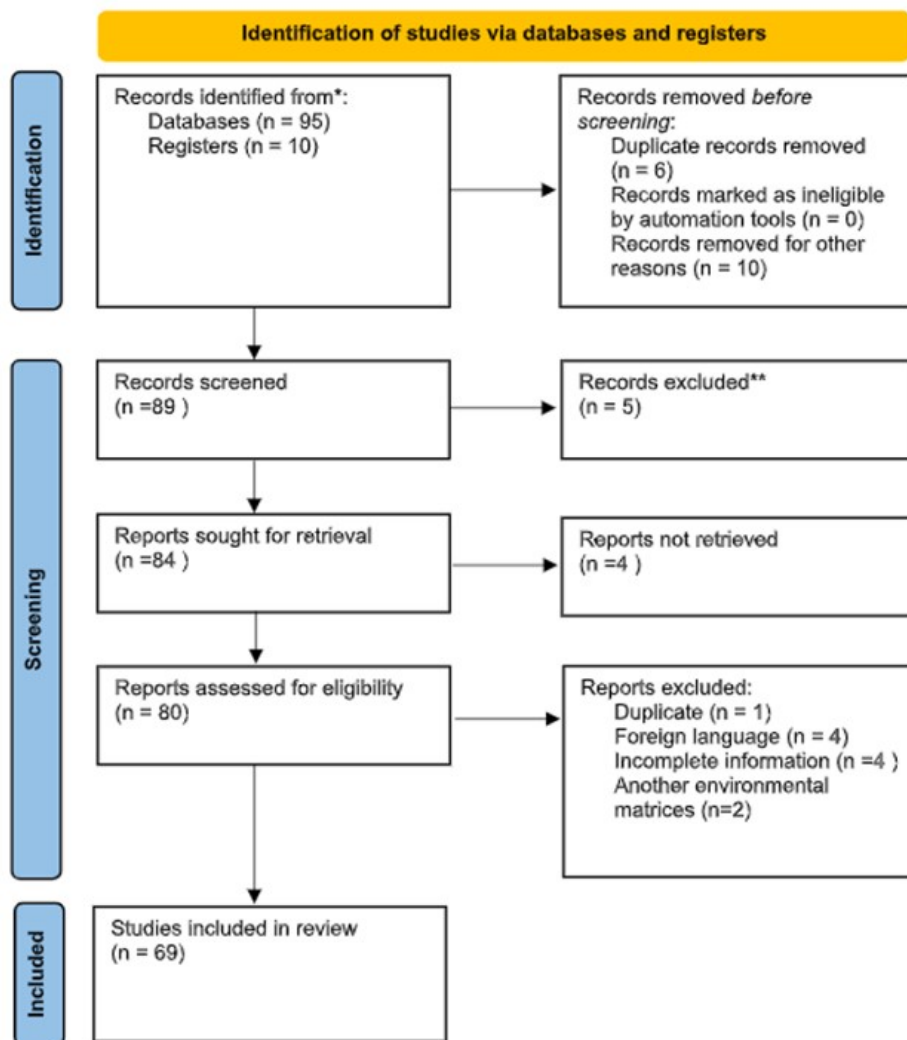


Figure 2.

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only.
From: Page et al. (2021).

3. RESULTS

Glyphosate: Chemical Properties, Action, and Degradation

This compound, a broad-spectrum, non-selective, post-emergent herbicide (Sterren et al., 2016; Raj, 2023), is an acid utilized in the form of isopropylamine salt of N-phosphonomethyl-glycine. Its molecular structure, as shown in Figure 3, comprises three polar functional groups: carboxyl, amino, and phosphonate (Huaracahuaraca, 2017). The mode of action of glyphosate is through the enzymatic disruption in the production of shikimic acid. Therefore, it disrupts precursors of important metabolites such as plant hormones (Junges et al., 2013; Benslama and Boulahrouf, 2016; in Chen et al, 2022). Glyphosate is also used to accelerate the ripening of forage cereals (Helander et al., 2012).

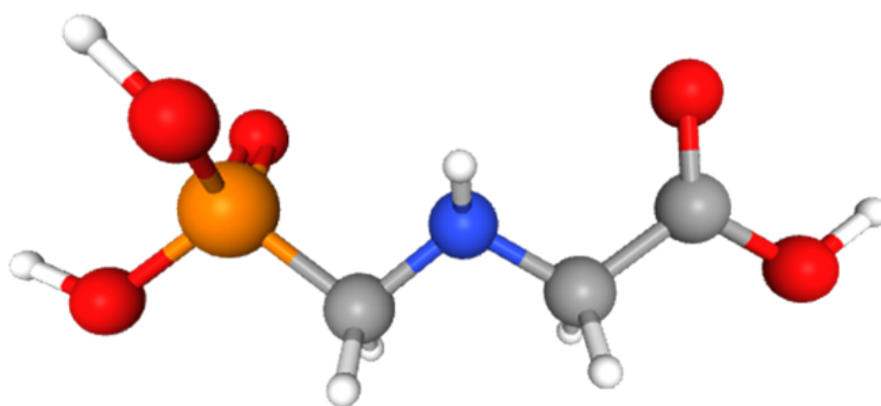


Figure 3.

Molecular structure of glyphosate. The red, gray, white, and orange spheres represent oxygen, carbon, hydrogen, and phosphorus respectively.

National Center for Biotechnology Information (2021).

Glyphosate, also known under the trade name “Roundup”, was developed by Monsanto Company in 1970, and commercialized since 1974 (de Castilhos et al., 2020; Lupi et al., 2015). Its popularity and application increased in 1996 when the Monsanto company developed a line of patented “Roundup Ready” seeds, which correspond to varieties of crops genetically modified to be tolerant to glyphosate inhibition (Torres & Romero-Natale, 2019). These resistance genes to this compound are transferred via bacteria or as the product of bioengineering techniques (Mantilla, 2020).

Concurrently, Chemical structure of Glyphosate is buildup of direct Carbon to Phosphorus (C-P) and Carbon to Nitrogen (C-N) bonds, these bonds can be split relatively quickly by the action of soil microorganisms (Ximenis, 2019). The principal degradation products are sarcosine and aminomethylphosphonic acid [AMPA] (Candela et al., 2010; Mesnage & Antoniou, 2018), whose degradability rate is usually slower (Table 1) (Sterren et al., 2016). Glyphosate is a highly soluble compound in water (Gros et al., 2017), with a low octanol-water partition coefficient ($\text{LogKow}=-3.2$); it presents a high affinity for organic carbon (Log KoC) and high absorption in the soil, which represents low mobility in this environmental compartment (ATSDR., n.d.; Lutri et al., 2020). Also, the adsorption of glyphosate in the soil will depend on its dissociation capacity (Huaracahuaraca, 2017) or constant dissociation ($\text{pKa: } 2.0 - 2.6 - 5.6$), which indicates that this compound will exist almost completely in zwitterionic form, i.e., tends to be adsorbed strongly in soils containing organic carbon and clay than its neutral counterparts (NCBI, 2021).

Glyphosate is applied by spraying and due its low sedimentation rate it is distributed through the air as aerosols (Torres & Romero, 2019). Approximately 75% of the compound remains in the field of which 12% reaches the ground through direct contact, leaf washing, root exudation, and treated plants, and one of the main factors that affect the environmental fate of glyphosate is the soil moisture (Villarreal, et al., 2020) The remaining percentage is lost to atmospheric drift, reaching non-target fields and plants, and a very small amount (3%) reaches bodies of water (Huaracahuaraca, 2017; Soares, 2019).

Table 1.

Physicochemical properties of Glyphosate, AMPA and Sarcosine.

Compuesto	Molecular formula	Relative molecular mass (g/mol)	K _{ow} at pH 7, 20 °C (LogP)	DT ₅₀ /DT ₉₀ (field), days Soil	Solubility in water at 20 °C (g L ⁻¹)	Henry's Law Constant (Pa.m ³ .mol)	Source
Glyphosate	C ₃ H ₈ NO ₅ P	169.07	-3.2 a 25 °C	23.79/ 169.68	10.5	2.1X10 ⁻⁹	(NCBI,2021), (Lewis, et al., 2016), (EPA,2015)
AMPA	CH ₆ NO ₃ P	111.0	-1.63	1000	1466.561		(Lewis, et al., 2016)
Sarcosine	C ₃ H ₇ NO ₂	89.09	-3.1	-	308		(NCBI,2021), (VCCLAB,2005)

authors.

Chemical persistence is quantified by the half-life ($t_{1/2}$, in days), representing the time required for a compound to decrease to half its initial concentration (Sterren et al., 2016). In the case of glyphosate, its persistence in soil ranges from 1 to 197 days, depending on various soil and experimental conditions (Ramírez-Haberkon et al., 2021; Yang et al., 2015). In addition, the number of days necessary for glyphosate to reduce to 90% of the initial concentration in this case, which for glyphosate varies between 40 and 280 days (Ximenis, 2019). According to the literature review, scarce data were found about the degradation times of glyphosate in aqueous media. However, as shown in (Table 2) $t_{1/2}$ at 20°C by aqueous hydrolysis is within the threshold established by England and the European Union (0.1 μgL^{-1}) (European Commission, 2009). Moreover, through aqueous photolysis, glyphosate in water achieves a half-life ($t_{1/2}$) of 69 days at pH 7, rendering it stable. Notably, glyphosate's half-life is influenced by pH changes, decreasing significantly to 33 days at pH 5 and extending to 77 days at pH 9. Its half-life, when subjected to aqueous hydrolysis in a water matrix at pH 7 and 20°C, remains stable within a pH range of 5 to 8 at 25°C (Lewis et al., 2016).

Table 2.
Glyphosate Persistence in Soil and Water

Degradation	Soil (aerobic degradation, in days)	In water (days)	Interpretation
$t_{1/2}$ (typical)	15.0	-	No persistent
$t_{1/2}$ (Lab, 20°C)	15.0	-	No persistent
$t_{1/2}$ (field)	23.79	13.82 – 301	No persistent in soil Moderately high persistence in rivers (EFSA, 2015)
Aqueous photolysis DT_{50} (days) a pH 7	-	69	Stable
Aqueous hydrolysis $t_{1/2}$ (days) a 20 °C y pH 7	-	Stable	Stable
Water-sediment $t_{1/2}$ (days)	-	74.5	Moderately fast
Water phase only $t_{1/2}$ (days)	-	9.9	Moderately fast

Adapted from Lewis, et al. (2016) and EFSA (2015).

Note. $t_{1/2}$: half-life.

The biodegradability of glyphosate can be affected by several factors, including bioavailability, soil composition, and soil microorganism activity, as stated by Guijarro (2019). Guijarro et al. (2018) found that the higher application rate of glyphosate compared to its dissipation rate favors its persistence in the environment. They also noted that agricultural management practices and land use can indirectly modify the structural and functional diversity of microbial populations as well as soil properties, thus affecting glyphosate's persistence.

Degradation Products of Glyphosate, Sarcosine and Aminomethylphosphonic Acid (AMPA)

Two primary metabolic pathways are involved in the breakdown of glyphosate in soil by microorganisms (Figure 4). The first pathway leads to the formation of sarcosine and inorganic phosphate through the action of a C-P lyase enzyme. The second pathway involves glyphosate oxidoreductases that convert glyphosate into aminomethylphosphonic acid (AMPA) and glyoxylate (Mantilla, 2020; Aslam, 2023). AMPA is a compound that is often found in the environment, particularly in water. It is formed when glyphosate and amino-polyphosphonates break down. The widespread use of these chemicals contributes to the prevalence of AMPA.

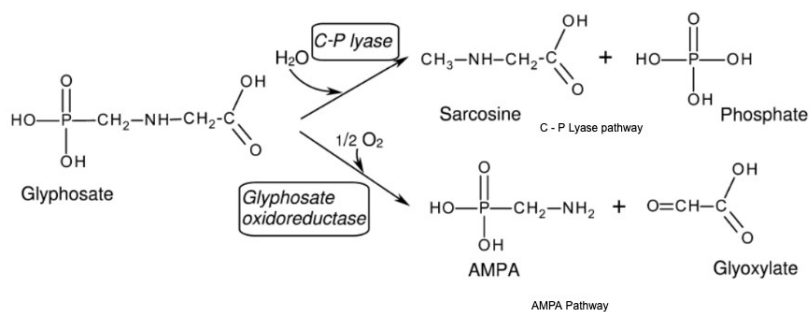


Figure 4.

Main metabolic routes of degradation of Glyphosate.

Adapted from: Pollegioni et al. (2011).

AMPA can also be a substrate for C-P lyase and subsequently oxidized to CO₂, and glyoxylate can be metabolized as a carbon source by microorganisms (Tauhata et al., 2020). The C-P lyase catalyzes the first metabolic pathway of breaking the C-P bond, releasing sarcosine and phosphate, which can be utilized as a phosphorus source by microorganisms. However, the metabolic conversion of glyphosate to sarcosine occurs less frequently in agricultural soils due to the high levels of phosphorus and the more stable C-P bond, which may be regulated by the concentrations of inorganic phosphorus in the soil. (Mantilla, 2020). Glyphosate is a commonly used herbicide that can be degraded through multiple metabolic pathways. For instance, AMPA can be broken down through C-P lyase and converted into CO₂, while glyoxylate can be utilized as a source of carbon by microorganisms (Tauhata et al., 2020). When the C-P bond is broken, sarcosine and phosphate are released, with the latter being utilized as a phosphorus source by microorganisms. However, due to the stability of the C-P bond and the abundance of phosphorus in agricultural soils, the metabolic conversion of glyphosate to sarcosine occurs less frequently and is regulated by the concentrations of inorganic phosphorus in the soil (Mantilla, 2020). The C-P lyase complex is only activated in response to intracellular inorganic phosphorus deficiency, which is not typical in natural environments. As a result, the degradation mechanisms through the C-P lyase and sarcosine pathways are still relatively unexplored, both molecularly and biochemically (Álvarez & Chávez, 2019). However, studies have found that in water sediment, the sarcosine degradation pathway is the first to occur, associated with microbial growth, while the AMPA pathway occurs later under starvation conditions and a lack of nutrients (Wang et al., 2016). In these environments, sarcosine is rapidly oxidized to glycine and incorporated directly into the microbial biomass, implying that the preferential degradation route is not necessarily through AMPA formation, as it is energetically more favorable for the cell to produce sarcosine (Okada et al., 2017).

Biodegradation processes of glyphosate vary according to the type of soil, and environmental factors (Ximenis, 2019). Some studies suggest that the bioavailability of the compound is also fundamental to the degree of total dissipation of the same in the soil (Guijarro, 2019). Glyphosate degrades more easily in soil, although its persistence can vary widely than its metabolite AMPA, which is more persistent ($t_{1/2}$ = 23-953 days) (Ximenis, 2019). Thus, the decomposition rate of AMPA is considerably lower than that of glyphosate, with a half-life of 3.5 times longer than the half-life of glyphosate (Ramirez Haberkon et al., 2021). Several studies indicate that the bioavailability of this compound plays an essential role in its complete dissipation in the soil (Guijarro, 2019). The dispersion behavior of glyphosate varies depending on the type of soil and environmental conditions. Although glyphosate degrades more easily in the earth, its persistence can show a wide variability compared to its metabolite AMPA, which proves to be more persistent ($t_{1/2}$ = 23-953 days) (Ximenis, 2019). Thus, the decomposition rate of AMPA is considerably lower than that of glyphosate, with a half-life that can be 3.5 times longer than that of glyphosate (Ramírez et al., 2021).

Furthermore, the factors that most affect the residence time of glyphosate and AMPA concentrations in the soil are related to their partition coefficients in the different environmental matrixes, that define, for example, the sorption characteristics on mineral particles, bioaccumulation potential, and tendencies to occur in the environment (Benintende, 2016). In general, other factors that favor pesticide dissipation processes also include organic matter content, type and proportion of soil minerals, cation exchange capacity, pH, pore space, and pore size distribution (Aparicio et al., 2015).

Transport and fate of Glyphosate in the environment

The apolar zones of the molecules facilitate rapid and strong absorption by soil particles, preventing their mobility and leaching (Torres & Romero-Natale, 2019). Furthermore, glyphosate has a low vapor pressure [1.94×10^{-7} mmHg at room temperature], giving the glyphosate the capacity to volatilize at a moderate rate (Maria et al, 2020; Carriquiriborde, 2021). Therefore, glyphosate can reach surface water sources in two ways, one is by spraying in the fields and another by erosion generated by the wind that drags the soil particles enriched with it. As shown in (Figure 5), the transport of glyphosate and AMPA follows a similar behavior (Mas et al., 2020). As well as runoff that also carries soil particles containing glyphosate into surface water ecosystems (Ximenis, 2019), especially in intense rain events, and if it happens just after the application of Glyphosate (the same happens with AMPA), even when these two substances tend to stay in the superficial part of the soil, this drag can occur (Grandcoin et al., 2017). In addition, factor that limits the leaching of Glyphosate to surface waters is biodegradation by soil microorganisms and the similarity between the chemical structures of glyphosate and other phosphate molecules, which establish competition between both compounds towards soil sorption sites (Carles et al., 2019).

Partition coefficients as octanol-watercoefficient (K_{ow}) which determines the hidrophobicity, the potential to bioacumulated in fatty tissues or the possibility to be biodegraded (Ximenis, 2019; Cumming & Rücker, 2017; Maria et al., 2020). For the case of glyphosate, as shown in Table 2, the $K_{OW}=-3.2$, shows that it is a highly hydrophilic compound, so the probability to bioaccumulation is insignificant, however, it often tends to be absorbed by sediments (Maria et al., 2020). That is, it has a greater affinity for organic matter that is generally retained in the sediments, therefore, it has less capacity to move through the aqueous phase (Carriquiriborde, 2021). As for AMPA and Sarcosine, they are expected to behave in a similar way as its parental compound (Lewis, et al., 2016). Despite the aforementioned, it is essential to emphasize that any chemical substance could exert detrimental effects on the environment if it is present in sufficiently high concentrations.

Based on its absorption coefficient K_{oc} range of 2,600 to 4,900, Glyphosate has slight mobility in soils (NCBI, 2021). However, erosive processes and runoff are the main transport processes of glyphosate to aquatic systems. The data in the table are European Union regulatory and evaluation data published by the European Commission (EC), European Food Safety Authority (EFSA) (Renewal Assessment Report (RAR), Draft Assessment Report (DAR) and conclusion files), and European Medicines Agency (EMA) (Lewis, et al., 2016). Regarding AMPA, it is also considered highly soluble (Criterion: $> 500 = \text{High}$). (Lewis, et al., 2016). Consequently, for example, studies carried out in the Suquía river basin in Córdoba, Argentina, found that the highest concentrations of glyphosate and AMPA were found in the sediments compared to the water (Bonansea et al., 2017). Although Glyphosate is soluble in water, due to its partition coefficient (K_{oc}) in aquatic systems it tends to transfer to sediments (Huaracahuaraca, 2017).

With an absorption coefficient K_{oc} range of 2,600 to 4,900; glyphosate demonstrates limited mobility in soils (NCBI, 2021). Nonetheless, erosion and runoff serve as the primary means of transporting glyphosate to aquatic systems. The data presented in the table originate from European Union regulatory and evaluation sources, including the European Commission (EC), the European Food Safety Authority (EFSA) (Renewal Assessment Report (RAR), Draft Assessment Report (DAR), and conclusion files), as well as the European Medicines Agency (EMA) (Lewis, et al., 2016). As for AMPA, it is also regarded as highly soluble (Criterion: $> 500 = \text{High}$) (Lewis, et al., 2016). Consequently, studies conducted in the Suquía river basin in Córdoba, Argentina, for instance, revealed that the highest concentrations of glyphosate and AMPA were found in sediments rather than in water (Bonansea et al., 2017). Although glyphosate is water-soluble, its partition coefficient (K_{oc}) in aquatic systems causes it to predominantly transfer to sediments (Huaracahuaraca, 2017).

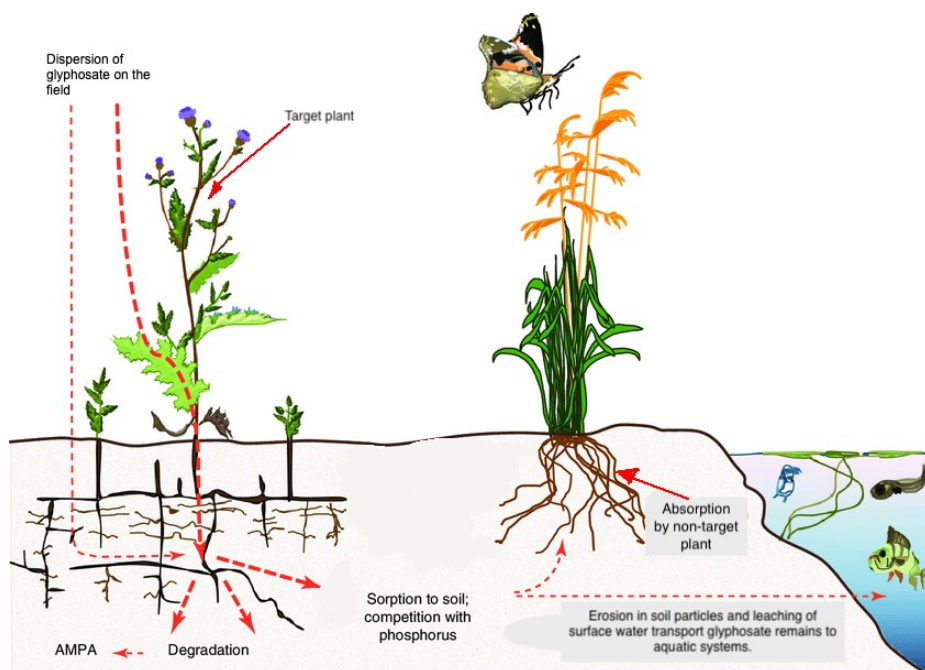


Figure 5.

The fate of glyphosate in the environment.

Source: Adapted from (Helander et al. (2012). (Red Dashed Lines) transport glyphosate from air, soil, and water. (Red Continuous Line) glyphosate Absorption in Plants.

A study conducted in China it was observed that Glyphosate and AMPA initially tend to be absorbed mostly in the upper 2cm of the soil, instead of being transported and absorbed deeper, however, residues of AMPA and glyphosate were found but in lower concentrations at as it went deeper into the soil (Yang et al., 2015). Glyphosate percolation into groundwater is very low given its strong affinity with the soil (Ximenes, 2019).

Another study carried out in seven states of the United States more than 90% of the samples from different watersheds were contaminated with pesticides, of which AMPA, glyphosate, and atrazine were found to have the highest presence, with a 33%, 21% and 18% of the samples respectively (Battaglin et al., 2016). Also, in Argentina, in the east of the province of Santiago del Estero, three types of water sources were monitored to determine the environmental fate of pesticides, and herbicides were found to be more prevalent. The most important compounds were glyphosate, atrazine, AMPA and hydroxyatrazine (HOA) (Mas et al., 2020).

Some studies conducted in the United States found glyphosate in air and rain in the range of 60 to 100% of samples collected over two growing seasons (Grandcoin et al., 2017). AMPA was also found in a range of 40-90% of the rain samples and 60-90% of the air samples. However, the proportion of applied glyphosate that is released into the atmosphere is unknown. (Grandcoin et al., 2017). In addition to this, wind erosion also has a great influence on the transport of glyphosate and AMPA with dust in suspension toward bodies of water, especially in semi-arid areas. For example, in Argentina, in the province of Chaco, studies were carried out on the sediment of bodies of water that confirmed that the material eroded by the wind contributes to the contamination of water with Glyphosate (Concentration found: $0.66-313 \mu\text{g kg}^{-1}$), and with AMPA

(Concentration found: $1.3\text{--}83 \mu\text{g kg}^{-1}$) (Mas et al., 2020). When the polluted particles reach the surface waters, they tend to adsorb to the bottom sediment; where the biodegradation of glyphosate is much slower (Zirena et al., 2018), the concentrations of glyphosate were found to range from 0.66 to $313 \mu\text{g/kg}$, while those of AMPA varied between 1.3 and $83 \mu\text{g/kg}$ (Mas et al., 2020). As contaminated particles enter surface waters, they tend to adhere to the bottom sediment, where the biodegradation of glyphosate occurs at a considerably slower rate (Zirena et al., 2018). Yang et al. (2015), in a study conducted at different slope gradients and application rates in plots with loess soil in the Loess Plateau in China, observed that the decomposition rate of glyphosate in this type of soil is rapid, and suggests that special care should be taken in areas with highly erosive rainfall due to off-site transport.

4. DISCUSSION: ECOTOXICOLOGICAL RISK ASSESSMENT

Table 3 presents a consolidated matrix of the ecotoxicological risk assessment of glyphosate in water, covering both chronic and acute tests. Acute tests on *Artemia franciscana* and *Microcystis aeruginosa* have shown that the continuous use of herbicides containing the isopropylamine salt of N-(phosphonomethyl) glycine can have highly toxic effects on zooplanktonic and phytoplanktonic organisms in aquatic environments (Solís-Gonzalez et al., 2019). Additionally, contamination with the commercial herbicide GLIFOPAC in water bodies has been found to have short- and medium-term toxic potential for organisms such as *Daphnia Magna* and *Artemia salina* (Huaracahuaraca, 2017).

Also, chronic exposure of goldfish (*Carassius auratus*) to glyphosate at low levels (34mgL^{-1}) altered metabolism in various tissues, producing oxidative stress (Li et al., 2017). As well as, in chronic tests on *Daphnia Magna* and *Ceriodaphnia Dubia*, they observed that *Daphnia magna* ephippia exposed to the substance exhibited modifications in their life cycle, as evidenced by the production of eggs that did not successfully develop. In exposed organisms where the net reproductive rate (R_0) < 1, presented a population decrease and possible local extinction in the environments disturbed by the evaluated herbicides (Reno et al., 2016). Likewise, acute tests on *Artemia franciscana* showed high toxicity (Category I: highly toxic, US EPA) at $\text{LC}_{50}(24) = 0.3054 \text{mgL}^{-1}$ and $24\text{h-NOEC} = 0.2488 \text{mgL}^{-1}$ (Solís-González et al., 2019).

Some acute tests on cyanobacteria *Microcystis aeruginosa* ($\text{IC}_{50}(72) = 53.95 \text{mgL}^{-1}$, form coefficient (CF) ≈ 1 , $72\text{h-NOEC} = 2.95 \text{mgL}^{-1}$, showed significant changes in its volume and cell surface $\text{IC}_{50}(72)$ of $7.69 \pm 1.69 \mu\text{m}^3$ with a 33% reduction in volume compared to the control cell and a category II toxicity (toxic) according to the US EPA classification (Solís-González et al., 2019). Another fundamental factor when evaluating the impacts on the ecosystem is the composition of Glyphosate, that is, its formulation with adjuvants, in particular surfactants such as polyoxyethylene amine (POEA) and MON 0818 (75% POEA) that can negatively impact the health of a variety of animals in the aquatic food chain, including protozoa, mussels, crustaceans, frogs, and fish, similar to the effects on terrestrial animals (Muñoz et al., 2020).

Because the composition of glyphosate is legally classified as confidential business information, confusion about the identity and concentrations of co-formulants is very common, raising concerns about the risks they may cause (Mesnage et al., 2019). In Argentina, one of the most used formulations in soybean production is Sulphosate Touchdown® (Syngenta Agro), a formulation based on glyphosate (potassium salt, 62%) (Fantón et al., 2020), and in a study carried out by Fantón et al. (2020), it was found that the presence of this herbicide in freshwater systems could pose a risk to the ecological role of *Notodiaptomus carteri* (copepods) in nature. On the other hand, Maria et al. (2020) indicated, according to the risk quotient, that glyphosate does not represent a risk for the survival or reproduction of microcrustaceans but can cause adverse effects on non-target and more sensitive organisms. However, AMPA did not present an apparent ecological risk, even with a toxic effect, probably due to its formation in low concentrations in the aquatic ecosystem. The maximum

environmental concentrations reached in the study of the aquatic microcosm (applying 100 Lha⁻¹) for the control of aquatic macrophytes did not show any risk for aquatic invertebrates. It is important to highlight in this study that glyphosate was applied in environments with the water surface completely covered by floating aquatic plants, a situation that could function as a filter to retain the surfactant, given that the commercial formulation of glyphosate is a mixture of substances that could make it more toxic than the active ingredient. In addition, within aquatic systems, copepods are dominant members of plankton communities and constitute a fundamental link in the food chain, they are small in size, sexually dimorphic and have a short life cycle, made up of twelve larval stages (*six nauplii* stages, five copepodite stages and adult stage) (Gutiérrez et al., 2011). In the case of *Notodiaptomus carteri* (freshwater copepod), chronic tests were carried out, in which it was found that glyphosate prevented copepods from reaching the adult stage, inhibited the growth of the first stage of copepodites and increased the activity of three enzymes. antioxidants, superoxide dismutase (SOD), catalase and glutathione-S-transferase (GST) in adult females. The lowest concentration of glyphosate increased the nauplii stages and the total development time (Fantón et al., 2020).

Finally, the evaluation of the acute toxicity of two commercial herbicides formulated with glyphosate and of a solution of the same (see Table 3. In section formulation a, b, c *) carried out by Álvarez et al., 2012, who compared to fish of the species *Poecilia reticulata* "lebigest" attributes to formulation B: Round-Up® (Conc. of glyphosate: 48% (480 gL⁻¹) – Excipients: POEA isopropylamine Ac. Orgánicos) the mortality of 100% of the specimens at 100 µL L⁻¹ (equivalent to 48 mgL⁻¹ of active ingredient) and 50 µL L⁻¹ (equivalent to 24 mgL⁻¹ of active ingredient); on the other hand, the solution formulated with pure glyphosate did not produce mortality even at concentrations of 400 mgL⁻¹. In the case of the chronic evaluation, using sublethal doses based on the data obtained in the acute toxicity test, it was possible to determine that in the long term, specimens of *Cyprinus carpio haematopterus* "carp koi" showed severe hematological and histological alterations compared to the experimental model used.

Table 3.
Ecotoxicological Risk Assessment Research of Glyphosate in Water

Endpoints	Organism studied (OE)	Type of Exposure	Concentrations	Effects	Tested formulation	Reference
Life history attributes and the population parameter (Ro)	<i>D. Magna</i> & <i>C. Dubia</i>	Chronic: 21 dias	0.15 y 0.62 mgL ⁻¹	<i>D. Magna</i> exposed experienced life cycle changes due to aborted eggs	Panzer Gold® (Dow Agrosiences)	(Reno et al., 2016)
			0.1 y 0.31 mgL ⁻¹	Exposed environments with Ro <1 showed population decrease and possible local extinction due to evaluated herbicides.	(Sultosate Touchdown® y Panzer Gold®)	
Median lethal concentration (CL50(24))	<i>Artemia franciscana</i>	Acute:25 h	CL50=0.3054 mgL ⁻¹ (0.2983-0.3151) NOEC=0.2488 mgL ⁻¹ (0.2370-0.2569)	US EPA Category I (highly toxic). [C50(72) of 7.69±1.69 µm ³ reduces volume by 33% and alters cell surface while remaining spherical.	Glyphosate with a purity of 97% [C ₃ H ₇ NO ₅ P] (La FAM®)	(Solis-González et al., 2019)
Population means inhibitory concentration (CI50) and the shape coefficient (CF)	<i>Microcystis aeruginosa</i>	Acute: 72 h	CI50=53.95mgL ⁻¹ CF=1, 72h-NOEC =2.95 mgL ⁻¹ .	Category II (toxic) according to the US EPA classification.		
Risk Quotients (RQ)= to assess chronic risk.	Fish <i>O. mykiss</i> Glyphosate	Chronic: NOEC=85 days	fish=9.6mgL ⁻¹	Chronic risk is high in sites with glyphosate plus AMPA (>60%) and ERQs> 1. At-OH, AMPA and flurochloridone were main contributors to low risk in other sites.	-	(Pérez et al., 2021)
	Fish <i>Pimephales promelas</i> AMPA	Chronic: NOEC=33 days				
	<i>D. magna</i> .	Chronic Invert: NOEC= 21 days.	Invert= 12.5 mgL ⁻¹			

Source: authors.

Note: C: Chronic; A: Acute; RQ: Risk Quotient, RQ<1: no ecotoxicological risk effects, RQ>1: ecotoxicological risk effects; UT: Toxic Units , $\sum UT < 1$ no acute effects, $\sum UT > 1$ acute effects; LC50: concentration of a substance that is lethal to 50% of the test organisms in a given time period; LC99 refers to the concentration of a substance required to cause 99% mortality in a population of test organisms; NOEC: highest concentration without adverse effects; LOEC: Lowest Observed Effect Concentration; IC50: the concentration of a substance that inhibits 50% of a biological activity or response in a test organism or cell line.

Tabla 3.
Continuation.

Endpoints	Organism studied (OE)	Type of Exposure	Concentrations	Effects	Tested formulation	Reference
Toxic Units (TU)= to assess acute risk	algae <i>Pseudokirchneriella subcapitata</i> (Growth inhibition)	Chronic: 96h EC ₅₀	Algae=2.0mgL ⁻¹			
	Fish <i>Oncorhynchus mykiss</i>	Acute: 96 h CL ₅₀	fish =38 mgL ⁻¹	$\sum TU < 1$, no acute ecological risks for 3 trophic levels studied (algae, daphnids, fish).		
	<i>Daphnia Magna</i> .	Acute Invert: EC ₅₀ 48 h.	Invert=40mgL ⁻¹			
Algae: <i>Pseudokirchneriella subcapitata</i> (Growth inhibition)	Acute: 72 h EC ₅₀	Algae= 19 mgL ⁻¹				
Acute toxicity tests. EPA, (1995)	<i>D. Magna</i>	AD: 48 h	LC ₅₀ : 27.4 mgL ⁻¹ LC ₉₉ :65.90 mgL ⁻¹ (IC, 95%)	<i>D. Magna</i> 2.5x more sensitive, 26% survival at 14.4 mg/L, NOEC=0.45 mg/L, LOEC=1.35 mg/L for fertility. >0.8mg/L: Reproductive effects.	GLIFOPAC	(Huaracahuara, 2017)
Mortality, survival, Reproduction	<i>A. salina</i>	AA: 48h	LC ₅₀ : 70.4 mgL ⁻¹ LC ₉₉ : 253.6 mg. L ⁻¹ (IC, 95%)	AMPA: toxic to Artemia in saline water. GLIFOPAC toxic to Daphnia and Artemia in aquatic environments.		
Method of exposure to toxicity with static renewal.	Golden fish (<i>Carassius auratus</i>)	Chronic: 90 days with water renewal 24 h	0.2 mmol/L (34mgL ⁻¹)	Glyphosate causes biochemical and organ dysfunction, and metabolism disturbances in fish.	Glyphosate "Nongteshi®" (Londgao, Chemical Industry Co., Ltd, Wuxi, China)	(Li et al., 2017)
Risk quotient (RQ) RQ > 1.0: High Risk	<i>D. similis</i> (Immobility and/or mortality)	Acute: 48 h	Glyphosate: 27.5 mgL ⁻¹	RQ: 0.52-No effect	(Roundup Original®)	(Maria et al., 2020)

Source: authors.

Note: C: Chronic; A: Acute; RQ: Risk Quotient, RQ<1: no ecotoxicological risk effects, RQ>1: ecotoxicological risk effects; UT: Toxic Units , $\sum UT < 1$ no acute effects, $\sum UT > 1$ acute effects; LC50: concentration of a substance that is lethal to 50% of the test organisms in a given time period; LC99 refers to the concentration of a substance required to cause 99% mortality in a population of test organisms; NOEC: highest concentration without adverse effects; LOEC: Lowest Observed Effect Concentration; IC50: the concentration of a substance that inhibits 50% of a biological activity or response in a test organism or cell line.

Tabla 3.
Continuation.

Endpoints	Organism studied (OE)	Type of Exposure	Concentrations	Effects	Tested formulation	Reference
Life Cycle Assesement Ontogenetic Development and the biochemical markers	<i>Notodiaptomus carteri</i> (freshwater copepod)	Chronic: 30 dias Chronic:10 dias	AMPA: 12.5 mgL ⁻¹	RQ: 0.01 - No effect	Sulfosate Touchdown® (Syngenta Agro)	(Fantón et al., 2020)
			Roundup: 13.6 mgL ⁻¹	RQ: 2.02		
			Glyphosate: 11.46 mgL ⁻¹	RQ: 1.59		
			AMPA: 20.25 mgL ⁻¹	RQ: 0.03 - No effect		
			Roundup: 20.16 mgL ⁻¹	RQ: 0.90 - No effect		
			Glyphosate: 27.5 mgL ⁻¹	RQ: 0.66 - No effect		
			AMPA: 12.5 mgL ⁻¹	RQ: 0.04 - No effect		
			Roundup: 3.6mgL ⁻¹	RQ: 5.06		
			Low glyphosate concentration increases nauplii and development time. High concentration inhibits copepod growth and antioxidant enzyme activity.			

Source: authors.

Note: C: Chronic; A: Acute; RQ: Risk Quotient, $RQ < 1$: no ecotoxicological risk effects, $RQ > 1$: ecotoxicological risk effects; UT: Toxic Units, $\Sigma UT < 1$ no acute effects, $\Sigma UT > 1$ acute effects; LC50: concentration of a substance that is lethal to 50% of the test organisms in a given time period; LC99 refers to the concentration of a substance required to cause 99% mortality in a population of test organisms; NOEC: highest concentration without adverse effects; LOEC: Lowest Observed Effect Concentration; IC50: the concentration of a substance that inhibits 50% of a biological activity or response in a test organism or cell line.

Tabla 3.
Continuation.

Endpoints	Organism studied (OE)	Type of Exposure	Concentrations	Effects	Tested formulation	Reference
Mortality	<i>Poecilia reticulata</i> "lebetes"	Acute: 96 h	Formulations A and B: 100 $\mu\text{g L}^{-1}$; 50 $\mu\text{g L}^{-1}$; 25 $\mu\text{g L}^{-1}$; 12,5 $\mu\text{g L}^{-1}$ Formulation C: 100 mg L^{-1} ; 200 mg L^{-1} ; 400 mg L^{-1}	Herbicide B is 4x more toxic than A, causing 100% mortality at 25 $\mu\text{g L}^{-1}$. Active ingredient not toxic at up to 400 mg L^{-1} .	*Formulation A is Glacoxan®, with a glyphosate concentration of 48% and no excipients. Round-Up® Formulation B contains 48% glyphosate and excipients such as POEA, isopropylamine, and organic acids. Formulation C - Conc. of glyphosate 0.6% (6g L^{-1}) - Excipients: Does not possess	(Alvarez et al., 2012)

Source: authors.

Note: C: Chronic; A: Acute; RQ: Risk Quotient, $RQ < 1$: no ecotoxicological risk effects, $RQ > 1$: ecotoxicological risk effects; UT: Toxic Units, $\Sigma UT < 1$ no acute effects, $\Sigma UT > 1$ acute effects; LC50: concentration of a substance that is lethal to 50% of the test organisms in a given time period; LC99 refers to the concentration of a substance required to cause 99% mortality in a population of test organisms; NOEC: highest concentration without adverse effects; LOEC: Lowest Observed Effect Concentration; IC50: the concentration of a substance that inhibits 50% of a biological activity or response in a test organism or cell line.

In recent times, there has been an increased interest in investigating the actual toxicological effects of glyphosate due to updated risk assessments of glyphosate exposure by the US Environmental Protection Agency (US EPA) (Carranza et al., 2019). Glyphosate and its degradation product, AMPA, were classified as a probable human carcinogen in Category 2A by the International Agency for Research on Cancer (IARC) in 2015. However, the US EPA found no evidence to support the classification of glyphosate as a human carcinogen (Benbrook, 2019). These recent regulatory reviews and reports have raised important questions regarding the potential health and environmental impacts of glyphosate, making it an important topic for further research and discussion in the scientific community.

The US EPA's disagreement with the IARC's conclusion on the probable carcinogenicity of glyphosate is well-documented. According to the EPA's official website, they relied on 15 approved carcinogenicity research found in the open literature, whereas the IARC based its classification only on eight animal carcinogenicity studies. However, the IARC maintains that its classification of glyphosate as a probable carcinogen (Category 2A) is based on limited evidence of cancer in humans exposed to realistic concentrations of glyphosate, as well as sufficient evidence of cancer in experimental animals exposed to pure glyphosate. The IARC classification was determined following rigorous procedures and criteria, which included independent experts compiling and reviewing all relevant and publically available studies.

On the other hand, Mas et al., (2020), in a study carried out in agricultural areas in Argentina, where rainwater has become an important source of drinking water, found that glyphosate and AMPA presented the highest concentrations in the monitored sites (dams, followed by cisterns and wells); although the risk assessment showed that pesticides from all sources presented a low potential risk to human health through the route of exposure to drinking water. Also, Mac Loughlin et al., (2022) also concluded in a study carried out in a Argentinian basin that finding glyphosate and AMPA in a basin used for horticulture indicates that

this herbicide is not limited to resistant crops anymore, but is now being used in other agricultural practices; even though Serra-clusellas et al., (2017) advised that for the development of technologies associated with the elimination of glyphosate, it is also necessary to consider the elimination of AMPA, since it has an even greater degree of persistence in water than glyphosate itself.

5. CONCLUSIONS AND RECOMMENDATIONS

Concerns have been raised about the high incidence of glyphosate in diverse ecosystems due to its widespread and indiscriminate use in the control of weeds. The scientific community is particularly concerned about communities exposed to unknown quantities of glyphosate and AMPA in water, particularly those that use untreated rain or surface water for human consumption, such as agricultural populations.

The environmental risk posed by glyphosate and its degradation products remains a topic of concern. Future research should focus on the transport and transformation of glyphosate, AMPA, and sarcosine in aquatic systems, with attention paid to their particularities. It is recommended that more studies be conducted to investigate the consequences of these degradation products on surface aquatic systems.

Finally, the contradictory results of recent glyphosate risk assessments performed by regulatory agencies such as the US EPA and the IARC highlight the need for additional research and discussion about the actual toxicological effects of glyphosate and its degradation product, AMPA. Potential health and environmental effects of glyphosate have significant effects on public health and environmental policy, underscoring the significance of continued research into the safety and dangers associated with its use.

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