

## Glyphosate: risk or threat to human health and wildlife

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### Abstract

Glyphosate is the most used herbicide worldwide in agriculture over the past 20 years. Its use has led to side effects with direct damage to ecosystems, wildlife, and human health. Much of the world's scientific community has joined the task of researching the effects of this herbicide and documenting sustainable alternatives to reduce its use. There are contradictions in the scientific literature in this regard, so it was proposed to carry out a meta-analysis of the scientific information in order to identify the effects derived from the use of glyphosate on human health and the environment. A systematic search of the Scopus database was conducted from 2014 to 2022. It was found that the main metabolite aminomethylphosphonic acid and the surfactant polyoxyethyl amine are present in the commercial formulation of Roundup<sup>®</sup> (glyphosate), are persistent and present toxicity in different tissues and organs of terrestrial and aquatic species, in addition to decreasing agricultural production by affecting the growth of seedlings. Other positions point out that glyphosate does not cause effects or exhibit toxicity. However, these claims lack credibility because of the type of compound used in toxicity bioassays. This review concludes that, for eminently precautionary purposes, it is necessary to reduce the use of this agrochemical in agriculture, the long-term effect of which puts human health and biota at risk. And, therefore, the need to generate regulations that control their use or restriction.

### Keywords:

environmental damage, glyphosate, herbicide.

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The active ingredient, glyphosate (N-(phosphonomethyl) glycine), is one of the most commercialized herbicides worldwide and its application is growing exponentially (CEDRSSA, 2020). This herbicide is broad-spectrum, and its use is primarily agricultural to combat broadleaf and narrow-leaved weeds, the purpose of which is to increase and improve agricultural productivity in addition to eliminating weeds that cause damage or compete for space and nutrients with the main crop. Its use is not necessarily agricultural, as it is also used for forestry purposes, urban or rural gardening, and along communication routes such as highways, roads, and tracks (Villamar-Ayala *et al.*, 2019).

The main residues generated in the degradation of glyphosate are methylphosphonic acid (MPA), N-methylglycine (Sarcosine) and aminomethylphosphonic acid (AMPA), the former are degraded more easily and quickly (half-life <40 days). However, the metabolite AMPA exhibits the same harmful effects as glyphosate but has a longer persistence in the environment (half-life of 76 to 240 days) (Chen *et al.*, 2022). The recent ban on glyphosate by the Mexican federal government (DOF, 2020) has sparked scientific interest. This debate centers on whether the evidence on this herbicide is entirely conclusive in determining its ban. The aim of this meta-analysis was to review the published scientific information regarding the effects of glyphosate in order to identify its effects and risks to human health and the environment.

The search for publications was carried out in the Scopus database; Boolean operators were used, in Spanish and English, whose search terms were glyphosate or 'roundup', the latter corresponds to the commercial name and 'use in agriculture', 'surface water', 'groundwater', 'toxicity', 'human health', and the publications from the period from 2014 to 2022 (last nine years) were reviewed. From the information collected, analyses were made of the main author, year of publication, origin in terms of country, study method or type of research, and most relevant conclusions.

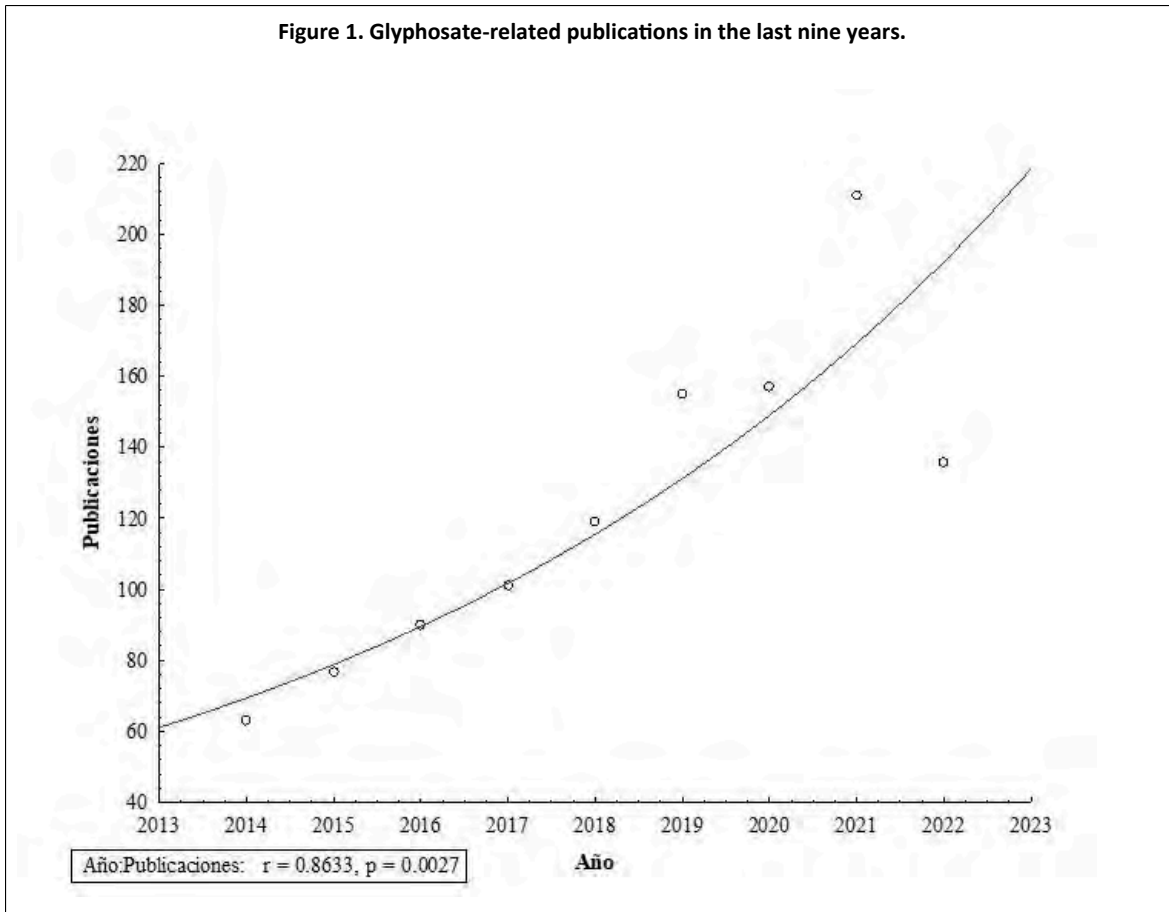
To facilitate the presentation of the results and their discussion, the information was generated through tables and graphs that expose the results and other considerations. The Statistica 7.0 software was used to generate graphs regarding the relevant topics, spatial-temporal analysis of the publications, as well as their correlation and areas with the highest number of publications. In addition, the VOSviewer software was used to visualize the co-occurrences of main terms, authors, and countries that provide evidence of the relevance of the topic.

In the search for publications in the Scopus database, only scientific articles were taken into consideration. A total of 1 109 publications were found. These publications were divided by areas, where the most represented areas are environmental sciences (61%), which includes toxicity studies in different environmental compartments such as public health, impacts on agriculture (24%) and other areas where detection and remediation methods are included (15%).

In 2021, a total of 211 articles were published and the accumulated up to the third quarter of this year represented 64.5% of the previous year's publications. This denotes that the issue is current and growing exponentially. To corroborate this, a correlation analysis was applied between the variables of years and number of articles published, finding that there is a strong degree of association between both variables ( $r= 0.8633$ ), which indicates that publications are increasing (Figure 1).



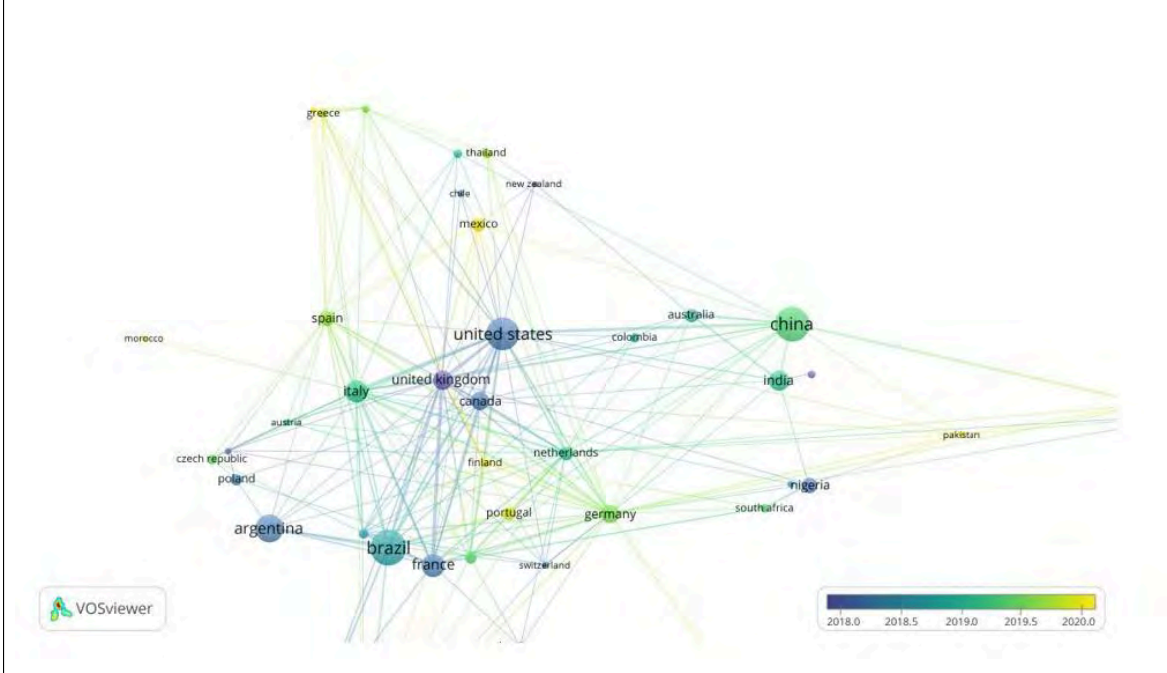
Figure 1. Glyphosate-related publications in the last nine years.



Mesnage *et al.* (2022) stand out among the main authors in glyphosate research. According to the co-occurrence network, the top ten countries that have published the most, in descending order, are Brazil, China, the United States of America, Argentina, Italy, France, the United Kingdom, India, Germany, and Canada. In the case of Mexico, it ranks thirteenth with 27 publications (Figure 2). It was found that there are countries with a lower number of publications, and, although they are not among the countries with the largest number of publications, their publications are the most recent, such is the case of Mexico, Spain, Germany, among others, and they can be visualized in the network of co-occurrences with a coloration from light green to yellow (Figure 2).



Figure 2. Network of co-occurrences from countries with a higher number of publications to countries with a lower number.



## Damage to biota and human health

There are reports indicating the potential harms related to the toxicity of glyphosate from direct and indirect exposure to this compound. Table 1 describes some of the most reported effects on biota (terrestrial and aquatic). It should be noted that some of these effects have been evaluated at the *in vitro* level, indicating that they may most likely occur in free-living species. For example, plasma concentrations of glyphosate were found in free-living manatees in South Florida, although an immediate effect has not been observed, it does pose a medium- and long-term risk (De María *et al.*, 2021).

Table 1. Reported effects of glyphosate on terrestrial and aquatic organisms *in vivo* and *in vitro*.

Organism	Effect	Result	Source
<i>Amaranthus rudis</i>	It decreases antimicrobial resistance of roots	Conclusive	Rosenbaum <i>et al.</i> (2014)
Juveniles of streaked prochilod ( <i>Prochilodus lineatus</i> )	Genotoxicity	Conclusive	Moreno <i>et al.</i> (2014)
Willow ( <i>Salix miyabeana</i> )	It decreases chlorophyll contents and photosynthesis	Conclusive	Gomes <i>et al.</i> (2016)
<i>Austrolebias nigrofasciatus</i>	Damage to reproduction and embryo development	Conclusive	Faria <i>et al.</i> (2021)
Mice	It affects locomotion and sociability, causes anxiety and cognitive impairment	Conclusive	Bali <i>et al.</i> (2019); Ait-Bali <i>et al.</i> (2020)

Organism	Effect	Result	Source
Soil bacteria	They modify microbial activity (decrease enzymatic capacity)	Conclusive	Chávez-Ortiz <i>et al.</i> (2021)
Tadpoles: <i>Boana faber</i> , <i>Leptodactylus latrans</i> , and <i>Dendropsophus minutus</i>	Genotoxicity, hepatotoxicity, mutagenicity, alteration of growth and mobility	Conclusive	Pavan <i>et al.</i> (2021); Lopes <i>et al.</i> (2021)
Honeybees/bumblebees	Alteration in their beneficial intestinal microbiota, it affects their ability to move and balance	Conclusive	Motta <i>et al.</i> (2018); Crall, (2022)
Embryos of zebrafish ( <i>Danio rerio</i> )	Mortality, it affects embryo growth and development, and causes cardiovascular damage	Conclusive	Lu <i>et al.</i> (2022)
Pigs' oocytes	It affects the maturation of pigs' oocytes	Conclusive	Xing <i>et al.</i> (2022)
Megalopas of <i>Callinectes sapidus</i>	Acute toxicity at high concentrations	Conclusive	Cházaro-Olvera <i>et al.</i> (2022)

In the studies taken as a reference, it was reviewed and verified that they had verifiable results through tests or experiments and that they were not only based on reviews, based on this they were considered conclusive and inconclusive. Glyphosate has been found to cause damage at the embryonic level, in reproduction, in addition to affecting the musculoskeletal system, and modifying the deoxyribonucleic acid (DNA), in both aquatic and terrestrial species.

However, one of the effects that can be observed immediately is the damage to the root system of the plant and the microbial activity of the soil and this is reflected in the mortality of seedlings and decrease in agricultural yield. It is important to note that small traces of glyphosate pass into foods, as has been reported in the case of corn tortillas, cereals, honey, and even formula milk (Liao *et al.*, 2018; Thompson *et al.*, 2019).

Of the reported effects of glyphosate on human health, it is pointed out as an endocrine disruptor. Therefore, it is inferred that the increased use of this herbicide, as well as its use without safety measures, is associated with the occurrence of many human diseases, including different types of cancer, kidney damage, and mental illnesses such as autism, attention deficit hyperactivity disorder, Alzheimer's disease, and Parkinson's disease (Young *et al.*, 2015; Fluegge and Fluegge, 2016; Fortes *et al.*, 2016).

Studies carried out in the human population indicate that the presence of glyphosate and metabolites in the body or its wastes is related to occupational exposure (Rendón von Osten and Dzul-Caamal, 2017); it has also been evaluated using human cells *in vitro* (Table 2).

**Table 2. Effects of the herbicide glyphosate and its metabolites evaluated *in vitro* and visually in population.**

Sample	Effect	Results	Source
Observational and analytical research in 40 patients	Serum protein S100B predicted neurological complications in patients intoxicated (poisoning) with glyphosate	Conclusive	Lee <i>et al.</i> (2017)
<i>In vitro</i> line IMR90-c4 iPSC ( <i>in vitro</i> blood-brain	Accidental exposure to a high level of glyphosate can cause	Conclusive	Martínez and Al-Ahmad (2018)

Sample	Effect	Results	Source
barrier based on induced pluripotent stem cells)	neurological damage and impaired glucose metabolism		
Population-based case-control study	It increases the risk of child autism spectrum disorder	Conclusive	Von Ehrenstein <i>et al.</i> (2019)
<i>In vitro</i> cell line SH-SY5Y	Cytotoxicity and neurotoxicity of glyphosate and AMPA	Conclusive	Martínez <i>et al.</i> (2020)
<i>In vitro</i> cell line: HepG2, A549, and SH-SY5Y	Inhibition of cell proliferation when commercial glyphosate (glyphosate + surfactant POEA) was administered, but not when glyphosate salt alone was administered	Conclusive	Hao <i>et al.</i> (2019)
Cross-sectional study of 288 smallholder farmers	Positive association between glyphosate exposure and visual memory impairment	Inconclusive	Fuhrmann <i>et al.</i> (2021)
<i>In vitro</i> cell line Caco-2 (human intestinal epithelial cell) and HepG2 cell lines (hepatocytes)	Cell necrosis and oxidative stress	Conclusive	Mesnage <i>et al.</i> (2022)
<i>In vitro</i> human lymphocytes	Genotoxicity at high doses in lymphocytes	Conclusive	Tarboush <i>et al.</i> (2022)

The highest-risk route of exposure for glyphosate is the direct route. That is, due to frequent exposures in handling and applications in agricultural fields. However, the indirect route is also a point of attention since mainly aquatic organisms are exposed to glyphosate through contaminated water and humans are no exception, since they access glyphosate through this route, by using contaminated water and even food with residues of this pollutant.

Water, being a vital element for all activities, must maintain optimal conditions in terms of its quality, but this is uncertain; the presence of glyphosate and AMPA has been reported in different bodies of water, both surface and underground (Grondona *et al.*, 2022; Lima *et al.*, 2022). So, if the reported concentrations are compared with the European regulations that establish a maximum permissible limit (LMP) of  $0.1 \mu\text{g L}^{-1}$ , these concentrations would be well above.

The presence of glyphosate in water bodies is related to its use in agriculture (as sources of diffuse pollution) and to a lesser extent, but significantly, through wastewater (as point sources). The contamination of water by glyphosate in both ways is alarming and in the particular case of our country, it should be added that the regulations applicable to the discharge of water, specifically of a residual type in receiving bodies, do not contemplate this type of pollutants.

The agreement establishing the ecological criteria for the quality of the water (CE-CCA-001/89) to be used as a source of drinking water supply, recreational activities, agricultural irrigation, livestock use, aquaculture or as a support for aquatic life does not include glyphosate either. This pollutant is only mentioned in the Mexican regulation NOM-127-SSA1-2017, which establishes a maximum permissible limit in water of  $25 \mu\text{g L}^{-1}$  for human use and consumption, well above the limit established by the European Union ( $0.1 \mu\text{g L}^{-1}$ ) but well below what is established by Canada ( $280 \mu\text{g L}^{-1}$ ) and the United States of America ( $700 \mu\text{g L}^{-1}$ ).

It is evident that one of the most important challenges today is to maintain and ensure water quality to be able to carry out any activity that allows for safety in the consumption of water and food free of herbicide residues, so it is necessary that in Mexico the regulations regarding the monitoring of surface and groundwater quality are applied and public policies around the preservation of natural resources free of pollutants are generated in order to have good human health and preserve natural resources and wildlife.

Glyphosate, despite being a pollutant with low bioaccumulation capacity, can remain for long periods since its half-life in water can be from 7 to 142 days, this depends on the volume of water and rainfall. However, during this period it is in contact with aquatic and terrestrial organisms and with human beings, causing internal changes and alterations that may not be observed in the short term. The presence of glyphosate in surface and groundwater bodies poses health and environmental risks and its occurrence in the environment and humans can increase due to excessive applications and doses higher than recommended (Seehaus *et al.*, 2020).

## Controversies surrounding the toxicity of glyphosate use

There are different positions regarding the toxicity of glyphosate, which leads to contradictory opinions in the scientific community, and this has led to confusion. Meftaul *et al.* (2020) point out that, if glyphosate is used in a controlled manner, following a correct preparation, dosage, and handling, it does not represent a high risk to the environment. However, they note that glyphosate-based formulations are accompanied by surfactants such as polyoxyethyl amine (POEA), which alone is highly toxic, which may be increasing the toxicity of glyphosate. For this reason, and for eminently precautionary purposes, it is recommended to restrict the use of this type of surfactants in formulations.

Since 2000, there have been positions that state that glyphosate, as well as its metabolites and surfactants do not cause adverse damage to the development, reproduction or endocrine systems of humans and animals. These evaluations have been based on studies carried out by regulatory agencies that establish the safety of the use of products, as well as the protection of the environment. References from Kier and Kirkland (2012); Mink *et al.* (2012) point out that there is no conclusive evidence indicating a positive association between glyphosate exposure and any type of cancer, and that glyphosate-based formulations have no toxic risk under normal human or environmental exposure conditions. It should be noted that some of the data taken by these authors as references are not recent.

The results of Cuhra *et al.* (2016) describe flaws and misinterpretations in reviews by regulatory agencies, as most of the reviewed research determining the toxicity of glyphosate used glyphosate technical acid instead of the original formulations, that is, commercial herbicide such as Roundup 'weed & grass killer concentrate plus', which contains glyphosate salt and the surfactant POEA.

Authors such as Mesnage and Antoniou (2017) indicated that in the reviews they point out, there was zero toxicity of glyphosate, there were also biases and interests of a commercial and ideological nature, and they even indicated that the published reviews are from consultants of companies engaged in the commercialization of this herbicide. Results such as those of Novotny (2022) support this position and add that to date there is a conflict of interest or 'revolving door syndrome', which leads to favoring regulations for the benefit of the commercialization of this herbicide by large consortia.

In other words, despite the existence of scientific evidence from independent researchers that reveal the damages, they are discarded and agencies such as the European Food Safety Authority (EFSA), the Environmental Protection Agency of the United States of America (EPA) and the European Chemicals Agency (ECHA) do not include the long-term toxicity of glyphosate-based formulations in their regulations. This calls into question the objectivity of these agencies.

According to this review, the studies that have been analyzed to determine the toxicity of glyphosate are not recent and some reports come from researchers with possible conflicts of interest. In addition, it should be considered that the commercialization of this herbicide represents large revenues for the producing countries and the large monopolies. Despite this, there are legal controversies of damage by glyphosate to human health. An example is the case of farmer Dewayne Johnson from the United States of America, who developed terminal cancer (non-Hodgkin's lymphoma) from frequent exposure to products such as Roundup® and Ranger Pro®, who won the lawsuit against Monsanto in 2018. This is just one of the cases that made it to trial. To date, the list

of lawsuits worldwide against this multinational is long, unfortunately several of these cases have not made it to trial.

## Conclusions

Although glyphosate is a compound that maintains or increases agricultural production and may not generate harmful effects that can be seen in the short term, it is important to point out that in the medium and long term, its constant use puts the health of wildlife and especially human health at risk directly or indirectly. The scientific evidence is mixed with reports from independent scientists and from those with conflicts of interest with the transnationals that market this product, generating a contradictory scenario of the toxicity of this agrochemical and the risk it represents. This review allowed us to identify that research on the impacts of glyphosate is on the rise and describes that glyphosate-based herbicides have toxicity with effects on terrestrial and aquatic organisms used mostly as laboratory tests.

This indicates that glyphosate does indeed represent a risk and can have irreversible effects on biota, natural resources, and even human health. For this reason, it is necessary that the international agencies in charge of restricting and prohibiting the use of this type of herbicides are independent and equitably fair, always ensuring human health. It is necessary to propose and implement sustainable alternatives for weed control in agricultural fields that allow food security to be maintained.

Likewise, it is also necessary to provide up-to-date information on the effects of pesticides, promote a change in awareness that leads to the care of natural resources and train agricultural workers in the safety and handling of all types of agrochemicals. Finally, it is important to regulate and reduce the use of agrochemicals in agriculture and to carry out environmental monitoring of pesticide concentrations in water bodies and to update current regulations. This point is key to ensuring the protection of ecosystems, food safety, and the supply of safe drinking water for human consumption.

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