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STUDY ON THE EFFECTS OF SYSTEMIC HERBICIDES ON THE GERMINATION OF CULTIVATED PLANTS

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ABSTRACT
Along the years studies have been made regarding the implications and the effects of glyphosate on the environment, on cultivated plants, on people and animals that come in contact with it. Although it is one of the most widely used herbicides, glyphosate can also have negative effects in various domains if the quantity surpasses the admitted limits. Herbicides based on glyphosate are famous worldwide and are used on a large scale to control the perennial herbage, such as the thistle, as well as acting like a support in harvesting which accelerates the culture. The use of glyphosate has a rather large area, being used mostly in agriculture, its use by agricultures becoming a routine. Due to the increased use of GBHs which are attributed to its wide range of application and the appearance of GRCs, the residues of glyphosate from the cultures have generated concerns among people regarding its impact upon health. In order to be able to observe the importance and the effects of glyphosate in other domains apart from agriculture, I have performed a statistic search of scientific works regarding the fields of work in which glyphosate is used bus also of the number of works obtained for each field. The obtained results highlight the fact that the number of areas in which the effects of glyphosate can be observed is increasingly higher, the number of scientific works is larger and larger, side effects being discovered.
KEY WORDS: *glyphosate, toxicity, cultivated plants, herbicide*

INTRODUCTION

Herbicides are used to improve the productiveness and the quality of the cultures by reducing or inhibitting the growth of herbage, along with the fact that they work as a dehidration agent for different crops, such as: cereals (wheat, barley, oats, corn, sorghum, etc.), oily seeds (soy, canola, etc.), legumes (beans, peas, chickpea, lentils etc) and pseudocereals (buckwheat, quinoa etc.). Out of various herbicides, the ones based on glyphosate (EBG s) are famous worldwide and are used on a large scale to control the perennial herbage such as the thistle as well as acting like a harvesting support that accelerates the crop (Bresnahan et al., 2003; Luchian et al, 2019; Boboescu et al, 2020; Florescu et al, 2020; Datcu et al, 2020). EBG s have become a routine application in the agricultural industry and are usually created under the form of salts or glyphosate esters [N- (phosphonomethyl) glycine] (Fig.1).

Glyphosate [N- (phosphonomethyl) glycine], discovered accidentally by Dr. Henri Martin in 1950, has not been recognized for its use in the agricultural industry until the beginning of the 1970s when Dr. John Franz identified and demonstrated the use of glyphosate as a herbicide (Dill et al., 2010). Glyphosate was researched for the first time as a patent by Lowell R. Smith in 1972, but this patent was not granted until 1977 (Baylis et al., 2000). Glyphosate under the form of an active ingredient in herbicides was brought out on the market for the first time in 1974 (Nandula, 2010).

Glyphosate was merchandised in 1974, when new ways of action for herbicides were introduced every 2 or up to 3 years (Duke et al. 2012). Before the introduction of CRG, the use of glyphosate was similar to the one of bipyridinium herbicides, which were traded for the first time more than a decade earlier that glyphosate (Calderbank et al, 1976).

Bipyridiniums and glyphosate are unselective herbicides used on a large scale, which are essentially active in the soil. Cole (1985) has demonstrated that affecting the plants' fito-chemical and physiologic processes by the glyphosate leads to the reduction of photosynthetic rhythms, the degradation of chlorophyll as well as the inhibation and oxidation of the vegetal auxin regulation hormone, which is responsible for the growth of plants. Critics have stated that CRGs (Glyphosate Cultures Resistant to Glyphosate) treated with glyphosate have suffered modifications in mineral nutrition and an increased susceptivity to plants'pathogen agents due to the capacity of glyphosate to chelate divalent metal cations, but the complete resistance of CRGs to glyphosate indicates the fact that some chelating metal cations do not contribute to the activity of herbicides or significantly affect the nutrition of minerals.

Glyphosate is toxic for some of the plants' pathogene agents and, consequently, it can act as a fungicide in CRG. The ultra-small doses of glyphosate stimulate the development of plants into glyphosate sensitive plants through unknown mechanisms (Duke, 2017). In the leaves the absorbtion of glyphosate is a biphasic process which implies a rapid initial penetration through cuticules, followed by a slow absorbtion through symplast (Monquero et al., 2004). The entrance in simplast can be made through a passive diffusion mechanism which is not affected by the PH (Gougler & Geiger, 1981) or by an endogene transport system, possibly a carrier of phosphate in the cellular membrane (Burton & Balke, 2012). The environmental factors are also known to affect the absorbtion of glyphosate. For example, the absorbtion of glyphosate is modulated by all factors that modify the water potential of the plants (such as the humidity of the soil and relative humidity) (Sharma & Singh, 2001), the production of cuticular wax (such as the reduced intensity of light) or the hidration and absorbtion of minerals through the perspiration speed which influences various factors (such as temperature) (Sharma & Singh, 2001). After the penetration of the leaves, glyphosate will reach active metabollic sites, such as the roots, after being translocated in the vascular tissues (Satchivi et al., 2000).

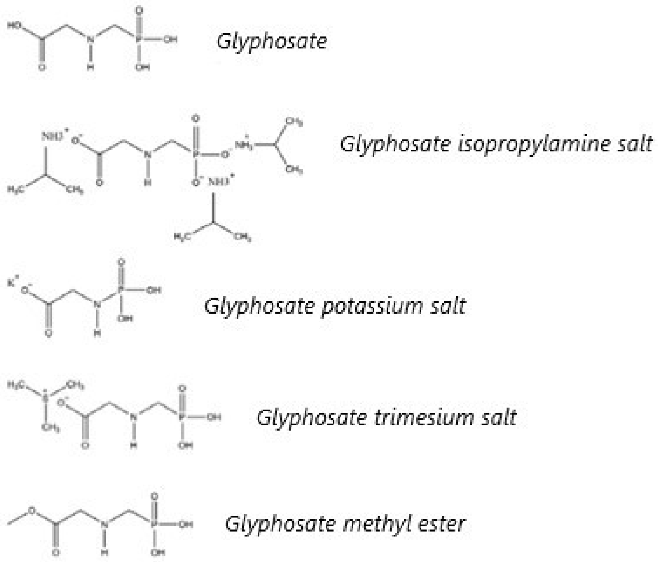


FIG. 1. Chemical structures of glyphosate and its derivatives (adapted from Jingwen Xu et al., 2019)

The application of GBHs in cultures controls the growth of weeds, but it can also alterate their development. As a result, an approach that uses transgenic engineering was the development of glyphosate-resistant cultures (CRG). Up to the present CRGs have been developed for soy, corn, cotton, canola, lucerne, beet and wheat, although many of these have not been approved for commercial use yet. (Dill et al., 2010). These CRGs gain attention rapidly due to their resistance to EBGs, which allows the agricultures to simply apply GBHs for a larger scale of applications (Solomon & Thompson, 2003).

Although it is more expensive than other herbicides, glyphosate presents more advantages. Contrary to other herbicides which have fast action, glyphosate has a slow action and is easily translocated in distant meristems of the treated leaves (Duke, 1988). Therefore, glyphosate is more efficient than other herbicides in preventing the re-growth in the meristems and, consequently, has an extraordinary advantage in killing perennial herbage. Finally, bipyridiniums are probably the most acid toxic herbicides for vertebrates, but vast investigations have shown that glyphosate and its aminnomethylphosphic acid (AMPA) degradation product have got a very low acute and chronical toxicity (Geisy et al. 2000; Williams et al., 2016).

Glyphosate can also have negative effects in various fields of work, if its quantity surpasses the admitted limits. At present, the classification of glyphosate as being potentially carcinogenic is controversial. The first statement comes from the International Agency for Research upon Cancer (IARC) that has evaluated the carcinogenic danger for the glyphosate herbicide and has reached the conclusion that glyphosate probably presents carcinogenic potential versus humans, based on the result of the faulty and incomplete summary of the evidence evaluated by the group of researchers (Tarone, 2018). This conclusion on behalf of IARC obtains afterwards the classification of glyphosate as a category 2A carcinogenic due to the sufficient evidence supplied through studies made on animals in junction with „limited evidence" in studies performed on a human community (Tarazona et al., 2017). All in all, EPA in the USA and the European Authority for The Safety of Foods (EFSA) has considered that it is least probable for glyphosate to present carcinogenic danger for humans, as IARC had previously stated (Portier et al., 2016). EPA and EFSA sustain that the lack of human evidence do not fulfill the requirements for the classification of glyphosate as a potential carcinogenic (Portier et al., 2016).

MATERIAL AND METHOD

A systematic search has been performed in the specialty literature using *Google Scholar*. Scientific works have been selected from the period of time between 2010 and 2020. For starters key words such as "glyphosate plants toxicity" have been used, resulting a number of 17700 scientific works. Then I performed a search on different topics using the following key words: "glyphosate effects and soil", "glyphosate effects on human health", "glyphosate effects on terrestrial animals", "glyphosate effects on aquatic organisms", "glyphosate effects on non-target organisms", "glyphosate effects in food contamination" and "glyphosate action in germination", glyphosate effects of human health", "glyphosate effects of terrestrial animals", "glyphosate effects of aquatic organism", "glyphosate effects of non-target organism", "glyphosate effects in food contamination" and "glyphosate action in germination". Patents and mentions have been excluded from this search.

RESULTS AND DISCUSSIONS

After performing the search on Google Scholar I have obtained:

- 18.900 articles using the key words "glyphosate effects and soil",

- 17.600 scientific works using the key words ”glyphosate effects of human health”,
- 7.100 scientific works using the key words ”glyphosate effects of terrestrial animals”, 13.00 scientific works using the key words”glyphosate effects of aquatic organism”,
- 7.910 scientific works using the key words ”glyphosate effects of non-target organism”,
- 18.100 scientific works using the key words ”glyphosate effects in food contamination”
- 12.100 articles using the key words ”glyphosate action in germination”**.** The results obtained can be observed in the chart below: (Fig.2):

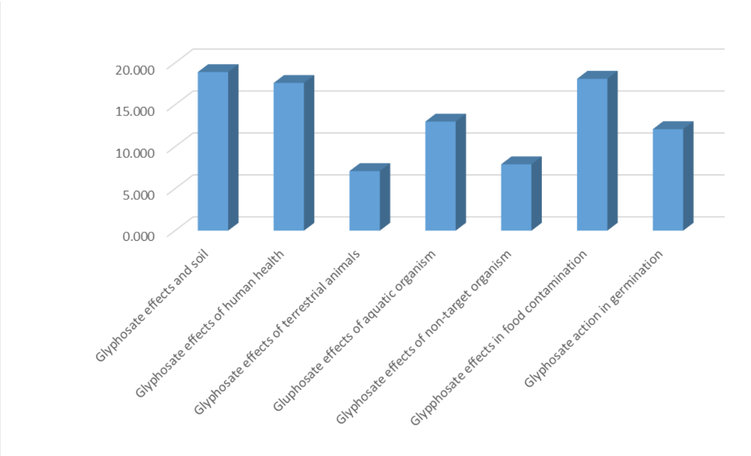


FIG.2. Search results for specialized papers by topic

From the chart one can observe that the majority of works in the entire period between 2010 and 2020 have been found by using the key words ”glyphosate effects and soil”, ”glyphosate effects of human health”, ”glyphosate effects in food contamination”.

After the search I have accesed between 5 and 10 articles on each investigated topic. The effects of glyphosate have been extremely studied, various studies being made upon the soil, upon plants and animals, upon humans. Various studies have been made abot the effects of glyphosate upon the soil (Bai & Ogbourne, 2016).

Correlations have been found between the increased use of glyphosate and a wide variety of human diseases, including forms of cancer, kidney lesions and menthal disorders such as ADHD, autism, Alzheimer and Parkinson (Fortes et al., 2016). The varieties and dermatological and respiratory diseases have been connected to the exposure to glyphosate during the campaigns of spraying the glyphosate in the air in order to eliminate the coca plants in Columbia (Camacho & Mejía, 2017).

Glyphosate and tensio-active agents like POEA and MON 0818 (75% POEA) can have negative effects upon the health of a variety of aquatic animals consumed by other species, including protozoans, mussels, shell-fish, frogs and fish, similar to the effects upon terrestrial animals (Li et al., 2017). Aquatic animals seem to be more sensitive to POEA than terrestrial animals. However, experimenting upon the effects of adjuvants upon health by independent entities has been rather limited due to the unique character of these chemical substances.

Similar to the effects of glyphosate on terrestrial animals (Table 1), pure glyphosate supresssed the activity of acetylcholinesterase at low concentrations values (1-676 mg l^{-1}) in brown mussels (*Perna perna*) and in several species of fish (Sandrini et al., 2013). Exposing embryos in some species of fish to higher concentrations of Roundup® (50 mg l^{-1}) brought about serious growth problems (Roy et al., 2016).

TABLE 1. Effects of chronic exposure of terrestrial cells and live animals and aquatic animals at low doses of Roundup (table adapted from Van Bruggen et al., 2018)

Terrestrial animals		**Aquatic animals**
Effects on cell cultures	Effects on live animals	Effects on live animals
Increase in reactive oxygen species (ROS)	Impaired neuronal cell development and axon growth in rats	Overproduction of ROS and oxidative stress in fish
Decrease in acetylcholinesterase activity	-impaired acetyl cholinesterase activity; -oxidative stress and glutamate excitotoxicity; -in the rat hippocampus; depressive-like behavior in offspring rats;	-suppression of acetylcholine-sterase activity in brown mussels and fish; -damage of motoneurons in fish; -developmental problems and brain damage;
DNA damage in leucocytes and decreased DNA methylation	-biochemical and anatomical liver damage; -liver and kidney damage and tumors in rats;	-disturbed metabolism and renal injury in fish; -changes in liver cells and mitochondria in carp
Deteriorated ovarian functions in cell cultures of cattle ovaries	Negative fertility effects in male rats	

In order to perform a more detailed analysis, and also using *Google Scholar* search engine, we used more key words to make the search more precise. We have looked for various studies on different plants, studies that highlight several effects of the herbicide on some crop plants but also on some weeds. The search time interval was set between years 2010-2020, excluding patents and mentions. Results were shown in table 2 in order to statistically observe on what species of plants the most scientific research was done in the chosen timeframe.

TABLE 2. Results of scientific research on the effects of glyphosate on crop plants and weeds

Glyphosate action in germination. Search period 2010-2020			
Crop plants. Positive effect		Weeds. Negative effect	
Plant species	Results	Plant species	Results
Effects of glyphosate on *Zea mays*	12.600	Effects of glyphosate on *Medicago lupulina*	3.700
Effects of glyphosate on *Glycine max*	10.900	Effects of glyphosate on *Chenopodium album*	3.010
Effects of glyphosate on *Triticum aestivum*	8.150	Effects of glyphosate on *Digitaria spp.*	2.840
Effects of glyphosate on *Brassica napus*	5.480	Effects of glyphosate on *Amaranthus retroflexus*	2.530
Effects of glyphosate on *Oryza sativa*	5.390	Effects of glyphosate on *Echinochloa crus-galli*	2.520
Effects of glyphosate on *Citrus*	5.040	Effects of glyphosate on *Sorghum halepense*	2.200
Effects of glyphosate on *Medicago sativa*	3.550	Effects of glyphosate on *Ambrosia trifida*	2.120
Effects of glyphosate on *Hordeum vulgare*	3.470	Effects of glyphosate on *Thlaspi arvense*	1.960
Effects of glyphosate on *Phaseolus vulgaris*	3.450	Effects of glyphosate on *Abutilon theophrati*	1.880
Effects of glyphosate on *Beta vulgaris*	3.330	Effects of glyphosate on *Sinapsis arvensis*	1.810
Effects of glyphosate on *Pisum sativum*	2.770	Effects of glyphosate on *Convolvulus arvensis*	1.740
Effects of glyphosate on *Solanum tuberosum*	2.250	Effects of glyphosate on *Cirsium arvense*	1.670
Effects of glyphosate on *Trifolium pratense*	1.690	Effects of glyphosate on *Amaranthus tuberculatus*	1.220
Effects of glyphosate on *Nicotiana tabacum*	1.660	Effects of glyphosate on *Polygonum convolvulus*	1.110
Effects of glyphosate on *Raphanus sativus*	1.590	Effects of glyphosate on *Taraxacum officinale*	1.050
Effects of glyphosate on *Solanum lycopersicum*	1.570	Effects of glyphosate on *Stellaria media*	1.020
Effects of glyphosate on *Salix*	1.350	Effects of glyphosate on *Plantago major*	907
Effects of glyphosate on *Allium cepa*	1.210	Effects of glyphosate on *Capsella bursa-pastoris*	766

Of all the plants, 18 species of crop plants and 18 species of weeds were selected for the search, these having been studied the most and showing the greatest number of effects of glyphosate. Thus, the crop plant that caused the most interest in research was corn (*Zea mays*), in the time interval 2010-2020 appearing in 12.600 articles and the most intensely studied weed was the small leaved clover (*Medicago lupulina*), for for the same time interval.

Glyphosate is toxic for both monocotiledonous plants (like herbage) and for dicotiledonous plants (broad leaved plants). Adaptation and translocation of glyphosate in plants is improved by the surfactants in the formulated product. The translocation process takes place both acropetal and basipetal (both up and down), so that glyphosate accumulates in the entire plant, including seeds and roots. Glyphosate and its decomposition product AMPA inhibit the activity of antioxidant enzyme and produce an accumulation of reactive species of oxygen (ROS) which produce physiological dysfunction and damage of cells (Gomes et al., 2016).

Plants treated with glyphosate do not produce aromatic by-products including antimicrobial phytoalexins that protect plants against pathogens. Thus, plants treated with glyphosate frequently die due to infection produced by pathogens universally present in soil (Rosenbaum et al., 2014).

The effects of glyphosate on corn (*Zea mays*)

Fifteen field experiments were done in the period 2009-2012 în Ontario, Canada and Michigan, SUA to determine the level of tolerance of *Zea mays* applied early (ears or in stages of 1 or 2 leaves) or late (8 or 10 leaves) applied in 900, 1800, 3600 or 7200 g ae ha^{-1} of glyphosate. Post emergence usages were evaluated to measure damage of corn, length and deformation of the shell, humidity of the crop at harvest time and yield in the absence of weeds. In the early use experiment no damage could be detected after usages of up to 3600 g ·ae·ha^{-1}; however, there was a 1,4% of damage at 4 weeks after treatment (WAT) when 7200 g ae ha^{-1} was applied to corn in stages of 1 to 2 leaves. In the late use experiment, the noticeable damage showed a tendency to grow as the glyphosate dose increased. In addition, for the corn treated with 7200 g ae ha^{-1} in the stage of 10 leaves, the procentage grew in time with 6%, 11% and, 12% for the noticed damage for 1,2 and 4 weeks of treatment (Mahoney et al., 2014).

As expected, in all moments of observation, the noticeable damage of the corn treated with 900 sau 1800 g ae ha^{-1} of glyphosate in stages of 8 or 10 leaves was similar to the untreated control crop. However, the noticeable damage had a tendency to grow once the dose of glyphosate was increased. The symptoms of damage included clorosis, necrosis, damage to the tissue of the leaves when applied, twisted leaves and reduced growth compared to the untreated control crop. Heck et al. (2005) raported up to 10% the malformation of corn when the doses of glyphosate were applied in the growth phase V4 and V8 in a total of 5040 g ae ha.

FIG. 3. Maize at physiological maturity after treatment with 900 g · a · ha - 1 of glyphosate in the 8-leaf growth stage (left-sighted ear) and 7200 g · ae · ha - 1 in the 10-leaf growth stage (ear with right view) (adapted from Mahoney et. al. 2014)

The effects of glyphosate on *Ambrosia*

In previous research on unguarded species it was proved that the efficiency of glyphosate is greater in sterile soils, compared to non-sterile soils and soil microorganisms have played an important role in the efficiency of glyphosate (Schafer et al., 2012).

A study on the response to the greenhouse dosis was made on two species of weed cultivated in sterile and non sterile soil and the answer to dry weight of the roots and shoots was measured. Some species of weeds responded differently to glyphosate when they were cultivated in sterile and non sterile soils, that is in the presence or absence of soil microorganisms. Soil microorganisms influenced the answer of the giant sensitive and resilient *Ambrosia* biotypes of the sensitive wild spinach. The different answers of the weeds to glyphosate with or without the presence of soil microorganisms proves that the interactions with the root zone are fundamental for the action of glyphosate. These discoveries suggest that the tolerance interval to glyphosate noticed in weeds and the evolution of resistence in weed biotypes can be also influenced by the interactions of the root zone. The type of soil used for the answer to dosis screening in order to identify the sensitive and resistent weed biotypes is very important (Ianovici, 2009; Schafer et al., 2012; Ciobanu & Ianovici, 2018; Alexan & Ianovici, 2018; Ianovici & Barsan, 2020).

A larger dose of glyphosate was necessary to kill the sensitive biotype when it is grown in sterile soil (Fig 5). SS surviving plants previously treated with a quantity of 0,84 and 1,68 kg ha^{21} of glyphosate were able to continue the growth out of axillary buds and seeds. However, SS plants which survived with a bigger dose than 3,36 kg ha were prevented from growing and producing seeds. The accumulation in dry weight of SU plants decreased after applying glyphosate of only 0,42 kg ha^{21} and the death of plants took place at a rate of 3,36 kg ha. At a frequently used rate of glyphosate in the field (0,84 kg ha), the sensitive biotype was capable to survive when it was grown in sterile soil, visually comparable to the resilient biotype grown in non sterile soil (Fig 6) (Schafer et al., 2012).

FIG.5. Control of susceptible giant ragweed biotype grown in sterile field soil (a) and non-sterile soil (b) 21 d after treatment with glyphosate (DAT) with 0, 0,21, 0,42, 0,84, 1, 68, 3.36 or 6.72 kg aH21 of glyphosate (adapted from Schafer et al., 2012)

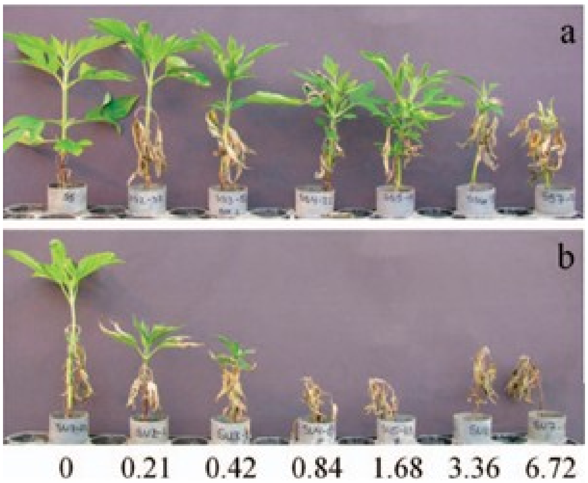
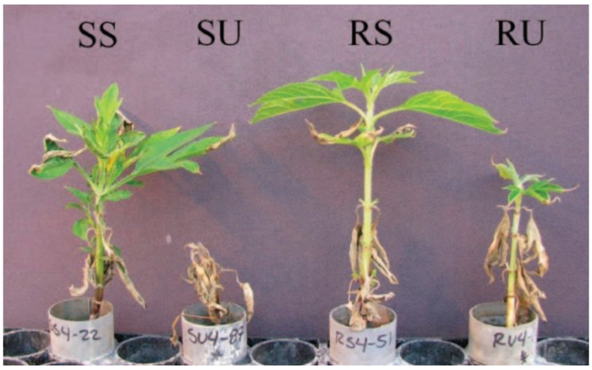


FIG. 6. Tissue response at 21 days after glyphosate (DAT) treatment on giant ragweed biotypes at 0.84 kg ae ha21 when grown in sterile and non-sterile field soil. SS: sensitive biotype grown in sterile soil in the field; SU: sensitive biotype cultivated in non-sterile field soils; RS: resistant biotype grown in sterile soil in the field; UK: resistant biotype grown in non-sterile field soils (adapted from Schafer et al., 2012)



Plant height and growth stage at the time of glyphosate application have been shown to affect the susceptibility and tolerance of wild spinach (Sivesind et al., 2011). Soil humidity differences affected glyphosate absorption and translocation (Waldecker & Wyse, 1985), and relative temperature and humidity influenced glyphosate absorption and translocation (Sharma & Singh 2001). The soil in which the plants were grown, in particular the presence or absence of soil microorganisms, appeared to play a key role in the efficacy of glyphosate on both giant ragweed biotypes and the sensitive wild spinach biotype.

CONCLUSION

Glyphosate is one of the most widely used herbicides, it toxicity and effects being of wide interest both for the researchers and for agricultures. After searching for articles about glyphosate I have reached the conclusion that this herbicide is used on a large scale. Its toxicity and effects in various categories being still rather studied. A lot of studies have analysed and reported the elimination or degradation of glyphosates, correlated with the post-harvesting processing, through washing or humid cleaning, milling, storage and by applying heat, such as in cooking or baking. Statistic results have shown that the effects of glyphosate appear not only upon cultivated plants and herbage but also have an action upon humans, terrestrial animals and aquatic animals. Connections have been found between the increased used of glyphosate and a wide variety of human diseases.

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