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# Glyphosate in Agriculture: Environmental Persistence and Effects on Animals. A Review

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**Abstract:** Glyphosate, in the history of agriculture, is one of the most commonly used herbicide, due to its high effectiveness and low cost compared to other solutions. Glyphosate based herbicides (GBHs), however, have been the subject of a long series of concerns and doubts, especially for public opinion and mass-media, concerning their possible toxic effects on humans and animals. In this paper, the most cited scientific literature about the persistence and possible toxicity of glyphosate and its formulations on animals has been revised. The different commercially-available formulations of GBHs contain diverse amounts of active ingredient (AI) and formulants. Therefore it is difficult to compare the many toxicity studies on animals, also because the researches currently available differ from each other, not only for the glyphosate formulations analyzed, but also for the animal's species tested, exposure times, for the treatment doses, etc. However, the results of this study, lead to the conclusion that there is a need for further research regarding the mechanisms of persistence in the environment of GBHs, the interference of glyphosate on reproductive cells and organs of animals, and the potential long-term toxic effects of this herbicide on animals.

*Keywords: animal production; herbicides; health; sustainable solutions; toxicity*

## Introduction

The weeds reduce severely crop yields causing considerable economic damage to farmers (Garde *et al*., 2018). Their presence can lead to losses that exceed 50% of the agricultural products (Oerke *et al*., 1994). Chemicals have the advantage of being much cheaper (that is, at a lower intensity of labor and financial investiment) than other solutions (i.e. deep or shallow plowing, manual weeding, weeding by fire, etc.) (Harris, 1991). This is allowing fast processing of large surfaces (by airplanes or other spreading ways), and are more effective than other systems (as, for example, high-density sowing, mulching, use of low-competition plants such as clover, etc.) (Vail, 2018). However, they can be toxic to humans and other non-target species; they may modify the soil ecosystem,

contaminate surface water and groundwater, and they can bring the development of herbicide-resistant weeds (Aktar *et al*., 2009). In fact, several authors agree about the progressive appearance of herbicide-resistant crops which cause farmers to increase the quantities use of chemical products: the use of GBHs, in the last twenty years, has caused the appearance of at least 34 invasive glyphosate-resistant plant species (Schütte *et al*., 2017).Despite all these aspects, often economic considerations prevail in the choice of the means to be adopted.
The indiscriminate use of pesticides my cause biodiversity imbalance and this heavily affects the assessment of the sustainability level of current farming practices (Bourguet and Guillemaud, 2016).

The introduction of glyphosate was reported in 1974, first in the United States and then in South America, Europe, and Asia (Chang and Delzell, 2016). This technique has changed the behavior of farmers, who have progressively abandoned other herbicides (phenoxy herbicides; diclofop-methyl; diquat; paraquat; imazethapyr; triazine herbicides; dinitroaniline; trifluralin; chlorsulfuron; and so on), particularly for the cultivation of some crops (corn, soybeans, cotton, etc.), and before harvesting, for crop desiccation (Monsanto International SARL and Monsanto Europe SA, 2017).

Glyphosate, first used in the herbicide Roundup by Monsanto, has certainly been one of the most commercially successful chemicals and the most commonly used herbicide in the world, thanks to its commercial formulations of high effectiveness and low cost (Baylis, 2000; Benbrook, 2016). The annual world consumption of glyphosate is estimated at about 8.6 million tons, sprayed at an average global quantity per hectare equal to 0.53 kg (Benbrook, 2016).

This situation is largely due to the fact that, since its first authorized use in 1974 by the US Environmental Protection Agency (EPA), it has been considered, by all regulatory authorities of the world, a low hazard potential herbicide (JMPR, 2016; Williams *et al*., 2000). Also for this reason, over the years, many genetically modified (GM) crops have been designed to be enzymatically resistant to its molecule (Funke *et al*., 2006).

However, the controversies on the toxicity of glyphosate and its commercial formulations still exist, especially for sustainable practices. The concerns regarding the safety of glyphosate-based herbicides (GBHs) have increased in recent years, mainly to risks arising for human health (Myers *et al*., 2016). Indeed, the World Health Organization's International Agency for Research on Cancer (IARC) independent 2015 report, which states that "glyphosate is a probable human carcinogen" (Category 2A) (IARC, 2015). In addition, the statement of the European Chemicals Agency (ECHA) in March 2017, reports that "the available scientific evidence did not meet the criteria in the CLP Regulation to classify glyphosate for specific target organ toxicity, or as a carcinogen, as a mutagen or for reproductive toxicity"( ECHA, 2019). This conviction had already been confirmed, in the previous year, also by the US-EPA (2016) that concluded that "the overall weight of evidence indicates that there is no convincing evidence that glyphosate induces mutations in vivo via the oral route". These different points of view are explained, according to some authors, by the use of different data sets of long-term carcinogenicity and toxicity in rodents (Tarazona *et al*., 2017).

In this general framework of uncertainty, the European Commission (EC), in November 2017, relying on a controversial 2014 report of the not-independent Federal German Institute for Risk Assessment (*Bundesinstitut für Risikobewertung*, BfR) (EFSA, 2015), approved the authorization for glyphosate for another 5 years, while the European Parliament (EP), opposed this resolution because, according to the EP, any decision on

pesticides must be based on scientific studies independent of the producers (Landrigan and Belpoggi, 2018).

In the paper, a distinction will be made between the different toxic effects of glyphosate as AI and the effects of its trade preparations (GBHs formulations).

**Glyphosate: herbicide details**

It is a Methylphosphonic amino acid (IUPAC: N-phosphonomethylglycine). The name is a contraction of glycinephosphonate. Its formula is the following (Franz, 1972; 1974):

$$HOOC\text{-}CH_2\text{-}NH\text{-}CH_2\text{-}PO_3H_2$$

It may be light-sensitive, while at first it was considered to be unaltered by sunlight (Rueppel *et al*., 1977). Later researches discovered glyphosate as being subject to photodegradation (Lund-Hoie and Friestad, 1986; Carlisle *et al*., 1988). In fact, it has been discovered that, while log wavelenght radiations have no effect on the photodegradation of glyphosate, UV rays quickly degrade this molecule (Kare and Hakon, 1986; Manogaran, 2018). Glyphosate salt formulations are no-selective herbicides, plant growth regulators, and fruit ripening accelerators (Travlos *et al*., 2017).

In several commercial products, glyphosate AI, to increase its intrinsic water poor solubility, it is synthesized as salts, for example, isopropylamine salt or an ammonium salt: glyphosate-trimesium, glyphosate-dimethylammonium, glyphosate-diammonium, glyphosate-potassium, glyphosate-isopropylammonium, glyphosate-sesquisodium, glyphosate-monoammonium.

Glyphosate, absorbed by the foliage of plants, is conveyed through the phloem by means of the plant accumulating itself in immature leaves, meristems, and underground tissues (Roberts, 1998). Across the cuticle the absorption is modest, and conveyance throughout the membrane of cells, compared to most other herbicides, is slow (Vencill, 2002). In plants, after the contact with the AI, there is an appreciable decrease in chlorophyll, changes in the morphology of the chloroplasts, and alteration of the activity of non-enzymatic oxidants (flavonoids and carotenoids) (Cardoso-Gustavson *et al*., 2018).

Glyphosate interferes with an enzyme used by plants (5-enolpyruvylshikimate-3-phosphate (EPSP) synthase) to produce aromatic amino acids (phenylalanine, tryptophan, and tyrosine) essential for plant growth and protein synthesis (Carlisle and Trevors, 1988; Tomlin, 2006; Goldfrank, 2002). Humans and other animals, as is known, cannot synthesize the amino-acids tryptophan and phenylalanine, as they lack the shikimic acid pathway (Metzler, 1977).

Food for humans provide these two important aromatic amino-acids (Funke *et al*., 2006). The shikimic acid pathway is exclusive for plants and certain micro-organisms (Bai and Ogbourne, 2016). Glyphosate, according to some authors, seems to have a low toxicity for non-target organisms owing to the absence of this pathway in mammals (WHO, 1996; Wu *et al*., 2006) and for this reason it is considered harmless (Monsanto Co., 1985).

A precursor of aromatic amino-acid synthesis, the phosphoenolpyruvate (PEP), can be inhibited by glyphosate that affects other important biochemical processes (Hartley and Kidd, 1991; Su *et al*., 1992; Lamb *et al*., 1998). For example, glyphosate disrupts cell membranes and chloroplasts; alters photosynthesis, respiration, protein, and nucleic acid

synthesis; and reduces chlorophyll, and other porphyrin compound synthesis (Hoagland and Duke, 1982).

Other studies have highlighted other pathways of action of glyphosate (Smedbola *et al*., 2018). Kitchen *et al*., 1981, have shown that the action of glyphosate in plants involves two enzyme pathways: one controlling the conversion of alpha-ketoglutarate to delta-aminolevulinic acid; the other controlling condensation of glycine with succinyl co-enzyme to form delta-aminolevulinic acid and carbon dioxide.

Glyphosate, however, appears to repress, in plants, prephenate dehydratase and/or chlorismate mutase inhibiting the biosynthesis pathway of aromatic amino-acid (Weed Science Society of America, 1983). According to "The Agrochemicals Handbook" (Hartley and Kidd, 1991) glyphosate acts, in plants, on some enzyme systems, interfering with other endogenous chemicals. For example, it inhibits acid invertase in sugarcane (Su *et al*., 1992).

Glyphosate affects the inhibition of enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and there is the suspicion that it can behave as a chelating agent, binding macro and micronutrients of the soil essential for the plant processes and for the resistance of pathogens and thus making them not available for non-target plant species (Mertens *et al*. 2018).

Glyphosate, in a leaf litter of some plants sprayed with GBHs, shows a half-life of 8-9 days (WHO, 1988). Lettuce, carrots and barley showed residues of glyphosate (Nicholls, 1990; McMullan *et al*., 1990), even after a year from the treatment of the soil with 0,681 kg/ha with the herbicide.

A very small quantity of glyphosate is metabolized, by hydrolysis, in plants, such as AMPA (Schuette, 1998), the only significant degradation product. In sugarcane, the main metabolites of glyphosate were identified as N-methylamino-bis-methylenephosphonic acid, amino-bis-methylenephosphonic acid, N-phosphonomethyl glycine, and N-methyl-N-phosphonomethylglycine (Menzie, 1980).

It seems that glyphosate is, for rats, a acetylcholinesterase (AChE) inhibitor and, consequently, could be toxic for non-target organisms, such as beneficial insects and mammals (Hietanen *et al*., 1983). The mechanism of toxicity for glyphosate consists be in an uncoupling of oxidative phosphorylation (Larsen *et al*., 2016).

## Adjuvants and their toxicity

The different commercially-available formulations of GBHs contain different amounts of active ingredient (AI). A huge number (over 750 products in the US only) of glyphosate-containing herbicides are available for sale on the world markets (Valavanidis, 2018).

In the different formulations of herbicides, the action of glyphosate is amplified or facilitated by the presence of other chemical compounds, that has focused the attention of scholars regarding their risks. While glyphosate and the aminomethylphosphonic acid (AMPA), its main metabolite, are easily detectable in the environment (Demonte *et al*., 2018; Gracieli *et al*., 2019), this is more difficult for the adjuvants of a specific formulation (Mesnage and Antoniou, 2017). On the contrary, some surfactants or other substances, added to certain glyphosate formulations, are toxic to animals and many aquatic species (Extoxnet, 2018).

The ingestion of the surfactant, together with the herbicide, causes a whole series of pathologies to animals (Sawada *et al*., 1988; Tominack *et al*., 1991; Talbot *et al*., 1991), such as nephrotoxicity and death (Wunnapuk *et al*., 2014). Thus, Richard *et al*., 2005,

examined the effects of a GBH formulation (Roundup containing 360 g/L acid glyphosate) and glyphosate at lower and non-toxic concentrations on the enzyme of the synthesis of estrogen, the aromatase. GBH formulations, as stated by other authors (Mesnage *et al*., 2015) too, disrupted the levels of mRNA and the activity of aromatase. These authors determined that GBH adjuvants, and not glyphosate, had toxic and endocrine effects in mammals enhancing the bioaccumulation and/or the bioavailability of glyphosate.

The most common adjuvant is polyethoxylated tallow amine (POEA), which is a surfactant used to improve the solubility of glyphosate and its plant penetration (Mesnage and Antoniou, 2017). Surfactants act as emulsifiers, detergents, foaming agents, wetting agents, and dispersants (Somervaille *et al*., 2011). Studies indicate that POEA, by oral way, is more toxic (up to five times) to animals than glyphosate itself (Bradberry *et al*., 2004; Williams *et al*., 2005; Cox, 1995; Altieri, 2009; Benachour and Séralini, 2009; Hedberg and Wallin, 2010; Zeliger, 2008). Most recent research (Mesnage *et al*., 2013) confirms that POEA-15 (15% of Roundup formulations) can cause cell necrosis.

Tests on animals have established that POEA presents low toxicity having an oral $LD_{50}$ in rats amounting to 1200 mg/kg bw/d, but in any case much higher than the toxicity of a Roundup formulation (41% IPAG, isopropylamine salt of glyphosate) (Williams *et al*., 2000; Cox,1995). A moderate intoxication does not cause permanent gastrointestinal symptoms, while acute poisoning results in gastrointestinal bleeding, hypotension, pulmonary dysfunction, and kidney damage (Talbot *et al*., 1991). More recent studies (Chłopecka *et al*., 2017; Defarge *et al*., 2018) confirmed the toxicity of glyphosate and POEA mixtures, which irreversibly influence the motor activity of the gastrointestinal tract of rats.

According to some authors, it seems that the GBH toxicity is not proportional to the quantity of glyphosate, but rather to the amount of POEA in the soil and its persistence in the environment (Tush and Meyer, 2016).

The surfactant X-77 Spreader, used in the formulation of the Rodeo, is one hundred times more toxic, than glyphosate alone, for aquatic invertebrates (Henry *et al*., 1994). Other surfactants or other compounds added to formulations based on glyphosate have been ascertained as being toxic to fish and other aquatic species (Extoxnet, 2018; Defarge *et al*., 2018). Another surfactant, the MONO818, used (at 15%) in the formulations of the Roundup, seems to interfere with the skin breathing apparatus of frogs and with the gills of tadpoles (Tyler, 1997; Tu *et al*., 2019). The same surfactant is very toxic for fish (Folmar *et al*., 1979; Servizi *et al*., 1987).

Furthermore, glyphosate is, very often, mixed with diquat, which is very toxic. This mixture is not used in the U.S. and Canada, but it is used in other countries, such as Great Britain. Many research studies (Schnick *et al*., 1979; Hornr, 1983; Slaughter *et al*., 2002; Zhang *et al*., 2011) indicate that the diquat at low concentration levels behaves as a chemotherapeutic substance while at high concentration it is a strong carcinogen. It is also worth remembering that mixtures with other herbicides such Paraquat, Dalapon, MSMA, Phenoxy, can modify or reduce the action of glyphosate (Weed Science Society of America, 1983). The possible dangerousness of other adjuvants should not be underestimated. Proxel (benzisothiazolin-3-one), e.g., used as a preservative can cause irritation and photo-contact dermatitis (Sullivan and Sullivan, 2012).

## Mechanism of immobilization in the soil and persistence in the environment

Once in the soil, glyphosate and its salts, being in ionic form, are converted, as primary decomposition, by certain species of *Pseudomonas* and other soil microbes components, to sarcosin, and form AMPA and glyoxylic acid (Dekker and Duke, 1995). AMPA, in particular, is much more persistent than glyphosate itself (Van Bruggen *et al*., 2018a; Van Bruggen *et al*., 2018b).

Even if it is a molecule with zwitterion structure, the adsorption of glyphosate increases with the ion exchange capacity of the soil and its clay content, and decreases with soil phosphorous content and its pH (Rueppel *et al*., 1977; Sprankle *et al*., 1975; Hance, 1976; Glass, 1987; Nomura and Hilton, 1977; Sheals *et al*., 2002; Miles and Moye, 1988; Borggaard, 2011).

Glyphosate and phosphate molecules compete in the soil in relation to the processes of adsorption and mobility: the presence of the phosphate group and the application of phosphate fertilizers change pH levels and reduce the retention power of glyphosate from the soil matrix (Munira *et al*., 2018).

It follows that glyphosate plant uptake, from soil, is negligible (Roberts, 1998). As soon as glyphosate touches the ground or is in contact with muddy water, the bonds with some soil components (cation-saturated clay minerals and humic substances) reduce or eliminate its herbicide capacity (*killing power*) (Sprankle *et al*., 1975; Hance, 1976; Nomura and Hilton, 1977): in particular, it is postulated that initial inactivation of glyphosate in soil is by reversible adsorption to clay and organic matter through the phosphonic acid moiety (Sprankle *et al*., 1975). It is absorbed by the soil, during the first hour (Sprankle *et al*., 1975). The strong adsorption by soil slows microbial degradation (Forlani *et al*., 1999; Guijarro *et al*., 2018) with formation of AMPA and $CO_2$, and thus glyphosate persists in aquatic environments and in the soil. The absorption of GBHs into plants delays soil-degradation and increases the persistence of glyphosate, in soil, by a factor of two to six times (Doublet *et al*., 2009). In particular, the percentage of glyphosate that undergoes mineralization varies depending on the size of plant residues and their distribution in the soil (Mamy *et al*., 2016).

Several investigators (Newton *et al*., 1984; Smith *et al*., 1986; Goldsborough and Beck, 1989; Goldsborough and Brown; 1993; Maqueda *et al*., 2017) have determined the persistence and degradation of glyphosate in aquatic environments under different conditions. It is moderately persistent in seawater with low-light conditions while it is highly persistent in the dark: Mercurio *et al*., 2014, in their study, demonstrate that glyphosate, under low light conditions in marine water, is significantly persistent: its half-life, at 25 °C in low-light, was 47 days, extending to 267 days, in the dark at 25 °C, and 315 days in the dark at 31 °C.

The average half-lives of glyphosate and AMPA in the soil range from 2 to 197 days, as a function of the soil characteristics, climate (above all, temperature and wind) (Bento *et al*., 2017) and, in water, they range from a few days to 91 days (Rueppel *et al*., 1977). Other studies (US EPA, 1993; Andréa *et al*., 2003; Li *et al*., 2017; Piccolo *et al*. 1994; Chlatter *et al*., 2017) report different persistence of herbicide in the soil or water by confirming that all depends on the conditions of the moment and place and of the type of bacterial comunity. In streams, the concentrations of glyphosate were maximum at post-treatment (US EPA, 1993). AMPA was found in the soil of some Swedish forests after two years from the application of glyphosate (Mamy *et al*., 2016; Torstensson *et al*., 1989; Albers *et al*., 2019; Ole *et al*., 2011), while the herbicide has been found in many foods, such as bread, potatoes, barley, etc. (HGCA, 2008).

The epidemiological studies cited below have highlighted the correlation between the onset, in the United States, of certain animals chronic diseases and the increasing consumption of GBHs. According to some authors, this situation may be due to the fact that the glyphosate molecule is very similar to that of glycine and therefore interferes with protein synthesis of mammals (Samsel *et al*., 2016).

The Toxicity Class of Glyphosate, assigned by the United States Environmental Protection Agency (EPA), is III for oral and/or inhalation exposure, on an I to IV scale (IV is the least dangerous) (Andréa *et al*., 2003).

Glyphosate salts, as they are highly soluble, have high impacts on aquatic wildlife; in fact, many recent reports have found massive glyphosate and AMPA presence in groundwater, rivers, rainwater and precipitation (Battaglin *et al*., 2014a; Battaglin *et al*., 2014b). Many streams, rivers, and lakes in the US (Battaglin *et al*., 2014b) and in the rest of the world have appreciable amounts of glyphosate (USGS, 2014). Glyphosate was identified in the Wuchuan River (China) surface water, at levels of 0.67-1.39 ng/L (Zhang *et al*., 2002).

As regards the many researches on the effects on animals, in the remaining part of this study, it is important to distinguish between those concerning only the AI and those concerning only the GB (glyphosate based) formulations.

### *Effects of glyphosate on land animals*

The long-term effects of glyphosate on land animals and aquatic species are still largely unexplored (Sheng *et al*., 2012; Helander *et al*. 2012; Sribanditmongkol *et al*., 2012). Besides, some studies conducted, until now, have been very often in contradiction (Pfeil *et al*., 2004; Torretta *et al*., 2018) among themselves, as we shall see shortly. Another controversial aspect is related to the absorption of the herbicide by animal organisms, with or without biotransformation.

According to other studies on experimental animals, the metabolism of this herbicide molecule shows that the AI is not biotransformed, because the dose is excreted as unchanged parent chemical (Torretta *et al*., 2018). It seems that glyphosate could be absorbed rapidly and cleared without any transformation, although there would be a minimum absorption by the organic fabrics of the commonly used species, including mammals, fish, and birds (Krieger, 2001). Most of the compounds would be eliminated via the feces (70-90%) and the remainder via urine (Curwin *et al*., 2007). Less than 0.5% would remain in the tissues, organs, particularly in the bones and spinal cord (Goldsborough and Brown, 1993; Krieger, 2001). According to Williams *et al*., 2000, glyphosate and AMPA, dosed by oral absorption are excreted fundamentally unmetabolized. Further, there were no adverse effects on endocrine systems, reproductive parameters, or fertility in mammals.

The total excretion of 14C-glyphosate in rats was, in feces, around 80-90% and in urine <10%, during a 14-day via the diet oral study (Goldsborough and Brown, 1993). This means that the total absorption levels, throughout the body and especially through bones, were about 15% or less, considering the secondary weight of the biliary elimination way.

These results are confirmed by other studies, meaning that glyphosate is partly metabolized by mammals, since traces of the metabolite (AMPA) were found in the gut flora of the animals observed (Goldfrank, 2002) some of which lived in a forest in Oregon (USA) (Newton et al., 1984). These residues of metabolites were untraceable

after 55 days. Residue levels of omnivores and carnivores viscera were anyway very high (over 5 mg/kg).

Kruger *et al*., 2013, have found the existence of a correlation between some altered parameters of the blood and the concentration of the herbicide in the urine of cattle; moreover, according to another research of the same working group (Kruger *et al*., 2014), the percentage of glyphosate residues traced in the urine of cows is lower than that found in human urine. On the contrary, other previous studies (US EPA, 1993) conducted on *beagle* dogs, treated for one year with varying doses of glyphosate, show the absence of alterations in the parameters examined. In this regard, it must be said that the exposure of pets to glyphosate, due to contamination of feed, is much higher than that of humans: 4-12 times higher per kg basis (Zhao *et al*., 2018).

Many type of researche have studied dermal irritation by contact with glyphosate. According to the above-mentioned Williams *et al*., 2000, paper, glyphosate had very low absorption and bioaccumulation by dermal penetration. Also by Krieger (2001) study on guinea pigs, dermal sensitization to glyphosate was not observed. Citing the US-EPA database (1993), there is a noteworthy dermal study on white rabbits skin of New Zealand over a period of 21-days, according to which effects (perceptible edema and erythema) were observed only in high doses. Shin *et al*., 2009, have noted that the use of glyphosate by man can lead, in particular situations, to the appearance of intestinal intoxication, as well as the growth of dermal multiform erythema. According to WHO (1988), no significant effect on growth and survival and no systemic toxicity (hematology and blood biochemistry, organ weights, gross pathology, limited histopathology) were found during glyphosate applications, at different doses, to rabbits for 3 weeks (6 h/day and 5 days/week). Minor dermal irritation was perceived at 5000 mg/kg b.w.

From a series of studies on laboratory animals reported by the National Toxicology Program (NTP) it seems that glyphosate is generally harmless and only slightly toxic at low doses (CAS: 1071-83-6). Batt *et al*., 1980, assert that there are no effects on the hatching of chicken eggs in a solution of 5% glyphosate. According to Sullivan and Sullivan's study (1979), black-tailed deer presented no dislike to foliage treated with glyphosate. According to Shore *et al*., 2001, mule deer exposed to woody browse treated with glyphosate (2.3 kg/ha) under field conditions showed no adverse effects on consumption or forage selectivity when compared to untreated areas. In another well-known study, Durkin and Diamond (2002) assert that glyphosate does not cause toxic effects on the immune system, nervous system, or endocrine function in experimental mammals. Claiming that glyphosate does not appear, for animals, an endocrine disruptor or immune toxicant is re-enforced by epidemiological studies on relationships between occupational farming and related exposure risks to glyphosate, such as fecundity, miscarriage and sperm quality.

Regarding the effects of glyphosate on the reproductive capacity of animals, there are many studies.

For example, Moxon (2000) stated that dietary concentrations of glyphosate, higher than 290 $mg \cdot kg^{-1}$ per day, to rats, over two generations, had no effect on fertility and sexuality. Also according to the California EPA and others (California Environmental Protection Agency 2018; European Chemicals Bureau, 2018), glyphosate dosed by ingestion has little toxicity to rats. In particular, male rats, despite a considerable reduction in sperm production, maintained sperm concentrations within normal levels; while for female rats the estrous cycle slightly lengthened. The study conducted by the European Chemicals Bureau (2018) reports that no sub chronic toxicity or effects on reproduction were noted when glyphosate was fed to Bobwhite quail for 22 weeks at dose

levels up to 1000 ppm. There were no effects, related to glyphosate treatment, on behaviour and reproductive success, food consumption, body weight and survival rates of embryos and adults. Potential effects of forest application of glyphosate on snowshoe hares in British Columbia were examined by Shore *et al*., 2001. The adult hares who were able to reproduce, were unaffected by the number of pregnancies, and survival were unaffected. Recruitment of hares on treatment and control areas was quite similar.

Other studies, however, have shown that chronic glyphosate treatment has a negative effect on semen characteristics and, in general, on fertility of mature white male rabbits in New Zealand (Yousef *et al*. 1995) and other animal organisms. Their conclusions were that a decline in body weight, libido, sperm concentration, semen osmolality, semen methylene blue reduction time and dead sperm had occurred. Walsh *et al*., 2000, have found, in rats cell culture, that glyphosate inhibited a testosterone production enzyme. More recently, Dai *et al*., 2016, confirmed the glyphosate toxicity, albeit slight, on the reproductive system of rats.

Minor health effects are reported by other authors. Burgat *et al*., 1998, by a retrospective analysis, between 1991 and 1994, of 482 glyphosate calls at the CNITV (France) assessed just 31 cases as highly probable direct ingestion of glyphosate with bland symptoms (diarrhea, hypersalivation and vomiting). In the aforementioned study conducted by USEPA (99) on gravid Charles River COBS CD rats, effects – like inactivity, decreased body weight, diarrhoea, red mouth and nose matter, breathing rattles, augmented serum glucose, increased serum potassium and phosphorus, serum alkaline phosphatase, pancreatic lesions and blood urea nitrogen (BUN) and deaths – were detected only at high-doses. Glyphosate also seems to affect other organs of animals with different consequences, as highlighted by many papers. Daruich *et al*., 2001, in a 2001 study have pointed out that glyphosate provokes, in pregnant rats and fetuses fed with a regular diet with glyphosate/ml drinking water, functional abnormalities.

Bolognesi *et al*., 1997, found, in lab mice, DNA and chromosomal injury in bone marrow, kidney and liver and lymphoid cells. Very similar effects were found in non-mammalian animals, including goldfish (Çavaş and Könen 2007), tilapia fish (Jiraungkooskul *et al*., 2003), sea urchins (Bellé *et al*., 2007), eels (Guilherme *et al*., 2010) and fruit fly (Michalkovà et Pekàr, 2009).

Many authors (Santillo *et al*., 1989; Mac Kinnon and Freedman, 1993; Kahn, 2005; Bergvinson and Borden, 1992) observed important alterations to mammals and birds of forests following large-scale glyphosate treatment. According to the same authors, acute lethal dose toxicity could derive from the decoupling of oxidative phosphorylation. Some investigators (WHO, 1988) reported, in mitochondria of rat livers after intraperitoneal doses, increased phosphatase activity and reduced respiratory control ratios. Kahn (2005) remembers that vomiting, nausea, hind-leg weakness and staggering have been detected in cats and dogs exposed to glyphosate on treated foliage.

Studies conducted on *Sminthopsis macroura* male marsupials presented, after exposure to glyphosate concentrations of up to 5000 mg a.i/kg feed, noteworthy body weight loss. Another study, published in 2010, asserted that glyphosate cause, in African clawed frogs (*Xenopus laevis*), craniofacial malformations and neural defects (US EPA, 1993).

Bergvinson and Borden (1992) indicated that glyphosate AI caused the invasion of *Dendroctonus ponderosae*, a mountain pine beetle, inhibiting the trees' defense reaction. In this case, it is obviously a secondary and not a direct effect of herbicide on the development of the larvae of this insect.

### *Effects of GBHs on land animals*

In recent years, many studies about the effects of glyphosate formulations on reproduction and on reproductive organs of rats, rabbits, birds, etc. have been conducted, but they are not easily comparable. For example, when adverse effects are detected, it is not always clear to attribute the herbicide toxic properties to glyphosate or its formulations.

Genotoxicity (30.4% glyphosate formulation) (Bolognesi *et al*., 1997) and consequences on the reproductive organs of some animals (Garry *et al*., 2002). have been reported by several authors. In this regard we recall that for about 30 years it was suspected that the environmental presence of pesticides might have undesirable effects on the sensitive mechanisms of male fertility (Eliason, 1985).

Gallegos *et al*., 2016, stated that the exposure – through drinking water to 0.2% or 0.4% of a commercial formulation of glyphosate (corresponding to a concentration of 0.65 or 1.30 g/L of glyphosate, respectively) – of rats offspring, *in utero*, to a GBHs, during pregnancy and lactation, affects the central nervous system and induces neurobehavioral alterations.

Researches conducted on female beagles (Goldsborough and Brown, 1993; Tai *et al*., 1990), about the joint effects of glyphosate (at plasma levels from 923 to 3450 mg/L) and surfactants, show that, by injection, both lead to cardiac depression, involving, primarily, increased myocardial contractivity. In particular, this research showed that glyphosate alone increases the rhythms of contraction of the heart, while the surfactants alone reduces significantly myocardial contractility. However, this cardiac involvement and the reason of the joint effect has not been specified in detail.

Gress *et al*., 2015, observed in rats and rabbits, to whom different doses of some GBHs (each with a declared AI between 36 and 48%) had been given, cardiac decompensations and arrhythmias so that the same authors even hypothesize – but this still remains to be demonstrated – that many deaths as a consequence of heart attack among farmers can be attributed to GBHs, hoping, for the future, extensive and depth epidemiological monitoring studies in hospitals.

Another research (Cassault-Meyer *et al*., 2016) investigated molecular effects of GBH (composed of 450 g/L glyphosate, 607 g/L isopropylamine salt and adjuvants) after acute exposure (0.5%), for an 8-day time frame, of 60-day male rats finding an increase in aromatase mRNA levels of at least 50% in rats treated, as well as the aromatase protein, and an increase in abnormal sperm morphology.

De Souza *et al*., 2017, analysing the hypothalamic-pituitary-thyroid (HPT) axis disruption by GBHs, showed that the perinatal exposure (from 5 mg/kg/day to 50 mg/kg/day) of groups of male rats to a GBH formulation (54% of glyphosate, as potassium salt) significantly reduced the thyroid-stimulating hormone (TSH) levels compared to the rats of the glyphosate AI control group.

A two-year experiment, evaluating toxicity in rats, feeding with GM NK603 Roundup maize – treated with Roundup, with 540 g/L of glyphosate, at 3 L/ha –, established that residual GBH in food caused changes in urine and blood parameters indicating renal and hepatic diseases (Mesnage *et al*., 2017). However, the same authors of this study admit that their results do not allow us to state with any certainty that GM maize consumption damages health or otherwise is not safe.

Regarding the intestinal microbiota, in a study of Lozano *et al*., 2017, significant dysbiosis in long-term Sprague-Dawley female rats, treated with 3 different doses (0.1 ppb, 400 ppm and 5000 ppm) of a GBH formulation (450 g/L glyphosate) were observed,

even when administered with low doses (400 ppm), and that the effects of GBH are different depending on the sex of rats; the authors after all recognize the need for further investigation.

A study on rats (Romano *et al*., 2010) found that administering commercial GBH formulation (540 g/L glyphosate) orally (at 0.25 ml/100 g of bw/d) to prepubescent rats affected testicle morphology and reduced the testosterone production, even if there were no effects on levels of corticosterone and estradiol.

Rainio *et al*., 2019, found several toxic oxidative effects of a GBH formulation (360 g/L glyphosate) on the larvae of a potato beetle (*Leptinotarsa decemlineata*), a non-target herbivore, randomly divided into three groups: 1) high concentration (100% GBH), 2) low concentration (1.5% GBH) and 3) control group (water) and exposed to GBH for different periods. In particular, the authors observed significant increases in oxidative peroxide levels already after two hours of exposure to the high concentration of GBH. It would have been very interesting to have a fourth group, treated with glyphosate alone, added to the three groups of larvae.

Schaumburg *et al*., 2016, – in a genotoxicity study of a glyphosate formulation (66.2% glyphosate) on the eggs of the tegu lizard (*Salvator merianae*), one of the species that lives in environments with contaminating effects – claimed that no teratogenic effects were observed in any exposed or control neonates and that no statistically significant difference was found in the size of the lizards at birth or after six months after exposure.

### *Effects of glyphosate and its formulations on aquatic species*

It seems that the glyphosate toxicity increases with increasing water temperature. In fact, a commercial GBH formulation (360 g/L glyphosate) was more toxic on specific aquatic species than glyphosate alone and surfactants alone and also twice toxic to bluegills at 27 °C than at 17 °C; to rainbow trout at 17 °C than at 7 °C (Folmar *et al*., 1980). These authors, however, conclude that, in natural environments (rivers and lakes) the applications of GBHs should not have any negative effect on the existing fauna in the water because the physical characteristics of the surface waters (low temperatures and neutral pH) quickly degrade the molecule in question.

GBHs are also recognized as being a significant source of water pollution. For example, Falace *et al*., 2018, exposing the *Fucus virsoides* to glyphosate and AMPA contained both in natural seawater, stated that these substances – but the authors have not indicated which of them – have a significant impact on the photosynthetic efficiency of this alga even for short exposure duration (24 hours). The same authors hypothesize that the bioavailability and toxicity of glyphosate on non-target species may be influenced by surfactants present in commercial formulations.

According to Giesy *et al*., 2000, during a ten-years research, glyphosate commercial formulation (54% glyphosate) was, for fish and amphibians in ecological exposures, not bioaccumulable and "practically non-toxic to slightly toxic".

Other researches have shown the opposite. Wan *et al*., 1989, stated that a GBH, with a 41% of N-(phosphonomethyl) glycine as Isopropyl-amine salt (30.5% glyphosate equiv.), had several concerns on young salmon. Fish, exposed to 5 mg/L of glyphosate for two weeks, were found to have gill damage; liver damage was observed at glyphosate concentrations of 10 mg/L (Neskovic *et al*., 1996). Particularly, Guilherme et al. (Guilherme *et al*., 2012) reported that anguillas had DNA damage after a GBH (containing isopropylammonium salt of glyphosate at 485 g/L as the active ingredient,

equivalent to 30.8% of glyphosate, and 16% of polyethoxylene amine as surfactant) exposition at 58 and 116 µg/L during one or three days. Another research (Modesto and Martinez, 2010) pointed out on the fish *Prochilodus lineatus*, highlighted that Roundup causes, after an of acute exposures (6, 24 and 96 h) to 10 mg/L, genotoxic effects in erythrocytes and gill cells, even if the origin of these abnormalities was not understood by the authors. Monitoring the activity of the enzyme acetylcholinesterase (AChE) in the same fish and at the same chronic daily exposure of GBH (41% of glyphosate) doses, these authors observed inhibition of acetylcholinesterase (AChE) in brain and muscle. However, these authors conclude that more work is necessary to discriminate what component of the formulated product, glyphosate or POEA, could be responsible for AChE inhibition.

Howe *et al*., 2004, studied GBHs acute toxicity for four North American amphibian species (*Bufo americanus, Rana clamitans, R. sylvatica, R. pipiens*) and the toxicity of POEA, glyphosate technical and five commercial formulations. No significant acute toxicity was observed with glyphosate technical material or the glyphosate formulations. *Rana pipiens* tadpoles, exposed to significant environmental concentrations of POEA, exhibited gonadal abnormalities, tail damage and decreased metamorphosis attributable to disruption of thyroid hormone signaling. The conclusions of this study are that the surfactant composition should be considered in the evaluation of the toxicity of GBHs.

More recently, Rzymski *et al*., 2013, affirmed, in their study on GBHs in a bathing area of Lake Lednica (Wielkopolska, Poland), that "glyphosate-based herbicides may have adverse effects on aquatic organisms including macroinvertebrates, thus their use in (or nearby) surface waters should be subject to strict limitation". The authors, however, do not specify what could be these effects and the mechanisms behind them.

In a recent study comparing the embryotoxicity of the AI and a GBH formulation in *Xenopus laevis*, Bonfanti *et al*., 2018, showed that while glyphosate was not embryoletal and that it involved simple edemas only at high concentration levels (50 mg a.e./L), the GBH, on the contrary, exhibited a 96 h $LC_{50}$ of 24.78 mg a.e./L.

The embryos of Xenopus laevis, incubated with 1/5000 dilutions of a commercial GBH (54% glyphosate), had shown alterations of neural and cephalic crest development and shortening of the anterior-posterior axis. Because embryos injected with pure glyphosate showed the same phenotypes, this fact led the authors to hypothesize that AI itself was responsible for the observed phenotypes, rather than another component of the commercial formulation (Paganelli *et al*., 2010).

Bach *et al*., 2018, evaluating and comparing sub-lethal histological effects of a GBH (containing 74.7% of the mono-ammonium salt of glyphosate, equivalent to 67.9% of glyphosate acid) and glyphosate AI on *Leptodactylus latrans* tadpoles, found joint adverse effects on the liver at concentrations normally found in the environment (from 0.37 mg a.e./L). Bach and its group confirmed too that formulation are more toxic than AI alone. Other similar studies are desirable to clarify on the adverse effects of these pesticides on animals at normal levels of environmental contamination.

## Discussion and conclusions

The degradation mechanisms and the persistence times of glyphosate and its metabilites, in soil and water, are very different depending on the many reactions that can occur between these chemicals and the biotic and abiotic components present in the environmental compartments. Once in the soil, glyphosate and its salts are converted by

certain species of *Pseudomonas* and other soil microbes components, to sarcosin, and form glyoxylic acid and AMPA that is much more persistent than glyphosate itself.

Glyphosate and phosphate molecules compete in the soil in relation to the processes of adsorption and mobility: the presence of the phosphate group and the application of phosphate fertilizers change pH levels and reduce the retention power of glyphosate from the soil matrix.

The persistence and degradation of glyphosate in aquatic environments depends on the lighting conditions: moderately persistent in seawater with low-light conditions while highly persistent in the dark.

Regarding the toxicity of glyphosate on animals, it depends, for the most part, on the adverse effects of its many commercial formulations, containing additives whose synergistic effects are still to be investigated. According to some authors, because the glyphosate molecule is very similar to glycine, it interferes with protein synthesis of mammals.

Glyphosate salts, as they are highly soluble, could have high impacts on aquatic wildlife; in fact, many recent reports have found massive glyphosate and AMPA presence in groundwater, rivers, rainwater and precipitation.

However, the studies on the above issues are still very few, often in contraddiction between them, and the long-term effects of glyphosate on land animals and aquatic species are still largely unexplored. Another controversial aspect is related to the absorption of the herbicide by animal organisms, with or without biotransformation.

Besides, current researches, concerning different animals, different health effects, different glyphosate formulations, different exposure ranges and different active concentrations are hardly comparable between them. About thiss point, it is important to distinguish between the possible adverse effects concerning only the AI and those concerning only the glyphosate formulations and metabolites.

It is therefore desirable for the future to be able to dispose of further studies about this herbicide and about the possible joint-effects on animals of the many GBHs at different doses.

A further and final consideration in terms of international development: now in Europe we are on the way to ban the use of glyphosate in all countries and this will perhaps pave the way for the use of other even more dangerous herbicides. Most likely, then, it would be more correct to control and limit the use of GBHs and not totally eliminate them. In general terms, in fact, it is the abuse of a product, even the most harmless, which determines its dangerousness.

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