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REVIEW ARTICLE

IS GLYPHOSATE REALLY HAZARDOUS FOR HUMAN HEALTH?

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Summary

Glyphosate [N-(phosphonomethyl) glycine] is one the world's most widely used agricultural herbicide. It allows farmers to spray a planted field, generally before the crops have sprouted, killing weeds but not the crops that will grow there. GMO critics claim glyphosate is linked to autism, cancer, gluten allergies, 'leaky gut' syndrome and other disorders. Concerns about glyphosate's possible health impacts increased in 2015 after the International Agency for Research on Cancer, a research arm of the World Health Organization, classified glyphosate as "probably carcinogenic". The ecological risk assessment indicates that there is potential for effects on birds, mammals, and terrestrial and aquatic animals. A joint panel from the World Health Organization and the Food and Agriculture Organization of the United Nations issued an summary evaluation of glyphosate in May 2016, concluding it poses no cancer risks as encountered in food and does not impact our genes. Although the European Food Safety Authority declared the evidence on glyphosate's carcinogenicity for humans to be "very limited", there is still some doubt as to whether all the studies have been made "lege artis" or whether they have not even been falsified.

Key words: glyphosate; Roundup; herbicide; human exposure; environmental health; risk assessment

ABBREVIATIONS AND SYMBOLS

AMPA	Aminomethylphosphonic acid	IL-1β	Interleukin 1 beta
DA	Dopamine	IPA	isopropylamine
CaMKII	Ca^{2+}/Calmodulin-dependent protein kinase II	LC_{50}	Median Lethal Concentration
CKDu	Chronic Kidney Disease of unknown cause	LD_{50}	Median Lethal Dose
D1	Dopamine receptor 1	LDLo	Lethal Dose Low
EFSA	European Food Safety Agency	NOAEL	No-Observed-Adverse-Effect Level
FAO	Food and Agriculture Organization	SAN	Sri Lankan Agricultural Nephropathy
GBHs	Glyphosate-based herbicides	TDLo	Toxic Dose Low
GMO	Genetically Modifes Organisms	TNF-α	Tumor Necrosis Factor alpha
IARC	International Agency for Research on Cancer	USEPA	U.S. Environmental Protection Agency
IFN-γ	Interferon gamma	WHO	World Health Organization

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INTRODUCTION

Glyphosate, commonly known by its original trade name Roundup, is the world's most widely used herbicide (Duke and Powles, 2008). Glyphosate has emerged to control annual and perennial weeds. Glyphosate-based herbicides are manufactured by many companies in many countries. This non-selective herbicide was initially targeted at the non-crop areas in agriculture and for industrial applications but, with the continuing development of minimum- and no-tillage agricultural practices, glyphosate also found usage in a number of crop outlets (Jordan et al., 1997). Most recently, glyphosate has found direct crop usage on plant varieties that have been genetically modified to be tolerant of glyphosate applications (Green, 2018). This massive use of glyphosate for decades has resulted in its ubiquitous presence in the environment, and poses a threat to humans and ecosystem.

The risk of glyphosate for human health has long been undermined, especially since almost all scientific studies have proven its safety. Because herbicidal action of glyphosate is primarily due to its capacity to block the production of essential amino acids in plants through a pathway called "shikimate", which is present only in plant. Thus, it was sold as "safe" for animals and humans (Amrhein et al., 1980). But when glyphosate residues began appearing in food, it caused a wave of outrage, and glyphosate became the subject of further studies on its safety (Giesy et al., 2000). It has been speculated that this herbicide may be responsible for many health problems and an increased number of some chronic diseases (Swanson et al., 2016). So, what is the safety of this plant killer and what are the hazards of the herbicide's residues in the environment for human health? This article attempts to answer this question.

HISTORY

The molecule of glyphosate (N-(phosphonomethyl)glycine) was first synthesized in 1950 by the Swiss pharmaceutical firm Cilag (Franz et al. 1997). Because the molecule showing no pharmaceutical perspective, the compound has not been investigated any further. Later it was transferred to the distributor of laboratory research chemicals, Aldrich Chemical Co., along with research samples of Cilag. In this time American company Monsanto developed water-softening agents on the basis of phosphonic acid derivatives and tested over 100 chemical substances related to aminomethylphosphonic acid (AMPA), among them N- (phosphonomethyl) glycine. Monsanto later extended the study of these compounds to herbicide activity testing, and observed their potential against perennial weeds (Dill et al., 2010). Herbicidal effect of N-(phosphonomethyl) glycine was described by Baird et al. in 1971, the subsequent patent (US 3799758) was claimed and obtained by Monsanto, and was introduced as a herbicide product Roundup® (formulation of the isopropylamine salt of glyphosate with a surfactant).

Upon the expiration of the patent protection in 2000, sales of generic preparations intensively expanded (Dow, Syngenta, NuFarm, and numerous Chinese chemical factories.), but the leading preparation producer remained Monsanto (Duke and Powles, 2008). At present, the global glyphosate production capacity is more than 1 million tonnes. The largest manufacturer is now China.

CHEMISTRY

Glyphosate, N-(phosphonomethyl)glycine (CAS 1071-83-6) is a phosphonomethyl derivative of the amino acid glycine. It is an amphoteric chemical substance (m.p. 200 °C) containing a basic secondary amino function in the middle of the molecule and monobasic (carboxylic) and dibasic (phosphonic) acidic sites at both ends (Fig. 1). This small molecule contais both donor (acidic) and acceptor (basic) functional groups with pKa 10.9, 5.9 and 2.3, respectivelly. Glyphosate can form a zwitterionic structure (Knuuttila and Knuuttila, 1979). This is reflected in very good water solubility (11.6 g/L at 25 °C) and poor solubility in organic solvents (Subramaniam et al., 2000). To further increase glyphosate water solubility it is often prepared in form of its salts.

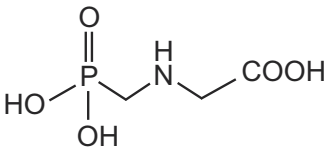

Figure 1. The chemical structure of glyphosate, N-(phosphonomethyl)glycine.

Synthesis

Glyphosate can be synthesized in several ways. Two main approaches are used to synthesize glyphosate industrially (Dill, 2010). The first synthesis is reaction of iminodiacetic acid with phosphorous acid and hydrochloric acid (Scheme 1) and second is a one-pot synthesis from dimethyl phosphite, glycine, and paraformadehyde (Scheme 2) (Fig. 2).

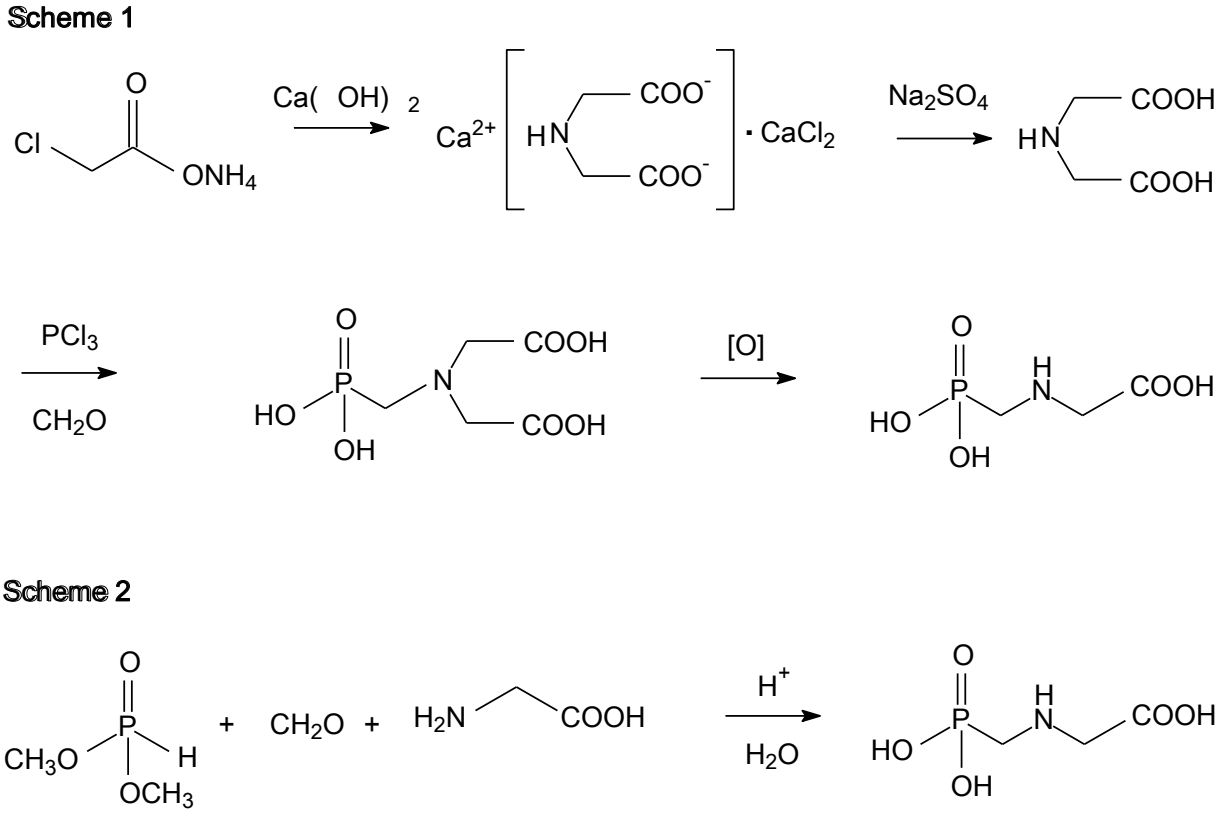

Figure 2. Scheme of the two most commonly used syntheses of glyphosate industrial production.

Degradation

Degradation of glyphosate takes place mostly by two processes: decarboxylation or dephosphorylation. The corresponding intermediate metabolites are AMPA or glycine, respectively. The first pathway (A) is catalyzed by oxidoreductases, the second (B) by C–P lyases cleaving the carbon-phosphorous bond. Both pathways occur in environmental matrices (water, soil) and plants, but the main metabolite in all cases is AMPA (Fig. 3). There are many reviews about the toxicity and fate of glyphosate and its major metabolite, AMPA (Parrot et al., 1995; Mañas et al., 2009; Daouk et al., 2013; Levine et al., 2015). However, there is lack of reviews on biodegradation and bioremediation of glyphosate (Zhan et al., 2018).

Glyphosate is moderately persistent in the marine water under low light conditions and is highly persistent in the dark. The half-life for glyphosate at 25 °C in low-light was 47 days, in the dark 267 days at 25 °C and 315 days in the dark at 31 °C (Mercurio et al., 2014). The degradation rate of glyphosate in the clay soil is also very slow, the half-life was 110-151 days and the kinetics of AMPA residues suggest that AMPA is more persistent than glyphosate (Bergström et al., 2011).

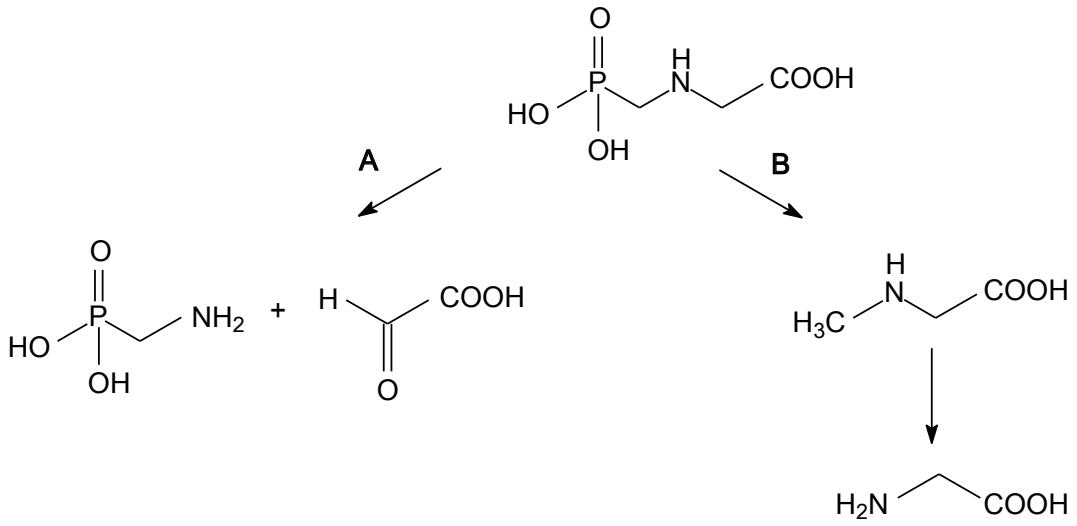

Figure 3. Two ways of degradation of glyphosate in environmental matrics. The first pathway (A) is catalyzed by oxidoreductases, the second (B) by C–P lyases cleaving the carbon-phosphorous bond. Major metabolite is AMPA.

TOXICITY

Glyphosate is a moderately toxic herbicide. Even though the LD_{50} values show the compound to be relatively non-toxic it can cause significant eye irritation. The toxicity of the technical product (glyphosate) and the formulated product (Roundup) is nearly the same. Published acute and subchronic toxic parameters of glyphosate are summarized in Table I.

Chronic Toxicity

Subchronic and chronic tests with glyphosate have been conducted with rats, dogs, mice, and rabbits in studies lasting from 21 days to two years. With few exceptions there were no treatment-related gross or cellular changes (Monsanto Company, 1985). In a chronic feeding study with rats, no toxic effects were observed in rats given doses as high as 31 mg/kg/day, the highest dose tested. No toxic effects were observed in a chronic feeding study with dogs fed up to 500 mg/kg/day, the highest dose tested (US EPA, 1992). Mice fed glyphosate for 90 days exhibited reduced body weight gains. The lifetime administration of very high amounts of glyphosate produced only a slight reduction of body weight and some microscopic liver and kidney changes. Blood chemistry, cellular components, and organ function were not affected even at the highest doses.

Reproductive and developmental toxicity

Different reports suggest that GBHs may act as endocrine disruptors. Dallegrave et al. (2007) described that exposure to glyphosate-Roundup may induce significant adverse effects on the reproductive system of male Wistar rats at puberty and during adulthood. Gasnier et al. (2009) documented that GBHs are toxic and endocrine disruptors in human cell lines using gene reporter tests. They therefore recommended to consider a real cell impact of glyphosate-based herbicides residues in food, feed or in the environment on human health.

Research suggests that glyphosate induce oxidative stress and induce harmful effects on reproductive parameters in fish and that this change would reduce the fertility rate of these animals (Hued et al., 2012; Harayashiki et al., 2013; Lopes et al., 2014; Uren Webster et al., 2014), like invertebrates (Chu et al., 2005; Schneider et al., 2009; Gaupp-Berghausen et al., 2015) and may also cause reproductive toxicity in mammalian systems (Yousef et al., 1995; Beuret et al., 2005; Benachour and Séralini, 2009) but results in laboratory animals showed that glyphosate alone has low toxicity on male reproductive system (Dai et al., 2016).

Table I. Published Acute and Subchronic Toxic Parameters of Glyphosate

Organism	Test Type *	Route	Reported Dose	Source **
honeybee	LD_{50}		> 100 µg/bee	Schuette, 1998
Daphnia magna	LC_{50}		930 ppm (48 hrs)	Schuette, 1998
rainbow trout	LC_{50}		38 ppm (96 hrs)	Schuette, 1998
quail	LD_{50}	oral	4,640 mg/kg	TOXNET, Glyphosate
bobwhite quail	LD_{50}	oral	3.851 mg/kg	Schuette, 1998
bluegill runfish	LC_{50}		78 ppm (96 hrs)	Schuette, 1998
mouse	LD_{50}	intraperitoneal	130 mg/kg	TOXNET, Glyphosate
mouse	LD_{50}	oral	5,600 mg/kg	Monsanto Comp. 1985
mouse	LD_{50}	oral	1,568 mg/kg	TOXNET, Glyphosate
mouse	NOAEL	oral (90-day)	500 mg/kg/day	EFSA 2015
mouse	NOAEL	dermal (90-day)	500 mg/kg/day	EFSA 2015
rat	LD_{50}	intraperitoneal	235 mg/kg	TOXNET, Glyphosate
rat	LD_{50}	oral	4,873 mg/kg	TOXNET, Glyphosate
rat	LD_{50}	oral	4,320 mg/kg	Schuette, 1998
rat	LD_{50}	oral	> 2,000 mg/kg	EFSA 2015
rat	NOAEL	oral (90-day)	414 mg/kg/day	EFSA 2015
rat	LD_{50}	dermal	> 2,000 mg/kg	EFSA 2015
rat	NOAEL	dermal	400 mg/kg/day	EFSA 2015
rat	LC_{50}	inhalation	12,200 mg/m^3	Hartley and Kidd, 1983
rat	LC_{50}	inhalation	> 5,000 mg/m^3	EC 2002
dog	NOAEL	Oral (90-day)	300 mg/kg/day	EFSA 2015
dog	NOAEL	Dermal (90-day)	300 mg/kg/day	EFSA 2015
rabbit	LD_{50}	oral	3,800 mg/kg	TOXNET, Glyphosate
rabbit	LD_{50}	dermal	7,940 mg/kg	Hartley and Kidd, 1983
man	LDLo	oral	2,143 mg/kg	Tominack et al., 1991
man	TDLo	oral	1,214 mg/kg	Menkes et al., 1991

* LD_{50} = Median Lethal Dose, LC_{50} = Median Lethal Concentration, LDLo = Lethal Dose Low, TDLo = Toxic Dose Low, NOAEL = No-Observed-Adverse-Effect Level
** TOXNET = Toxicology Data Network, EFSA = European Food Safety Authority, EC = European Commission.

Despite the relative safety of glyphosate, various adverse developmental and reproductive problems have been alleged as a result of exposure in humans and animals. Although toxicity was observed in studies that used glyphosate-based formulations, the data strongly suggest that such effects were due to surfactants present in the formulations and not the direct result of glyphosate exposure. Because human exposures are extremely low, the estimated exposure concentrations in humans are >500-fold less than the oral reference dose for glyphosate of 2 mg/kg/day set by the U.S. Environmental Protection Agency (USEPA 1993). In conclusion, the available literature shows no solid evidence linking glyphosate exposure to adverse developmental or reproductive effects at environmentally realistic exposure concentrations (Williams e al., 2012).

Many studies have focused on reproductive and developmental toxicity on glyphosate-based herbicide, but few evidence exists to imply the male reproductive toxicity of glyphosate alone in vivo (Romano et al., 2012; Uren Webster et al., 2014). Based on these studies, it may be conclude that glyphosate alone has low toxicity on male

rats reproductive system (Dai et al., 2016) and that the available literature shows no solid evidence linking glyphosate exposure to adverse developmental or reproductive effects at environmentally realistic exposure concentrations (Williams et al., 2012; Kimmel et al., 2013).

Teratogenicity

The very first examples of observed teratogenicity of glyphosate preparations have been linked to amphibians. Perkins and coworkers (2000) observed a formulation dependent teratogenic effect of glyphosate on embryos of the frog species *Xenopus laevis*. Lajmanovich and co-workers (2003) studied the effects of a glyphosate preparation on the tadpoles of *Scinax nasicus*, and found that a 2-4-day exposure to 3 mg/l glyphosate caused malformation in more than half of the test animals, but the treatment was carried out nearly at the LC_{50} level of glyphosate. Similary Dallegrave and co-workers (2003) found fetotoxic effects on rats treated with glyphosate at very high, 1000 mg/L concentration on the 6^{th}-15^{th} day after fertilisation. Nearly half of the newborn rat progeny in the experiments were born with skeletal development disorders.

Marc and co-workers (2004) observed a collapse of cell cycle of the embryos of the sea urchin of glyphosate preparations and consider the sea urchin biotest they developed as a possible experimental model for testing teratogenicity effect. Similar teratogenic effects were seen on embryos of chicken (Paganelli et al., 2010), but certain conclusions were chalenged by other authors (Mulet, 2011; Palma, 2011; Saltmiras et al., 2011). In his answer, Carrasco (2011) emphasised their opinion that the company representatives ignore scientific facts supporting teratogenicity of glyphosate and he also emphasized that of 180 research reports of Monsanto, 150 are not public, or have never been presented to the scientific community.

It is important to note that the bulk of the data provided during the evaluation stages of glyphosate safety were provided by the industry. Given the recent history of the endocrine disruptor field with low dose effects observed in numerous academic laboratories but not in industry-funded studies (Myers et al., 2009), it is clear that a reasonable corpus of independent studies is necessary to fully evaluate the effects of agrochemicals on human health. Animal experiments show that glyphosate is toxic to the mother and induces morphological impairments and developmental retardation of the fetal skeleton (Dallegrave et al., 2003; Yusof et al., 2014) but in humans there is little evidence of glyphosate teratogenicity.

The direct effect of glyphosate on early mechanisms of morphogenesis in vertebrate embryos opens concerns about the clinical findings from human offspring in populations exposed to glyphosate in agricultural fields (Paganelli et al., 2010). Its residues are found in the environment (Carlisle and Trevors, 1988), major crops, and food items that humans, including pregnant women, consume daily (Parvez et al., 2018). The new risk assessment must take into account all the data on the toxicity of glyphosate and its commercial formulations, including data generated by independent scientists and published in the peer-reviewed scientific literature, as well as the industry-sponsored studies.

Mutagenicity and Genotoxicity

Glyphosate was not mutagenic in Salmonella, and did not induce micronuclei in mice. The compound does not cause mutations in microbes. The tests on eight different kinds of bacterial strains and on yeast cells were all negative. The compound poses little mutagenic risk to humans (Stevens and Sumner, 1991). The no-observed-adverse-effect level (NOAEL) for the salivary gland lesions was 3125 ppm in the diet for mice. A NOAEL could not be determined from the rat study (Chan and Mahler, 1992). The potential genotoxicity of glyphosate was tested in a variety of well-established in vitro and in vivo assays including the Salmonella typhimurium and Escherichia coli WP-2 reversion assays, recombination (rec-assay) with Bacillus subtilis. Chinese hamster ovary cell gene mutation assay at the hypoxanthine/guanine phosphoribosyl transferase gene locus, hepatocyte primary culture/DNA repair assay, and in vivo cytogenetics assay in rat bone marrow (Li and Long, 1988).

Toxicity and genotoxicity studies indicate that glyphosate is not harmful, although several investigations suggest that it can alter various cellular processes in animals (Monroy et al., 2005). As demonstrated by the study Mañas et al. (2009) genotoxic may be the major metabolite of glyphosate, AMPA. In human lymphocytes was found

statistically significant clastogenic effect AMPA at 1.8 mM compared with the control group. *In vivo*, the micronucleus test rendered significant statistical increases at 200-400 mg/kg.

The present review of subsequent genotoxicity publications and regulatory studies of glyphosate and glyphosate-based formulations (GBFs) show the glyphosate and typical GBFs do not appear to present significant genotoxic risk under normal conditions of human or environmental exposures. (Kier and Kirkland, 2013; Kier, 2015).

In 2015, the International Agency for Research on Cancer (IARC) published a monograph concluding there was strong evidence for genotoxicity of glyphosate and GBFs and moderate evidence for genotoxicity of the AMPA. These conclusions contradicted earlier extensive reviews supporting the lack of genotoxicity of glyphosate and glyphosate formulations. The Expert Panel reviewed the genotoxicity and oxidative stress data considered in the IARC Monograph, together with other available data not considered by IARC and concluded that glyphosate, glyphosate formulations, and AMPA do not pose a genotoxic hazard. With respect to carcinogenicity classification and mechanism, the Expert Panel concluded that evidence relating to an oxidative stress mechanism of carcinogenicity was largely unconvincing and that the data profiles were not consistent with the characteristics of genotoxic carcinogens (Brusick et al., 2016).

Organ Toxicity

Kidney and liver are the main target organs for glyphosate with studies showing disruption of gene expression, alterations of enzyme levels, interference in mitochondrial metabolism, oxidative damage and, in the case of the kidney, tumours (Lamb et al., 1998; Peixoto, 2005; Gasnier et aal., 2010; Jasper et al., 2012; Larsen et al., 2012; Séralini et al., 2014).

In humans, ingestion of Roundup results in nephrotoxicity. Patients who die from glyphosate ingestion usually have developed acute kidney injury (Wunnapuk et al 2014). So called chronic kidney disease of unknown cause (CKDu) also called Sri Lankan Agricultural Nephropathy – SAN) amongst farmers in Sri Lanka is probably connected with glyphosate. It is characterised by tubular interstitial nephritis associated with mononuclear cell infiltration, glomerular sclerosis, tubular atrophy, and tubular proteinurea (Jayasumana et al., 2014, 2015). As showed Mesnage et al. (2013) roundup killed human embryonic kidney cells at levels 200-fold lower than those recommended for agricultural use. The LC_{50} was 57.2 mg/L.

There are limited data on the central nervous system (CNS) effects of glyphosate toxicity especially as regards the entry of glyphosate into the brain through the blood-brain barrier (Astiz et al., 2012)

Carcinogenicity

The carcinogenicity of glyphosate has been reviewed by several national and international agencies (Ibrahim 2015). Rats and dogs and mice fed glyphosate over a wide range of doses showed no cancer related effects directly due to the compound (Forest Service, 1984). EPA has stated that there is sufficient evidence to conclude that glyphosate is not carcinogenic in humans (USEPA, 1992). Unfortunately, the study carried out by Swedish oncologists showed that glyphosate may induce cancer of the lymphatic system (Hardell and Eriksson, 1999). The results of the Swedish study have changed our opinion about "safety" of this herbicide (Pieniazek et al., 2003). Investigations concerning both its accumulation and toxic effect in animals and plants are now under way in many laboratories.

Carcinogenicity in animals

There is only one published study on the carcinogenicity of the active substance glyphosate in rats (Chruscielska et al. 2000), which showed no significant increase in tumour incidences in any treated group. Two additional published studies on glyphosate formulations, the first one on initiation-promotion in mice (George et al. 2010) and the second one, a study of rats (Seralini et al. 2014) that was retracted and republished creating some controversies (Fagan et al. 2015), were considered inadequate by IARC and EFSA for carcinogenicity assessment (European Food Safety Authority 2012; IARC 2015). Consequently, industry-sponsored studies, required by several jurisdictions worldwide, have constituted the basis for the assessment of animal carcinogenicity by both IARC and EFSA.

Carcinogenicity in humans

Glyphosate has been rigorously and extensively tested for carcinogenicity by administration to mice and to rats and Most authors have concluded that the evidence does not indicate a cancer risk to humans. The International Agency for Research on Cancer (IARC), however, evaluated some of the available data and concluded that glyphosate probably is carcinogenic to humans (Williams et al., 2016).

The most up-to-date review of human epidemiological studies on glyphosate offered study IARC (2015). Positive evidence regarding an association between exposure to glyphosate and non-Hodgkin lymphoma, observed in some case-control studies but not confirmed by cohort studies, was considered sufficient by IARC to conclude on "limited evidence" in humans. IARC concluded that the limited evidence in humans was supported by sufficient evidence of carcinogenic potential in animals and strong mechanistic evidence for genotoxicity and oxidative stress. In the absence of conclusive human evidence, and despite some views suggesting the need for re-assessing its relevance (Beyer et al. 2011; Marone et al. 2014; Osimitz et al. 2013), rodent long-term toxicity/carcinogenicity studies are used for predicting carcinogenicity in humans (Doktorova et al. 2012).

Neurotoxicity

Neurotoxicants can produce neurologic effects by several general mechanisms, as detailed by Fonnum (1999): Damage to nerve cells from free radicals, disruption of nerve fibers, disruption of myelin, interference with ion channels, interference with uptake, release, or metabolism of neurotransmitters, and disruption of neuroglia cells. Some recent studies demonstrate that glyphosate exposure is associated with oxidative damage (Kašuba et al., 2017; Tang et al., 2017) and that glyphosate might lead to excessive extracellular glutamate levels and consequently to glutamate excitotoxicity and oxidative stress in rat hippocampus (Cattani et al., 2014).

Animals

The former toxicology studies of glyphosate has been examined in subchronic, chronic, and multigeneration studies in rodents and in subchronic studies in dogs and did not find evidence of neurotoxicity. More recently Coullery et al. (2016) report about impaired neuronal development caused by glyphosate exposure. They observed that the initial axonal differentiation and growth of cultured neurons is affected by glyphosate and that glyphosate led to a decrease in Wnt5a level, which is a key factor for the initial neurite development and maturation, as well as inducing a down-regulation of CaMKII activity. The results of Hernández-Plata et al. (2015) indicate that repeated glyphosate exposure of Dawley rats results in hypoactivity accompanied by decreases in specific binding to D1-DA receptors in the NAcc, and that acute exposure to glyphosate has evident effects on striatal DA levels.

Humans

The clinical case literature of acute glyphosate intoxication is reasonably extensive but does not provide evidence for glyphosate being an acute neurotoxicant in humans. Large-scale controlled epidemiological studies of glyphosate exposure and neurological outcomes have not been reported. In the hundreds of reported cases of glyphosate poisoning suggests that any neurologic symptoms associated with glyphosate exposures were secondary to other toxic effects (Sawada et al., 1988; Menkes and Temple, 1991; Talbot et al., 1991; Tominack et al., 1991; Temple and Smith, 1992; Hung et al., 1997; Pushnoy et al., 1998; Sorensen and Gregersen, 1999; Lee et al., 2000; Williams et al., 2000).

Immunotoxicity

Immunotoxicity of glyphosate was studied by Chinese authors (Ma et al., 2015) in fish. The results of this study indicate that glyphosate causes immunotoxicity on common carp (*Cyprinus rarpio*) via suppressing the expressions of immunoglobulin M, complement C3, and lysozym and also via damaging the fish kidney. In other experiment (Ma and Li, 2015) the acute toxicity tests showed that the 96 h LC_{50} of glyphosate for common carp was 520.77 mg/L and sub-acute exposure of glyphosate altered the contents of IFN-γ, IL-1β, and TNF-α in fish immune organs. In addition, glyphosate-exposure also caused remarkable histopathological damage in the fish liver, kidneys,

and spleen. These results suggest that glyphosate-caused cytokine alterations may result in an immune suppression or excessive activation in the treated common carp as well as may cause immune dysfunction or reduced immunity.

The toxic effects of glyphosate on Chinese mitten crab (*Eriocheir sinensis*), were assessed using immunotoxicity and genotoxicity biomarkers in the study of Hong et al. (2017). The results showed that 24 h and 96 h LC_{50} values of glyphosate for *E. sinensis* were estimated as 461.54 and 97.89 mg/L, respectively, and the safe concentration was 4.4 mg/L. Using immunological assays, it was found that glyphosate has evident toxic effect on *E. sinensis* by immune inhibition that is possibly due to the haemocyte DNA damage and a sharp decline in haemocyte numbers, which subsequently induced changes in activities of immune-related enzymes and haemocyte phagocytosis.

This studies does not provide information that is adequate for determining whether the reported immune responses were due to a direct effect on the immune system or secondary effects associated with cytotoxicity.

HUMAN POISONING

Glyphosate is one of the most commonly used herbicides worldwide, which is minimally toxic to humans. Clinical toxicologists occasionally encounter cases of severe systemic toxicity. Cost reported cases have followed the deliberate ingestion of the concentrated glyphosate-based formulations (Jyoti et al., 2014; Thakur et al., 2024). There is a reasonable correlation between the amount ingested and the likelihood of serious systemic sequelae or death (Bradberry et al., 2004).

Glyphosate and commercial glyphosate-based formulations

Commercial glyphosate-based formulations most commonly range from concentrates containing 40 % glyphosate to 1 % glyphosate formulations marketed for domestic use. They generally consist of an aqueous mixture of the iso-propylamine (IPA) salt of glyphosate, a surfactant, and various minor components. It is problem that commercial formulations contain surfactants, which vary in nature and concentration, and many surfactants probably contribute to the acute toxicity of glyphosate formulations. Therefore, it is difficult to separate the toxicity of glyphosate from that of the formulation as a whole or to determine the contribution of surfactants to overall toxicity. Accidental ingestion of glyphosate formulations is generally associated with only mild, transient, gastrointestinal features.

Potential risks of glyphosate to human health via food contamination

The US EPA classifies glyphosate as 'practically non-toxic and not an irritant' under the acute toxicity classification system. This classification is supported by the majority of scientific literature on the toxic effects of glyphosate. However, in 2005, the FAO reported that glyphosate and its major metabolite AMPA, are of potential toxicological concern, mainly as a result of accumulation of residues in the food chain (Bai and Ogbourne, 2016). Because current safety assessments rely heavily on studies conducted over 30 years ago and human exposures to glyphosate are rising. It is probably that current safety standards for glyphosate are outdated and may fail to protect public health or the environment. To improve safety standards, the following are urgently needed: (1) human biomonitoring for glyphosate and its metabolites; (2) prioritisation of glyphosate and GBHs for hazard assessments, including toxicological studies that use state-of-the-art approaches; (3) epidemiological studies, especially of occupationally exposed agricultural workers, pregnant women and their children and (4) evaluations of GBHs in commercially used formulations, recognising that herbicide mixtures likely have effects that are not predicted by studying glyphosate alone. (Vandenberg et al., 2017).

GLYPHOSATE AND MICROBIOME

Glyphosate has been known to have negative effects on microorganisms in the soil (Carlisle and Trevors, 1988), but it influenced also microorganisms in the human digestive system (microbiome). A number of recent studies show that glyphosate can cause imbalances in the normal microbiome, increasing vulnerability to pathogenic bacteria, as well as influencing the response to antibiotics and intestinal functioning, in humans and animals. In an *in vitro* study on poultry gut microorganisms, the highly pathogenic bacteria (*Salmonella entritidis*, *Salmonella gallinarum*, *S. typhimurium*, *Clostridium perfringens* and *C. botulinum* were found to be highly resistant to glyphosate while

most of beneficial bacteria (*Enterococcus faecalis*, *E. faecium*, *Bacillus badius*, *Bifidobacterium adolescentis*, and *Lactobacillus* spp.) were moderately to highly susceptible to it. This indicates poultry feed containing residues of glyphosate may be a predisposing factor in increased risk of pathogens in poultry and subsequently foodborne illness in humans (Shehata et al 2013, 2014).

In an *in vitro* study on rats, glyphosate impaired small intestinal motility at concentrations that are reported to be present in human blood (3 – 14 mg/L) (Chlopecka et al 2014). The authors suggest that the repetitive presence of glyphosate in low doses in intestine cells might play a crucial role in recurrent intestinal dysmotility. Samsel and Seneff (2013) have hypothesised that glyphosate residues in food may be linked to increasing coeliac disease in North America and Europe, because of glyphosate's adverse effect on the balance between beneficial and pathogenic gut biota, its ability to chelate metals, and its inhibition of some cytochrome P450 enzymes.

REGULATION

An independent review by the International Agency for Research on Cancer (IARC) found that glyphosate is a "probable human carcinogen". A review by the EFSA found no evidence of carcinogenic hazard. These differing findings have produced regulatory uncertainty.

Reflecting this regulatory uncertainty, the European Commission on November 27 2017, extended authorization for glyphosate for another 5 years, while the European Parliament opposed this decision and issued a call that pesticide approvals be based on peer-reviewed studies by independent scientists rather than on the current system that relies on proprietary industry studies.

On November 27 2017, the European Commission extended the authorization for glyphosate for another 5 years. The European Parliament, however, opposed this decision and issued a call for pesticide approvals to be based on published peer-reviewed studies by independent scientists instead of the current system, which is largely based on unpublished proprietary studies. Regulatory uncertainty and debate are extensive (Portier et al., 2016; Vandenberg et al., 2017).

CONCLUSIONS

The herbicide glyphosate, N-(phosphonomethyl) glycine, has been used extensively in the past 40 years, under the assumption that side effects were minimal. However, in recent years, concerns have increased worldwide about the potential wide ranging direct and indirect health effects of the large scale use of glyphosate. In 2015, the WHO reclassified glyphosate as probably carcinogenic to humans. Although the acute toxic effects of glyphosate and AMPA on mammals are low, there are animal data raising the possibility of health effects associated with chronic, ultra-low doses related to accumulation of these compounds in the environment. Independent research is needed to revisit the tolerance thresholds for glyphosate residues in water, food and animal feed taking all possible health risks into account.

Finally, it would be useful to answer the question posed in the article title. IS GLYPHOSATE REALLY HAZARDOUS FOR HUMAN HEALTH? A critical review of the recent studies on glyphosate can clearly lead to the conclusion that this herbicide poses a major threat to the environment and to humans. Its massive use should be stopped as quickly as possible and should only be used to a limited extent where it can not be replaced by other total herbicides.

COMPETING INTEREST

The authors declare that they have no competing interests.

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