ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 10, Issue, 06(C), pp. 32865-32869, Jun, 2019

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

GLYPHOSATE TOXICITY, OXIDATIVE STRESS, CARCINOGENICITY AND REPRODUCTIVE EFFECTS: A REVIEW

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DOI: http://dx.doi.org/10.24327/ijrsr.2019.1006.3558

ARTICLE INFO

Article History:
Received 15th March y, 2019
Received in revised form 7th April, 2019
Accepted 13th May, 2019
Published online 28th Jun, 2019

Key Words:

Glyphosate, neural death, carcinogenicity, reproductive toxicity.

ABSTRACT

Introduction: Glyphosate (GLY) is the most commonly used herbicide which is applied to a wide variety of crops. Farmers are exposed more to the toxic effects of GLY than any other group of people, through consumption of contaminated food and water, among other sources. **Purpose:** To carry out a comprehensive review of studies on the effects of GLY at the level of oxidative stress, neurotoxicity, carcinogenicity and reproductive toxicity. Methods: A literature review was conducted using combinations of the keywords: glyphosate, oxidative stress, neurotoxicity, carcinogenicity and reproductive toxicity. The free data scientific databases used were PUBMED, FreeFullPDF.com and Google Scholar. **Results:** The search revealed that GLY reduces levels of glutathione (GSH) in epidermal cell lines, and increases the liver and kidney levels of GSH and glutathione peroxidase (GPx). It activates the antioxidant defense system and produces hepatic leakage of enzymes such as aminolevulinic acid synthase (ALA) and aspartate amino transferase (ASAT).

It acts through a mechanism similar to that of glutamic acid (Glu) agonists, which bring about neuronal death through receptor activation. It is positively associated with non-Hodgkin lymphomas, and shows clear evidence of tendency to induce renal tubule carcinoma, hemangiosarcoma, pancreatic islet adenomas and estrogen-dependent breast cancer. Glyphosate (GLY) inhibits the synthesis of testosterone and reduces fertility up to 20 % in humans. **Conclusion:** GLY activates oxidative mechanisms, produces neuronal death, causes reproductive damage, and has carcinogenic potential. However, its classification as a harmful agent is thought to be mainly due to its indiscriminate use.

INTRODUCTION

General Aspects of GLY

Glyphosate (GLY) is a post-emergence, broad-spectrum and non-selective herbicide which is absorbed by leaves and stems, and distributed to the roots (systemic toxicity). Thus, it kills all weeds, which makes it the most widely used herbicide worldwide[1]. In 2014 alone, 825,000 tons of GLY were used. However, 40% of the GLY on sale is a Roundup® brand which has been banned in some countries such as France, Holland and Denmark. Unfortunately, in Mexico, it is still one of the most used brands. This brand inhibits the synthesis of amino acids which a plant cannot synthesize by itself for its nutrition and growth, thereby leaving it lifeless[2]. It is microbiologically degraded to a toxic metabolite, aminomethylphosphonic acid (AMPA), which is found together with GLY in surface water[1]. These waters become contaminated through runoff after applying the product, and cause toxic effects on phytoplankton communities such as microalgae (considered resistant to chemical compounds) and freshwater fish (Figure 1), through oxidation of lipids and DNA[3–5]. In humans, GLY generates a series of clinical manifestations due its effect on various organs. These effects range from respiratory symptoms, gastrointestinal, dermal neuromuscular, and psychological problems, to conjunctivitis, dizziness and malaise. It is also known that GLY generates lethal effects associated with atrioventricular blocks, lung disease and kidney damage[6]. The objective of this study was to review the effects of GLY at the level of oxidative stress, neurotoxicity, carcinogenicity and reproductive toxicity.

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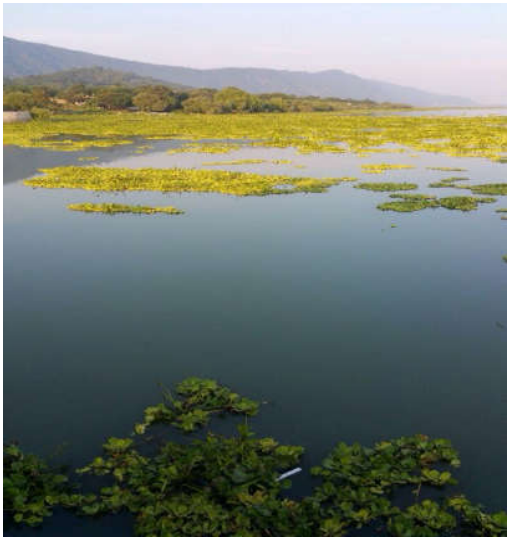

Figure 1 Surface waters of Lake Chapala seen from Mezcala Jalisco Mexico. This is one of the lakes that are considered contaminated with GLY due to soil runoff; GLY is also applied in the same lake for lily removal.

Oxidative stress and GLY

Studies in rats exposed to GLY and AMPA have shown lipid peroxidation-induced renal and hepatic damage, as well as DNA oxidation due to reactive oxygen species (ROS) and reactive nitrogen (RNS)[7,8]. These species (ROS and RNS) are the main causes of cell aging. They bring about damage DNA which subsequently leads to cancer. In 2015, the International Agency for Research on Cancer (IARC) classified GLY as a "probable human carcinogen" (Group 2A). This classification was based on available evidence on its effects on humans and lower animals[9].

The Acquavella team[10] performed an epidemiological analysis where they evaluated the correlation between GLY exposure and the development of different types of cancer, including multiple myeloma. However, the results are still insufficient, making it necessary to follow up with further studies. On the other hand, knowing that the skin is one of the routes of exposure and absorption of pesticides and herbicides, experiments have been conducted on human epidermal cell lines (HaCaT) exposed to Roundup®. The results revealed decreases in reduced glutathione (GSH) levels[11]. Reduced glutathione (GSH), which is a coenzyme synthesized by the liver, is very important reductant for combating cellular aging. Its deficiency leads to cell damage or death from oxidative stress, in addition to serious pathologies. Similar studies have determined that exposure to high concentrations of GLY leads to cardiovascular, respiratory, nephrotoxic and hepatotoxic damage. Ingestion of GLY may be lethal (oral $LD_{50} \geq 500$ mg/kg; inhalation $LD_{50} \geq 3400$ mg/L). It has been reported that it is possible to detect GLY in the urine. Exposure through consumption of contaminated food and water at a daily intake of 0.1 to 3.3 mg/kg may resulted in urinary GLY concentrations of 0.8 - 1.35 µg L^{-1} [12]. In some studies, urinary levels of GLY and AMPA were higher in men than in women[13]. The herbicide also contaminates susceptible human populations such as farmers and their families, even by contact with contaminated work clothing[14]. Results from the determination of GLY doses and their harmful effects to human health have so far been variable and controversial. A summary of the findings on doses and effects of GLY in murine models is presented in Figure 2.

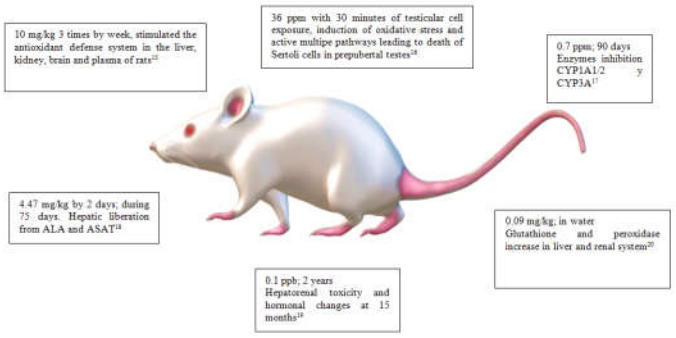

Figure 2 Representative diagram of the various effects of GLY in murine models at different doses and with different routes of administration.

Neurotoxic effects of GLY

Glyphosate (GLY) is a derivative of glycine. It interferes with the activity of serine hydroxymethyltransferase, an enzyme that catalyzes the synthesis of glycine from intracellular serine[21]. Since glycine and Glu act as neurotransmitters and play important roles in brain function, it may be reasonably assumed that GLY could have neurological effects. Its potential as a neurotransmitter is supported by its structural similarity to a glutamic acid receptor agonist, 2-amino-3-phosphonopropionic acid. It has also been shown that exposure to GLY induces neuronal death through the activation of Glu receptors in the hippocampus of immature rats, a region whose main function is maintenance of long-term memory[22]. In humans, this condition could cause movement disorders resembling symptoms of Parkinson's disease. Indeed, the Eriguchi team in 2019 reported an isolated case of a patient who developed Parkinson's disease four years after ingesting glyphosate[23]. This effect was related to dysfunction of mitochondrial activity and generation of oxidative stress, although the mechanism involved has not yet been elucidated. On the other hand, it has been reported that GLY exposure in male Wistar rats decreased levels of serotonin, dopamine and norepinephrine content in brain regions of the striatum, hippocampus, prefrontal cortex, hypothalamus and mesencephalon, thereby bringing about changes in the serotonergic, dopaminergic and noradrenergic pathways[24]. In Sprague-Dawley rats, exposure to GLY affected the dopaminergic pathway and decreased the locomotor activity, particularly with direct damage to the striatum and nucleus accumbens which are highly vulnerable to the toxic effect of GLY[25]. It has also been shown that the brains of rats treated with GLY at 10 mg/kg showed increased peroxidation of lipids and proteins, forming ROS and RNS as nitrites, with decreased levels of alpha-tocopherol, a fat-soluble compound that is part of vitamin E[15]. In another study using the same dose of GLY, a decrease in cardiolipin was observed, which affected the mitochondrial transmembrane potential of brain neurons[7].

Carcinogenicity of GLY

Some studies on the carcinogenicity of GLY in rats and mice have been carried out as part of toxicity evaluation of GLY. These toxicity studies were performed according to the criteria of the Organization for Economic Cooperation and Development (OECD) with doses ranging from 3 to about 5000 mg/kg. The experiments started in young adult animals and were completed after 2 years (before the aging of the rodents).

In general, the authors concluded that there was no evidence of a carcinogenic effect related to GLY treatment[26]. In contrast, IARC has considered four key epidemiological studies that reported incidents of non-Hodgkin's leukemia (lymphoma). Three of these studies were of cases and controls, and they indicated a positive and statistically significant association between GLY and lymphomas. The last was a cohort study in agricultural health which showed no association, although it did not contradict the results from previous studies. The IARC panel also considered the report that GLY induced renal tubule carcinoma and hemangiosarcoma in male mice[27]. In two other studies, it was observed that GLY also increased adenoma (benign tumor) of pancreatic islet cells in male rats. In addition, evidence of carcinogenicity in humans has been supported by a meta-analysis of occupational exposure to agricultural pesticides, which showed a positive association between exposure to GLY and non-Hodgkin lymphoma subtypes[28]. A Roundup® herbicide formulation was tested in rats using environmentally relevant exposure level of 0.1 ppb in water[19]. The results showed that GLY exerted a non-linear and hormone-dependent tumorigenic effect on the rat mammary glands. An *in vitro* study showed that GLY promoted the growth of estrogen-dependent human breast cancer cells at a dose of 0.1 ppm[29]. In this study, the transcription of factors sensitive to estrogen increased from 5 to 13 times in the presence of GLY. It is worth mentioning that the concentrations used here were within the range of environmental exposures of GLY. However, the reproducibility and relevance of this finding in *in vivo* situations must be confirmed through further studies. Alterations in estrogenic gene expression have also been demonstrated in another study that investigated the transcriptome responses of mammary cells to GLY at higher exposure levels[30]. Moreover, there have been accentuations in DNA damage and chromosomal aberrations in Ecuador and Argentina, which are populations occupationally exposed to glyphosate-based herbicides (GlyBH). In Argentina, several reports showed increases in the incidence of different types of cancer and tumors[31], especially in the young population[32]. However, these findings still need to be adequately complemented by epidemiological studies.

Reproductive toxicity of GLY

Glyphosate (GLY) inhibits cytochrome P450 which is responsible for the metabolism of xenobiotics and drugs in human cells[33] and rats[34]. It also inhibits testosterone synthesis *in vitro* in testicular cells isolated from adult rats[35]. Some epidemiological studies have investigated the potential reproductive effects of GLY, and reported a reduction in fertility of at least 20% of female residents of Ontario farms[36]. Other studies suggest that spermatogenesis might be vulnerable to numerous environmental toxicants, including GLY[37,38]. Glyphosate (GLY) also induces alterations in hormonal regulation that induce adverse effects on the male reproductive system during the prenatal and neonatal periods[39]. Exposure of young rats to HBGly induces changes in the progression of puberty and testicular morphology, and reduces testosterone production[40]. In adult rats, GLY induces alteration of normal testicular architecture, decreases testosterone secretion, and causes abnormalities in properties of sperm[41]. It has also been shown that exposure of rats to HBgly (Roundup®) at a dose of 50 to 450 mg/kg/day during pregnancy and lactation, produced adverse effects on serum testosterone levels and sperm parameters[42,43]. In these studies, it was also shown that HBgly (Roundup®) caused changes in sexual behavior in male offspring, while perinatal exposure to GLY (but not to HBGly) affected the weight of testicles, morphology of the seminiferous epithelium and the level of circulating testosterone in the short term[43].

Main findings

PARAMETER	EFFECT OF GLYPHOSATE
Oxidative stress	GLY oxidizes lipids and DNA in the kidneys and liver of murine models due to ROS and RNS increase. Exposure to GLY decreases both GSH level and superoxide dismutase (SOD) activity in liver and kidney of Sprague-Dawley rats. In Sprague-Dawley rats, exposure to GLY increased levels of malondialdehyde (MDA), the enzymatic activity of catalase and therefore the levels of hydrogen peroxide.
Neurotoxicity	GLY acts inhibits the enzymatic activity of hydroxymethyltransferase in its binding with serine Similarity of the GLY structure with 2-amino-3-phosphonopropionic, glutamate receptor agonist. GLY activates receptors for Glu, an effect which induces neuronal death GLY decreases levels of serotonin, dopamine and norepinephrine in the striatum, hippocampus, prefrontal cortex, hypothalamus and mesencephalon. GLY induces mitochondrial damage and increases oxidative stress, a mechanism possibly related to Parkinson's disease development. GLY decreases levels of alpha-tocopherol and cardiolipin.
Carcinogenicity	In a case-control study, a positive association was found between GLY and the presence of non-Hodgkin lymphomas. Exposure to GLY induces a positive trend for the development of renal tubule carcinomas and hemangiosarcoma. GLY is a risk factor that increases adenomas of cells in pancreatic islets of rats. GLY promotes the growth of breast cancer cells in humans from 0.1ppm.
Reproductive toxicity	GLY inhibits the synthesis of testosterone in testicular cells of rats. A 20% reduction in fertility was found in women exposed to GLY. Exposure to GLY alters testicular architecture, weight of testicles and in the level of circulating testosterone.

CONCLUSION

Despite the existence of multiple evidence on the toxic effects of GLY, the scientific community continues to insist that these effects are mainly due to its indiscriminate use, especially in view of the paucity of evidence in humans. Indeed, some industrial associations are of the view that the GLY risk is comparable to that of alcohol consumption in humans. However, it has been shown that chronic exposure to GLY is sufficient for detection of its residues in blood and urine samples. These residues induce generation of ROS and RNS, leading to oxidative stress, cell damage, neurotoxic effects and germ cell damage, with inhibition of the synthesis of testosterone. Due to these findings, we suggest that the GLY should be considered a highly toxic substance that can damage the genetic material and cause some degree of cancer.

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How to cite this article:

Silva-Madera Rosa Jazmin *et al*., 2019, Glyphosate Toxicity, Oxidative Stress, Carcinogenicity and Reproductive Effects: A Review. *Int J Recent Sci Res.* 10(06), pp. 32865-32869. DOI: http://dx.doi.org/10.24327/ijrsr.2019.1006.3558

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