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Glyphosate and Microbiota: controversies and truths

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ABSTRACT

Glyphosate-based herbicides (GBHs) are currently the most widely used herbicides in the world, and for many years, they have been considered safe for human health due to the assumption that human cells are presumably not directly affected by glyphosate, given the lack of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme. However, the effect of glyphosate on the host-associated microbiota has been recently suggested in several studies. The manuscript is a critical review of the existing literature on the topic, with the aim to focus on the confirmed effects of glyphosate and co-formulants on gut microbiota as the target of potential harmfulness for humans. Moreover, if future evidence will confirm that glyphosate may act as one of the drivers for antibiotic resistance, its global impact on human health will need to be reassessed.

KEYWORDS

GLYPHOSATE
MICROBIOTA
HERBICIDES
HUMAN HEALTH

INTRODUCTION

Glyphosate-based herbicides (GBHs) are currently the most widely used herbicides in the world. During the past fifty years, million tons of GBHs have been sprayed globally due to affordable price, effectiveness and broad-spectrum ability to kill weeds. Glyphosate has excellent properties of fast sorption in soil, biodegradation and less toxicity to non-target organisms. However, glyphosate has been reported to increase the risk of cancer, endocrine disruption, celiac disease, autism, effect on erythrocytes, leaky-gut syndrome, etc. The reclassification of glyphosate in 2015 as 'probably carcinogenic' by the International Agency for Research on Cancer has been broadly circulated by anti-chemical and environmental advocacy groups claiming for restricted use or ban of glyphosate. In contrast, some comprehensive epidemiological studies involving farmers with long-time exposure to glyphosate in the USA and elsewhere, coupled with available toxicological data, showed no correlation with any kind of carcinogenic or genotoxic threat to humans[1].

Glyphosate can influence microbial survival directly as it inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) of the shikimate pathway, which produces essential amino acids (tyrosine, tryptophan and phenylalanine) in both plants and the majority of microbes[2].

This causes a shortage in aromatic amino acid synthesis and consequent death. As this shikimate pathway only exists in bacteria, fungi and plants, but not in

vertebrates, glyphosate was thought to impose minimal risks to mammals, including humans. However, emerging evidence suggests that glyphosate or GBHs, can adversely affect mammalian biology via multiple mechanisms. The gut microbiota has emerged as a possible link between glyphosate and adverse health effects reported in humans.

GBHS AND THEIR EFFECTS ON MICROBIOTA IN ANIMALS

Several studies have reported the detrimental effects of GBHs on animals. It has been reported that GBHs exposure can alter the microbiota in honeybees. Glyphosate increased, whereas the GBHs decreased gut microbiota diversity, indicating that negative effects are attributable to co-formulants. Healthy core microbiota have been shown to protect bees from parasite infections, change metabolism, and decrease mortality. Thus, the heavy use of GBHs may have implications on bees and ecosystems[3]. Other studies demonstrated that glyphosate and co-formulations caused profound changes in the caecum microbiome of rats, and this might influence the long-term toxicity, carcinogenicity and multigenerational effects of GBHs[4]. Recent evidence has shown an association between gut microbiota alteration and male reproductive toxicity induced by glyphosate in rats[5]. In another rodent model[6], the urine metabolite profile and gut microbiota were found to be significantly altered in dams and pups after exposure to commonly used GBHs at a currently acceptable human exposure dose.

Several studies have also suggested the possible link between GBHs exposure and abnormality in neurodevelopment. Pu et al[7] found abnormal composition of gut microbiota and short-chain fatty acids in fecal samples of juvenile offspring after maternal glyphosate exposure, correlated to an increase in autism spectrum disorder (ASD)-like behavioral abnormalities. A number of studies have demonstrated the profound relationship between gut microbiota alterations and behavioral changes. Subchronic and chronic exposure to GBHs were found to induce an increase of anxiety and depression-like behaviors in mice[8]. In addition, GBHs significantly altered the gut microbiome composition, with a decrease of *Corynebacterium, Firmicutes, Bacteroidetes* and *Lactobacillus* in treated mice, thus reinforcing the essential link between gut microbiome and GBHs toxicity.

Avian species are largely exposed to glyphosate via their food. Disturbance of cecal microbiota associated with plasma oxidative stress and accumulation of glyphosate in metabolic tissues in response to dietary glyphosate-based herbicides have been observed in exposed broiler hens, although most of these effects were reversible[9].

GLYPHOSATE AND ITS EFFECT ON HUMAN MICROBIOTA

Humans may be exposed to glyphosate directly, as farm workers, or indirectly via drinking water and foodstuffs containing glyphosate residues. Human cells are presumably not directly affected by glyphosate due to the lack of the EPSPS enzyme, but the effect of glyphosate on the host-associated microbiota has been suggested in several studies. Overall, there is limited experimental evidence available for the effects of glyphosate on the human gut microbiome. The gut microbiome is known to be a key player in modulating host metabolism, with multiple studies describing strong correlations between gut microbiome and host metabolites. GBHs exposure may have the potential to modify the human microbiota, which, in turn, influences host metabolic functions.

Human exposure might be possible through the ingestion of several glyphosate-contaminated foods of agricultural origin. Evidence has shown that traces of herbicide can be found in formula milk, honey, cereal grains or soy. The gastrointestinal tract can absorb a limited part of glyphosate, a minimal proportion (<0.7%) of the ingested dose. It is subsequently metabolized by hydrolysis to aminomethylphosphoric acid (AMPA), its main metabolite, and the rest is rapidly eliminated through urine and faeces[10]. Moreover, experimental studies initially failed to demonstrate the toxicity of GBHs, so they concluded that they didn't represent a risk to human health[10]. Despite this, the latest evidence suggests that glyphosate-induced dysbiosis may represent the link to explain the effects of GBHs in humans. Most gut bacteria do not possess a complete shikimate pathway, and this pathway is mostly transcriptionally inactive in the human gut microbiome[11]. This suggests that gut bacteria are mostly aromatic amino acid auxotrophs and, thus, relatively resistant to potential growth inhibition by glyphosate. However, Mesnage and Antoniou[11] classified *E. coli* EPSPS enzyme homologues as class I (sensitive to glyphosate) and class II (resistant to glyphosate). Among 44 subspecies reference genomes, accounting for 72% of the total assigned microbial abundance in 2144 human fecal metagenomes, 9 subspecies have class II EPSPS.

Previous results evidenced that highly pathogenic bacteria as *Salmonella Enteritidis, Salmonella Gallinarum, Salmonella Typhimurium, Clostridium perfrin-*

Effect of glyphosate on human health

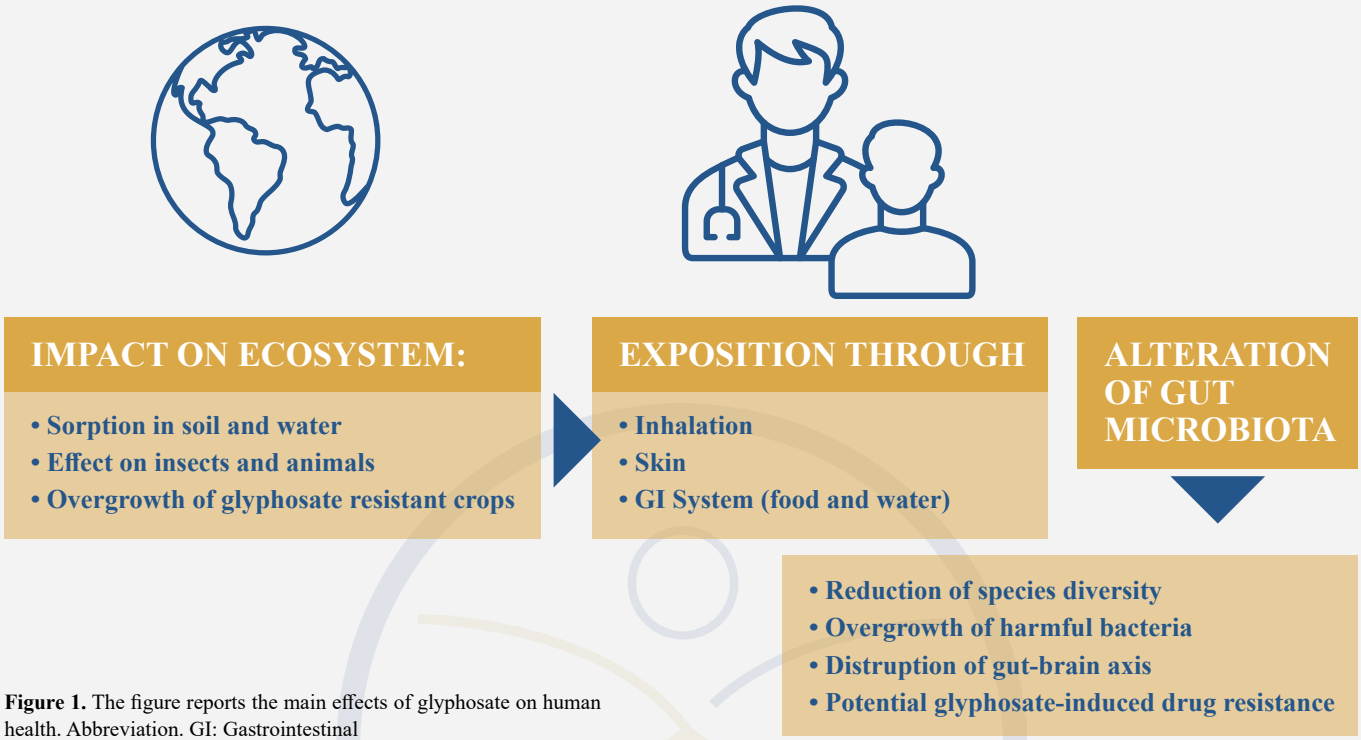

Figure 1. The figure reports the main effects of glyphosate on human health. Abbreviation. GI: Gastrointestinal

gens and *Clostridium botulinum* are highly resistant to glyphosate. In contrast, most of beneficial bacteria as *Enterococcus faecalis, Enterococcus faecium, Bacillus badius, Bifidobacterium adolescentis and Lactobacillus spp*. were found to be moderate to highly susceptible[12]. The consequent glyphosate-induced gut dysbiosis and the toxicity of glyphosate to the most prevalent *Enterococcus spp*. could be a significant predisposing factor associated with the increase in C. botulinum-mediated diseases. The overgrowth of bacteria such as clostridia generates high levels of noxious metabolites in the brain, which can contribute to the development of neurological deviations. A recent review[13] evaluated the impact of glyphosate-induced intestinal dysbiosis on the central nervous system, focusing on emotional, neurological and neurodegenerative disorders due to the disruption of the gut-brain axis.

Conversely, in a recent experimental study[14], the metabolism of glyphosate by the human fecal microbiota *in vitro* has been investigated, and neither a degradation of glyphosate nor a formation of AMPA was detected under the conditions used in the study, hinting at the assumption that transformation of glyphosate by the gut microbiota seems to be negligible in humans.

Although all these divergent reports leave the discussion about the safety of glyphosate and co-formulations still ongoing, increasing concerns raised about glyphosate may affect microbe-mediated ecosystems[15]. A microevolutionary analysis of the EPSPS enzyme showed that phylogenetics best explains bacterial sensitivity to glyphosate, *Firmicutes* being significantly more resistant to glyphosate than *Proteobacteria*, whereas *Actinobacteria* the most sensitive group to glyphosate. Furthermore, bacterial lifestyle has been found to be associated with sensitivity, i.e., facultative host-associated bacteria being more sensitive to glyphosate than free-living bacteria, probably because the latter are directly exposed to glyphosate spray at high concentrations. Thus, the heavy use of glyphosate may have a strong impact on the species diversity and composition of microbial communities via (i) the purifying selection against sensitive bacteria, (ii) the rapid adaptation of some bacterial groups to become resistant to glyphosate, and (iii) the potential glyphosate-related multidrug resistance in bacteria[6]. Several investigations confirmed that the surfactant, polyethoxylated tallow amine (POEA), contained in the formulations of glyphosate, is toxic by itself and is likely to increase the toxicity of glyphosate. Moreover, after the evolution of genetically modified glyphosate-resistant crops and the extensive use of glyphosate over the last 45 years, about 38 weed species developed resistance to this her-

bicide. Consequently, its use in recent years has been restricted or banned in 20 countries[17].
Finally, the latest research confirmed the herbicide's potential to disrupt healthy microbiomes, including the human microbiome. In addition, intensive glyphosate use seems to act as a stress factor, inducing glyphosate-resistance in bacteria and increasing antibiotic resistance to antimicrobial agents[18]. Although it remains an unanswered question how much these mechanisms could globally impact ecosystems and human well-being, further investigations and revisiting thresholds for glyphosate residues in water, food, and animal feed, are needed[19].

CONCLUSIONS

More empirical studies are needed to assess the effect of glyphosate and co-formulants on the healthy human microbiota, and additional epidemiological studies are needed to determine these proposed effects of glyphosate-based products on ecosystems, with potentially negative consequences on environmental health, ecology and sustainability.
Moreover, if future evidence will confirm that glyphosate may act as one of the drivers for antibiotic resistance, its global impact on human health will need to be reassessed.

Conflict of Interest
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MAZ, GL and IB revised scientific literature and contributed to the writing of the paper draft. SC, CC and AP conceptualized the idea and critically revised the manuscript. All the authors accepted the final version of the manuscript.

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