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Review

REVIEW

Glyphosate-based herbicides and cancer risk: a post-IARC decision review of potential mechanisms, policy and avenues of research

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Abstract

Since its initial sales in the 1970s, the herbicide glyphosate attained widespread use in modern agriculture, becoming the most commercially successful and widely used herbicide of all time as of 2016. Despite a primary mechanism that targets a pathway absent from animal cells and regulatory studies showing safety margins orders of magnitude better than many other, more directly toxic herbicides, the safety status of glyphosate has recently been brought into question by a slow accumulation of studies suggesting more subtle health risks, especially when considered in combination with the surfactants it is usually applied with. Current, official views of respected international regulatory and health bodies remain divided on glyphosate's status as a human carcinogen, but the 2015 International Agency for Research on Cancer decision to reclassify the compound as Category 2A (probably carcinogenic to humans) marked a sea change in the scientific community's consensus view. The goal of this review is to consider the state of science regarding glyphosate's potential as a human carcinogen and genotoxin, with particular focus on studies suggesting mechanisms that would go largely undetected in traditional toxicology studies, such as microbiome disruption and endocrine mimicry at very low concentrations.

Introduction

Glyphosate's herbicidal capacity was initially discovered by John Franz in 1970 (1). The patent was assigned to his employing corporation, Monsanto, in 1974, and first introduced to market under the brand name formulation Roundup. Usage of glyphosate-based herbicides (GBHs) increased with the introduction of glyphosate-resistant genetically modified (GM) crops. By the turn of the century, glyphosate resistance was the most common GM trait in agriculture (2). GBHs, now manufactured by many chemical companies beyond the original patent holder, are the most commonly used herbicide class worldwide, accounting for more than half of agricultural herbicide use in the USA alone (3,4). A GBH contains an aqueous solution of glyphosate salt as well as other adjuvant compounds, including surfactants that increase penetration and efficacy but may carry their own effects (5). GBHs, through their synergy with glyphosate tolerant GM crops, are major contributors to the economic benefits GM crops provide in the US agricultural sector. Farmers utilizing these combinations see crop yield increases of up to 22%, and profit increases of up to 68% over non-GM crops (6).

Glyphosate's mechanism of herbicidal action is the inhibition of the shikimate pathway, an aromatic amino acid metabolism pathway absent in animal cells but critical to the growth of most plants (7). Specifically, glyphosate inhibits the enzyme enolpyruvylshikimate-3-phosphate synthase (8,9). As such, glyphosate's mechanism of action is a particularly desirable trait for an herbicide to which animals may be collaterally exposed. Importantly, though, this pathway is also present and necessary for growth in some bacteria and fungi (10). The proprietary data

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Abbreviations

AMPA	aminomethylphosphonic acid
EPA	Environmental Protection Agency
GBH	glyphosate-based herbicides
GM	genetically modified
IARC	International Agency for Research on Cancer
NHL	non-Hodgkin's lymphoma
SCFA	short-chain fatty acids

submitted to regulators in the 1970s for initial registration of the compound reported low toxicity. No significant risk of long-term effects such as elevated cancer risks were determined, and many studies in the intervening decades concurred with that assessment (11,12). However, the last several years have seen the publication of research advancing evidence that long-term risks, especially from chronic exposure, may in fact exist. However, in recent years, some studies have reported findings that evidence of long term risks, especially from chronic exposure, may in fact exist. The resulting discordance between toxicity proponents and skeptics has divided the scientific community in one of the most heated scientific debates in recent memory.

One of the most controversial studies to report carcinogenic effects from glyphosate in mice was published by Gilles-Éric Séralini in 2012 (13). The study was widely criticized on a number of different fronts. There were concerns raised about appropriate evaluation of tumor types observed, the small number of animals tested, gross mistakes regarding pathology and animal welfare compliance issues. In addition, particularly anomalous conclusions were reached about the effects of the ingestion of GM plants themselves, as opposed to glyphosate (14). Other groups took issue with Séralini's statistical methods and claimed that no significant elevation in tumor incidence is observed if more traditional statistical analysis is used with the data (15). In the end, the Séralini affair concluded with the journal that first published the group's article retracting it, despite finding no evidence of misconduct. The central stated reason for retraction was the significant concerns raised regarding the statistical methods, although the author raised concerns that double standards were being applied (16). The Séralini group republished their article without further review in a second journal (17).

The results of the Séralini group's 2012 article are now generally discounted by mainstream scientists, but allegations of bias have been aimed at their detractors as well. The appointment of a former Monsanto employee to a newly created position on the editorial board of *Food and Chemical Toxicology* immediately preceding the Séralini retraction raised questions of potential impropriety (18). Many authors writing leading reviews that push a toxicity-skeptic viewpoint acknowledge funding from corporate entities with a vested interest in glyphosate as well (12). Other researchers point out that much of the original data supplied by manufacturers during the regulatory process, upon which initial safety assessments were based, is still considered to be proprietary and not open to public review (19). Another anti-glyphosate author whose lack of background in microbiology often invites skepticism, Stephanie Seneff, claims that these initial assessments were based on improperly combined experimental and historical control data (20).

The International Agency for Research on Cancer's (IARC) 2015 reclassification of glyphosate as a Category 2A (probable carcinogen) belies the continued debate around the status of this compound (21). Critiquing groups maintain that the overall weight of evidence still shows no significant risks (22). Another subdivision of the World Health Organization, the Joint Food and Agriculture Organization, maintained in 2016 that glyphosate was unlikely to pose a carcinogenic risk (23). Many regulatory agencies, including the European Food Safety Authority and European Chemicals Agency, continue to hold the official viewpoint that glyphosate does not pose a genotoxic or carcinogenic risk to humans (24). In contrast, some scientists contended that the IARC evaluation was in fact more rigorous by relying solely on publicly available, peer-reviewed data to make its assessment, while European Food Safety Authority and European Chemicals Agency regulators factored in proprietary information from registrants closed to comment from the wider scientific community (25). Other groups expressed concern with these agencies' potential bias toward registrant entities (26).

In the USA as of early 2018, glyphosate is currently undergoing a reregistration review process in accordance with the Federal Insecticide, Fungicide and Rodenticide Act, but the Environmental Protection Agency (EPA) still maintains that there is no demonstrable link to carcinogenicity (27,28). The studies cited in the recently released, *Revised Glyphosate Issue Paper*, though, give a mixed message. Of the three occupational exposure epidemiology studies given a 'High Quality' ranking, one reports a strong statistically significant association with non-Hodgkin's lymphoma (NHL) incidence (29). Another suggests a strong trend toward association with multiple myeloma in the initial study, with a 10-year follow-up study showing a trend toward association with acute myeloid leukemia in the same cohort, although neither trend reached significance (30,31).The third, an investigation into the effects of many pesticides, finds no association between glyphosate and prostate cancer, but does not focus on glyphosate or even mention the compound outside of supplemental data tables (32).

Review methods

A great number of reviews have sought to aggregate the information available about glyphosate's long-term toxicity potential, often drawing conflicting conclusions based on interpretations about the validity of the studies they examined (19,22,28,33). Acute toxicity studies were excluded from our search. For this review, we sought to focus largely on more subtle mechanisms that could operate to promote carcinogenesis through low-dose exposure. Literature searches were conducting using the Science Direct, PubMed and Google Scholar platforms. Keyword combinations were used (glyphosate AND keyword) to screen articles for each section. For the exposure limits section, co-keywords included 'regulation', 'environmental AND exposure' and 'application'. For the direct carcinogenesis section, co-keywords included 'carcinogen', 'cancer' and 'genotoxicity'. For non-monotonic effects, we used 'nonmonotonic' and 'endocrine', whereas for microbiome effects we used 'microbiome', 'bacteria' and 'microbiota'. Articles that were not available in full to our institution or not available in English were not used.

Exposure methods, levels and limits

Glyphosate remains the most widely used herbicide both in the USA and in the world. Rates of use continue to increase each year. Grube *et al*. estimate that in 2007, 180 million pounds were applied to US crops, whereas Benbrook *et al*. estimate that that amount increased to 270 million pounds by 2014 (34,35). Nearly 93% of the soy crop and 85% of the US corn crop are treated with GBHs, with 2 pounds of the active ingredient applied on average to each treated acre of corn (36). Multiple factors, beyond simple expansion of the agriculture industry, drive this great increase in applied glyphosate. The growing emergence of glyphosate-resistant weeds demands increased herbicide levels to maintain

the same level of control (37). The compound itself is being used in new roles, as well. The process of preharvest crop desiccation, for example, involves the deliberate spraying of glyphosate sensitive crops with the chemical to speed cessation of growth and prepare the crop for harvest in a more controlled manner—a process that often leaves glyphosate residue on the desiccated crops (38,39). As of 2009, glyphosate was the only herbicide registered to be used in this manner in the USA (40). Some 250 000–300 000 acres of sugarcane were desiccated in this manner across the state of Louisiana in 2005 (41). Glyphosate can accumulate in treated plants, so the EPA has set allowable levels (shown in Table 1) for most food products (42). The chronic reference dose, also set by the EPA, is currently 1.75 mg/kg/day for the USA, although the European Union's acceptable daily intake is set much lower, at 0.3 mg/kg/day (19).

Fortunately, most experimentally measured environmental concentrations fall below both of these hard limits. Bøhn *et al*., for example, reports 3.3 mg/kg of glyphosate and 5.7 mg/kg of its metabolite aminomethylphosphonic acid (AMPA) in GM soybeans (44). Seneff reports levels up to 1 mg/kg in rat chow and 0.3 mg/kg in dog food—well within regulated levels, although the endogenous levels in rat chow should merit special consideration from animal researchers, as this would set a control value floor in animal experiments (20).

Glyphosate's water-soluble nature does present a runoff risk. The compound can accumulate in streams and especially irrigation ditches near to treated areas. In areas directly adjacent to treated fields, Coupe *et al*. measured water concentrations of glyphosate as high as 0.86% (~5×10^{-5} M) (45). Areas further from treatment sites are still at risk as well. Over a third of US lakes, ponds and wetlands were tested positive for glyphosate and AMPA, with concentrations up to 0.3 ppm (~1.77×10^{-9} M) (46).

Most human and animal studies also show detectable amounts of glyphosate eliminated via the primary pathway of urination. Krüger *et al*. found an average concentration of 15 µg/ml (~8.87×10^{-8} M) in the urine of European human volunteers eating a conventional diet (38). In a review of eight studies, Niemann *et al*. estimate an average intake between 0.1 and 3.3 µg/kg of body weight per person per day, well below limits currently imposed by regulators (47).

Evidence of direct genotoxicity and/or carcinogenicity

The IARC decision to reclassify glyphosate as a Category 2A (probable carcinogen) was largely based on four studies showing elevated frequencies of NHL in occupationally exposed workers (21,29,31,48). The exact mechanism by which glyphosate may increase NHL risk, though, is still unclear. Bolognesi *et al*. demonstrated slightly increased levels of micronuclei (MN) derived from chromosome breakage events in exposed workers from Colombia (49). These events, however, might not result from direct genotoxic effects from glyphosate itself. Many common genotoxicity assays, such as the Ames assay, show no significant induction of DNA damage by glyphosate exposure alone (50). A stronger association is seen with the application of complete GBH. Bolognesi *et al*. demonstrated that both glyphosate alone and the full GBH formulation resulted in an increase in bone marrow MN in mice exposed at 300 mg/kg, in addition to single strand chromosome breaks induced in human lymphocytes. In this study, glyphosate alone induced a significant, but low, increase in measurable breaks, whereas the full Roundup formulation was far more potent (51). A follow-up study using radioactive P-32 post-labelling of DNA adducts found that full GBH exposure led to an increase in radiodetectable adducts, providing a mechanism of action for the previously observed strand breaks. However, glyphosate alone led to no detectable increase in adducts using this method (52). Evidence suggests that the surfactants present in the application mixture, especially POE-15, are a major driver of GBH-induced DNA damage, as well as lethality. These surfactants have been shown to be, on their own, toxic to human embryonic and placental cells at levels as low as 1 ppm (53). The full Roundup formulation was more than twice as effective at inducing lethal toxicity in human placental cell lines, albeit at levels much higher than environmental concentrations (54). Guilherme *et al*. showed increases in double-strand breaks (DSB)-detecting Comet Assay and MN assay lesions after 1 day at 56 µg/l Roundup in eels (0.05 ppm), which is well within environmental exposure levels (55). Çavaş and Könen showed dose-dependent increases in the same criteria in goldfish, but starting at the higher dose of 5 ppm (56). These data suggest that it is crucial to analyze and regulate GBHs as a mixture, rather than assume no synergy and set levels based on each component's toxicology alone.

Glyphosate's main degradation product, AMPA, seems to induce genotoxicity as well. Guilherme *et al*. showed DSB induction by this compound in the eel model at just 11.8 µg/l (57). Mañas *et al*. showed that this compound induces measurable breaks at 2.5 mM in human lymphocyte culture and at 200 mg/kg in mice (58). Studies seeking to measure glyphosate residues both in organisms and the environment must also take this degradation product into account when calculating total exposure.

The IARC also cited animal cancer studies showing elevated risk of hemangiosarcoma, renal tubule carcinoma and pancreatic cell islet adenoma, as well as skin tumor promotion in a two-hit mouse model (21). At the same time, many other studies demonstrate no direct increase in risk of carcinogenesis (22). In most observations, direct toxicity is not well observed until above current regulatory levels. If glyphosate and its metabolites are carcinogenic, it is likely that much of this risk can only be measured outside of the standard Paracelsian dose-response model.

Table 1. EPA allowable glyphosate residue for selected crops (43)

Crop	Tolerated ppm (mg/kg)
Sugarcane (cane)	2 ppm
Sugarcane (molasses)	30 ppm
Non-grass animal feed	400 ppm
Barley Bran	30 ppm
Soy	20 ppm

Endocrine disruption and non-monotonic effects

The majority of toxicology characterization studies used by worldwide regulatory agencies assume that the agent in question will behave monotonically. Testing of a compound at many doses is used to define an upper toxicity effect level (E_{max}) and a no or lowest observed adverse effect level), between which a gradation of effects is usually assumed (59). However, for a growing variety of compounds, particularly those which mimic or disrupt aspects of the endocrine system, this classical dose-response assumption is no longer sufficient to fully characterize risk. For example, the well-known estrogen mimic bisphenol A decreases tumor latency and metastasis only at very low doses in a mouse breast cancer model (60). The non-monotonic dose-response relationships of these compounds mean that effects may be present below a previously set no or lowest observed adverse effect level (61,62).

Cell line studies give different results depending on the line, dose and formula used. The full Roundup formulation is often associated with a more linear dose profile, suggesting that toxicity from adjuvant compounds is largely monotonic (63). In estrogen receptor-reporter transfected HepG2 cells, glyphosate alone had a non-linear effect at under 0.05% concentration, whereas the full Roundup formulation reduced androgen receptor–induced transcription linearly at lower doses (64). Testosterone-producing Leydig cells provide another model for endocrine disruption effects both *in vivo* and *ex vivo* (65). Walsh *et al*. reported significantly disrupted progesterone production, with no parallel decrease in total protein synthesis, linearly from 25 μg/ml, but only for the complete Roundup formulation (66). Full Roundup formulation significantly changed progression of puberty and decreased serum testosterone in prepubertal Wistar rats exposed from 5 mg/kg once per day.

Thongprakaisang *et al*. noted the non-monotonicity of glyphosate alone on human hormone-dependent breast cancer cell line proliferation, observing a greater effect at concentrations of 10^{-8} to 10^{-9} rather than 10^{-6} M. This effect was mediated by the estrogen response element and could be blocked by the addition of an estrogen receptor antagonist (67).

Jin *et al*. recently observed a non-monotonic effect on estradiol levels in male Delta Smelt, with significant elevations after exposure to 0.46 and 4.2, but not 45 and 570 μM glyphosate (68). Armiliato *et al*. detected significantly increased expression of steroidogenic factor-1 and oocyte growth in zebra fish exposed to water concentrations as low as 65 μg/l (69). In other fish, such as trout, no significant association with endocrine disruption has been shown. Glyphosate did not show any estrogenic activity in yeast with a recombinant trout estrogen receptor at concentrations of 10^{-8} to 10^{-4}, nor did it increase levels of plasma vitellogenin in young rainbow trout themselves at 0.11 mg/l (70,71). In larval amphibians, environmentally relevant aqueous concentrations of glyphosate were associated with a greater perturbation of behaviors, such as movement frequency, than that associated with higher concentrations. If a non-monotonic mechanism underlies the response, the results suggest that subtle effects on the nervous system may be possible at very low doses (72). Despite these recent observations, the effects of glyphosate at very low concentrations may be underinvestigated. Via endocrine mimicry, very low levels of glyphosate might potentiate human carcinogenesis, even if under regulatory limits currently considered to be safe.

Microbiome disruption

In recent decades, the microbiome has grown to be a major new frontier in the field of human health. The composition of our gut microbiota has been compared with a 'second genome' due to its far-reaching effects on nearly every aspect of human health. Determination of the species inhabiting the human gastrointestinal tract, and their relative numbers, is multifactorial and dynamic. Even within an individual, this composition can change greatly over a lifetime in response to health, diet and antibiotic exposure, among other factors (73).

Probiotic, or beneficial, bacteria, benefit human health via a number of mechanisms from the gastrointestinal tract. Pathogenic bacterial adhesion and toxin efficacy are inhibited both by antimicrobial compounds generated on site, as well as direct competition for real estate. For example, the presence of *Bifidobacteria* directly inhibits the ability of *Salmonella* species to bind and cause disease (74). Beneficial bacteria balance the gut's immune response by modulating the ratio of inflammatory to anti-inflammatory cytokine production, which effects levels of inflammation both in the gut and systemically (75). Lactic acid bacteria, although they represent a very small portion of the total microbiome, have been demonstrated to be of particular importance with regards to gut homeostasis (76,77). The acidic pH products that they generate as waste inhibit the growth of many strains of pathogenic bacteria, and the short-chain fatty acids (SCFAs) they produce are a direct source of energy for human gut epithelial cells, improving gut integrity and colonic function (78,79). Beyond this metabolic benefit, the production of SCFAs by bacteria in the lumen is essential for the regulation of inflammatory state through a number of different mechanisms (80). For example, butyrate generation can directly ameliorate pro-inflammatory signaling by inhibiting histone deacetylase activity in local T-cell populations (81). Their production can also contribute to the inhibition of pro-inflammatory pathogenic strains. Probiotic production of the SCFA acetate is capable of directly reducing adhesion and toxin translocation by enterohemorrhagic *Escherichia coli*. SCFAs are generally derived from the ability of lactic acid bacteria and other beneficial bacteria to metabolize otherwise indigestible dietary fiber (82). Deficiency in these strains is often associated with inflammatory states such as celiac disease (83,84). The loss of gut homeostasis associated with increases in pathogenic strains decreases in beneficial strains, and inflammation is termed gut dysbiosis.

Inflammatory states associated with gut dysbiosis lead to decreased integrity between epithelial cells junctions, making the barrier 'leaky' and creating a feedback loop by impairing nutrient uptake and pathogen defense (85). The 'leaky gut' dysbiotic phenotype has been linked to negative effects ranging from inflammatory bowel disease to depression (86,87). All chronic inflammatory diseases of the gut have been strongly linked to increased risk of cancer (88, 89). Pathogen-induced inflammation in particular is closely associated with cancers of local tissues. For example, mucosal-associated tissue lymphomas are associated with bacterial inflammation and colitis (90), whereas the stomach pathogen *Helicobacter pylori* is especially well linked to stomach inflammation and gastric cancers (91,92). It should be noted, however, that there is no substantial evidence indicating a connection between glyphosate exposure and intestinal cancers in humans as of yet. Importantly, evidence also exists that local inflammation can lead to systemic inflammation, thereby increasing global carcinogenesis rates (93). Intestinal inflammation can lead to increased hematopoiesis and genotoxicity, presenting a plausible link between perturbations on site and the eventual formation of NHLs observed in human epidemiology (94). Much individual NHL risk is associated with initiating germline mutations, so inflammation-induced lymphocyte population expansion and genotoxicity could serve as a driver of promoting mutations through a number of mechanisms, including loss of heterozygosity and changes in regulatory RNA expression, to result in mature NHL (95–97). Thus, exposure to glyphosate has the potential to affect the human gut microbiome profile and function, which might lead to decreased pathogen defense and inflammation, both in the intestine and systemically. This represents, then, another pathway through which carcinogenicity could be induced by glyphosate and GBHs.

Glyphosate has long been known to have antibiotic function through its inhibition of enolpyruvylshikimate-3-phosphate synthase (10,98). Several studies have also shown greater effects of glyphosate on strains generally considered to be 'beneficial' than those considered to be 'pathogenic'. Table 2 describes levels of glyphosate reported as growth inhibitory for different pathogenic and beneficial gut bacteria. Shehata *et al*. found

Table 2. Studies demonstrating inhibitory concentrations of glyphosate on gut microbiota

Study	Bacterial strains	Role	Glyphosate tolerance (MIC)	Notes
Shehata *et al.* (99)	*Clostridium* sp.	Pathogen	High, 5 mg/ml	Chicken gut isolates
	Salmonella sp.	Pathogen	High, 5 mg/ml	
	Escherichia Coli	Commensal/pathogen	High, 1.2 mg/ml	
	Staphylococcus sp.	Commensal/pathogen	Med, 0.3 mg/ml	
	Lactobacillus sp.	Beneficial	Med, 0.6 mg/ml	
	Bifidobacterium sp.	Beneficial	Low, 0.075 mg/ml	
	Enterococcus sp.	Commensal/beneficial	Low, 0.15 mg/ml	
Schulz *et al.* (105)	*Pseudomonas aeruginosa*	Pathogen	High, ~1 mg/ml	
Ackermann *et al.* (106)	*Clostridium botulinum*	Pathogen	High, >1 mg/ml	Botulism toxin production increased at this level
	Ruminococcus sp.	Ruminant fermenter	Low, 0.01 mg/ml	
Krüger *et al.* (100)	*Enterococcus* sp.	Commensal/beneficial	Low, 0.1 mg/ml	Capacity to inhibit *C. botulinum* toxin production decreased at this level
	C. botulinum	Pathogen	High, >1 mg/ml	

over 50 times greater tolerance to glyphosate in disease-causing *Clostridium* species than that in *Bifidobacteria* species considered to be largely beneficial (99). Glyphosate may also indirectly lead to increases in pathogenic bacteria. For example, exposure to glyphosate concentrations that do not inhibit *Clostridium* growth was associated with a decrease in *Enterococcus*-derived inhibition of Clostridial production of toxins, such as botulinum (100). At levels over regulatory limits, but beneath application concentrations, glyphosate also induced antibiotic resistance mechanisms in *Salmonella* species (101). *Staphylococcus aureus*'s enolpyruvylshikimate-3-phosphate synthase is insensitive to glyphosate inhibition, which might enable a disproportionate growth of this species compared with others, including more beneficial species (102). Although most inhibitory concentrations appear to be well above regulatory limits, differential sensitivity, and the multifactorial nature of microbiome composition stability, could mean a dysbiosis that favors increased risk of inflammation and potential carcinogenicity. It should be noted, however, that there is no substantial evidence associating glyphosate with intestinal cancers specifically. Another mechanism by which this could occur is the inhibition of microbially derived SCFAs described previously. For example, Nielsen *et al.* report that a brief 2-week exposure of young mice to food containing glyphosate had no effect on the microbiome based on DNA sequencing. However, a significant, dose-dependent decrease in fecal pH was observed, suggesting impaired SCFA production by beneficial strains (103). In a follow-up study, the Nielsen group found that glyphosate's potential to effect gut bacterial community composition was limited by the presence of sufficient aromatic amino acids in the diet of the test animal. However, even in these cases, decreased levels of detectable SCFAs, in particular acetic acid, were observed with increasing glyphosate exposure (104). As longer studies are completed, it will be interesting to determine whether these short-term effects measurably impact long-term health outcomes.

The shikimate pathway inhibited by glyphosate is also important for bacterial folate production (107). Probiotic bacteria are a major source of folate, producing the vitamin on site in the gut (108). Folate deficiency in humans has been directly linked to genotoxicity, carcinogenicity and chromosome breakage events (109,110). Deficiency increases the frequency of ionizing radiation-induced DNA strand breakage in human lymphocytes (111). Thus, this is another mechanism by which glyphosate exposure could directly affect cancer risk.

The adjuvant surfactants and emulsifiers present in GBHs contribute to microbiome disruption more than glyphosate alone. Clair *et al.* report that Roundup inhibits the growth of the lactic acid bacteria *Lactococcus cremoris* at concentrations of 200 ppm, whereas glyphosate alone does not (112). Emulsifiers and surfactants alone have been shown to induce colitis in a mouse model, a condition associated with increased rates of colon carcinogenesis (113, 114). Interestingly, one of the mechanisms by which emulsifiers such as Tween 80 appear to cause dysbiosis is by creating an environment favorable to flagellin-expressing pathogenic bacteria, rather than directly harming beneficial strains (115). Dietary grade emulsifier exposure in this model led to population increases in bacteria directly associated with chronic, low-grade inflammation (116). Results from these surfactant studies raise the interesting possibility that GBHs could potentially have a synergetic, two-pronged effect on the gut microbiome from the action of their ingredients in tandem. Emulsifying agents could be inducing a pro-pathogen environment, while at the same time glyphosate itself inhibits the growth and antipathogen properties of beneficial strains, increasing risk of inflammation and its sequelae. Although most existing studies show strong inhibition of beneficial bacteria by glyphosate only at levels above that to which the gut would be exposed, based on current regulation and exposure estimates, this does not rule out potential effects resulting from differential inhibition in the gut, a far more complex environment than growth media. An external push from even a small factor, such as a change in diet, might also change the relative ratios of strains to move the gut ecosystem closer to a dysbiotic state—even if no single factor alone is directly responsible for the decrease in a strain population (117). For other strains, glyphosate could potentially be a primary driver of inhibition. Ackermann *et al.* showed that the glyphosate threshold dose for inhibitory effects on the ruminant fermenter, *Ruminococcus*, falls very close to some predictions of dietary glyphosate intake (106).

Most studies showing changes have been conducted in animal models, and it remains unclear whether observed differences can be relied upon as predictive for human health risks given the differences between the types of genes present in mouse and human microbiota. Nonetheless, intestinal

microbiota perturbation deserves further evaluation. Future studies should investigate whether relative ratios of gut bacteria, dysbiosis, inflammation, or even diarrhea associated with such glyphosate-resistant bacteria as *Clostridia* are associated with occupational or dietary glyphosate exposure. In particular, the potential of glyphosate to change microbiota populations at levels below media minimum inhibitory concentrations merits dedicated study in humans.

Recommendations

The IARC has classified glyphosate as a probable human carcinogen, but its status as one is far from decided in the eyes of the international scientific community. There is much work to be done in the foreseeable future to elucidate the mechanisms by which it may cause human health risks. Despite the economic benefits these compounds provide to the agriculture industry, we feel that the potential risks glyphosate and GBHs present to public health merit the following policy recommendations:

1. In light of reports of possible genotoxic, effects of glyphosate on the human body, we suggest the USA invoke the Food Quality Protection Act's enforcement of a 10-fold safety margin for pesticides or herbicides without reliable data showing no risk to children (118). Current glyphosate chronic reference dose levels of 1.75 mg/kg/day should be reduced to 0.175 mg/kg/day, bringing the value beneath the 0.3 mg/kg/day level used by the European Union and closer to that recommended by multiple research groups (19,119). The vast majority of human urine samples collected from herbicide workers still falls well below this level, so enforcement to this standard is not unreasonable (47). Debate over the European Union's reapproval of glyphosate, and whether to change the acceptable daily intake levels, is still ongoing near the close of 2017.
2. Much of the debate over glyphosate's safety is marred by accusations of politically and economically motivated study findings. Each party has accused the other of disregarding and withholding data that do not fit the set of conclusions they seek to promote. We hold the opinion that the principle of free and open peer review is the best method to put these issues to rest. We call on researchers of glyphosate toxicity and carcinogenicity to place extra effort into keeping all raw data publicly available for perusal and comment. In particular, we call on corporate entities that maintain proprietary datasets, especially those used to comply with government registration and regulation processes, to voluntarily make this information freely available for independent review.
3. Given that glyphosate inhibits the shikimate pathway, which is critical to the metabolism of many species in the human gut microbiome, and that adjuvants present in GBHs may induce other changes in the microbiota profile, both direct toxicity and epidemiology studies should be conducted to evaluate the potential for GBH consumption through the diet to increase cancer risk. Studies should include the effects of GBH-treated and GBH-free food diets on the composition of the human microbiome, as well as the secondary gut and systemic inflammatory conditions at doses relevant to anticipated exposure levels.
4. The testing of different formulations of GBHs should occur alongside and in addition to the testing of glyphosate alone at every level, and especially at the regulatory stage. Many regulatory agencies do not require retesting of chemical combinations, especially those at levels deemed 'safe' on their own (120). Despite this policy, it is well accepted that surfactant compounds can act to increase the rate of cell entry or systemic absorption of glyphosate, which may have relevance to the potential carcinogenicity of GBHs (53).
5. If glyphosate is a human carcinogen, the mechanisms by which it acts are probably obfuscated behind such complex mechanisms as non-monotonic endocrine mimicry and indirect initiation of inflammation and genotoxicity through microbiome mediators. These events can require large studies to elucidate with significant statistical power. Therefore, relying solely on the often used, three-tier system for genotoxicity risk assessment (generally Ames test, *in vitro* mammalian cell mutation and *in vivo* chromosomal aberration) is insufficient. This approach is currently favored by such bodies as the organization for economic cooperation (OECD) and the US EPA (121). Results that do not adhere to this accepted framework are given less weight by both regulatory agencies and scientists associated with glyphosate-registering corporations. Additional investigations of glyphosate with regards to specific mechanisms of toxicity in specialized models must be completed. For example, the deletion (DEL) assay, a yeast-based test that drastically outperforms the traditional Ames test in carcinogen detection, could be used to examine induction of DSBs in exposed cells (122,123).
6. With regards to carcinogenesis itself, animal results are often taken less seriously if they do not adhere to standard dose and number criteria such as those advanced by the OECD (124). Although this may be warranted in some situations, these criteria could cause low-dose, non-monotonic responses, such as those observed in cases of cases of endocrine disruption, to remain overlooked. New regulatory testing protocols must be established to determine whether a given compound has a non-monotonic dose response (61,62,125). In addition, the scientific community should continue to critically examine every carcinogenesis study by its own merits in consideration of the totality of evidence, rather than disregarding studies that do not meet current criteria for standardized carcinogenicity testing for reasons such as sample size (126).

Conclusions

Economically, glyphosate is one of the most important chemical compounds in use worldwide, with increased agricultural yields resulting from its use. From this perspective, the skepticism shown toward results suggesting that its use carries long-term risks to public health is both rational and reasonable. The use of glyphosate, and restrictions placed upon that use, must be carefully measured against economic, environmental and health repercussions.

Over the last two decades, there has been increasing concern that GBHs may present a carcinogenic risk. Farmers are now using greater amounts of glyphosates than in the past, at more time points during year and in new roles, such as preharvest desiccation. As a result, levels of glyphosate and its degradation product AMPA continue to increase in both our food and our water supply.

Glyphosate's potential for carcinogenic effects is probably complex in nature. If glyphosate is a true carcinogen, mechanisms of action are most likely to include effects such as endocrine or microbiome disruption. Traditional carcinogenicity testing methods may no longer be relevant for evaluating a substance with such effects. Much of the framework used by

international regulatory agencies is also tailored to set 'safe' levels only for compounds that function via classical dose-response mechanisms, allowing potentially non-monotonic carcinogen effects, such as in the case of glyphosate, to be overlooked. These agencies must modernize their standards of testing and regulation in order to properly respond to new science.

The potential ramifications of glyphosate use are significant enough that careful, measured and unbiased peer-reviewed research is necessary to ascertain the magnitude of its effects. All possible mechanisms of action should be under investigation. In no cases should we assume that relying solely on past data is acceptable, especially when such data were gathered while understanding of the far-reaching effects of hormone mimicry and the microbiome was incomplete. The scientific and regulatory communities must reach consensus in an open manner that results in an appropriate response to any risk posed by glyphosate, as well as establish a better, more comprehensive framework for herbicide safety assessment in the future.

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