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 evincing more insidious 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 IARC 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 which would go largely undetected in traditional toxicology studies, such as microbiome disruption and endocrine mimicry at very low concentrations.

Summary: In this review, we examine the current regulatory limits on and environmental concentrations of this compound, discuss studies suggesting toxicity that could directly or indirectly affect human cancer rates, and assess their relevance in light of modern understanding of subtle mechanisms of carcinogenesis. In particular, we suggest that regulatory framework and future studies should address these complex, multistep mechanisms before reaching a conclusion of safety or non-safety.

Background

Glyphosate Based Herbicides and Cancer Risk

Glyphosate's herbicidal capacity was initially discovered by John Franz in 1970 (John 1974). 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 (Carpenter and Gianessi 1999). 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 United States alone (Atwood and Paisley-Jones 2017; Coupe and Capel 2016). 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 (Folmar et al. 1979). GBHs, through their synergy with glyphosate tolerant GM crops, are major contributors to the economic benefits GM

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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 (Klümper and Qaim 2014).

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 (Holländer and Amrhein 1980). Specifically, glyphosate inhibits the enzyme enolpyruvylshikimate-3-phosphate synthase (EPSP) (Boocock and Coggins 1983; Steinrücken and Amrhein 1980). 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 (Amrhein et al. 1980). The proprietary data submitted to regulators in the 1970s for initial registration of the compound reported low toxicity. No significant risk of long term effects like elevated cancer risks were determined, and many studies in the intervening decades concurred with that assessment (Greim et al. 2015; Williams et al. 2012). 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. 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 Giles-Éric Séralini in 2012 (Séralini et al. 2012). 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 (Schorsch 2013). Other groups took issue with Séralini's statistical methods, and claim that no significant elevation in tumor incidence is observed if more traditional statistical analysis is used with the data (Panchin and Tuzhikov 2017). In the end, the Séralini affair concluded with the journal that first published the group's paper retracting it. despite

finding no evidence of misconduct. The central stated reason for retraction were the significant concerns raised regarding the statistical methods, although the author raised concerns that double standards were being applied (G Séralini et al. 2014). The Séralini group republished their manuscript without further review in a second journal (G-E Séralini et al. 2014).

The results of the Séralini group's 2012 paper 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 (Robinson and Latham 2013). Many authors writing leading reviews that push a toxicity-skeptic viewpoint acknowledge funding from corporate entities with a vested interest in glyphosate as well (Williams et al. 2012). 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 (Myers et al. 2016). 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 (Samsel and Seneff 2015).

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 (Fritschi et al. 2015). Critiquing groups maintain that the overall weight of evidence still shows no significant risks (Williams et al. 2016). Another subdivision of the World Health Organization (WHO) , the Joint Food and Agriculture Organization, maintained in 2016 that glyphosate was unlikely to pose a carcinogenic risk (FAO 2016). Many regulatory agencies, including the European Food Safety Authority (EFSA) and European Chemicals Agency (EChA) continue to hold the official viewpoint that glyphosate does not pose a genotoxic or carcinogenic risk to humans (Juncker 2017). 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 EFSA and EChA regulators factored in proprietary information from registrants closed to comment from the wider scientific community (Portier et al. 2016). Other groups expressed concern with these agencies' potential bias towards registrant entities (Robinson et al. 2013).

In the United States as of early 2018, glyphosate is currently undergoing a re-registration review process in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), but the EPA still maintains that there is no demonstrable link to carcinogenicity (Code 1985; EPA 2017). The studies cited in the their 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 (Eriksson et al. 2008). Another suggests a strong trend towards association with multiple myeloma in the initial study, with a 10 year followup study showing a trend towards association with acute myeloid leukemia in the same cohort, although neither trend reached significance (Andreotti et al. 2018; De Roos et al. 2005).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 (Koutros et al. 2012).

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 (EPA 2017; Mesnage et al. 2015; Myers et al. 2016; Williams et al. 2016). 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 nonmonotonic effects, we used "nonmonotonic" and "endocrine", while 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 U.S. and the world. Rates of use continue to increase each year. Grube et al. estimate that in 2007, 180 million pounds were applied to U.S. crops, while Benbrook et al. estimate that that amount increased to 270 million pounds by 2014 (Benbrook 2016; Grube et al. 2011). Nearly 93% of the soy crop and 85% of the U.S. corn crop are treated with GBHs, with 2 pounds of the active ingredient applied on average to each treated acre of corn (Fernandez-Cornejo et al. 2014). 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 (Powles and Preston 2006). The compound itself is being used in new roles, as well. The process of pre-harvest 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 (Krüger et al. 2014; Soltani et al. 2013). As of 2009, glyphosate was the only herbicide registered to be used in this manner in the U.S. (Dalley and Richard Jr 2010). Some 250,000 to 300,000 acres of sugarcane were desiccated in this manner across the state of Louisiana in 2005 (Legendre et al. 2005). Glyphosate can accumulate in treated plants, so the EPA has set allowable levels (shown in **Table 1**) for most food products (Johal and Huber 2009). The chronic reference dose (CrfD), also set by the EPA, is currently 1.75 mg/kg/day for the U.S., although the E.U.'s acceptable daily intake (ADI) is set much lower, at 0.3 mg/kg/day (Myers et al. 2016).

Fortunately, most experimentally measured environmental concentrations fall below both of these hard limits. Bøhn, for example, reports 3.3 mg/kg of glyphosate and 5.7 mg/kg of its metabolite aminomethylphosphonic acid (AMPA) in GM soybeans (Bøhn et al. 2014). 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 (Samsel and Seneff 2015).

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) (Coupe et al. 2012). Areas further from treatment sites are still at risk as well. Over a third of tested U.S. lakes, ponds, and wetlands tested positive for glyphosate and AMPA, with concentrations up to 0.3 ppm (~1.77×10^{-9} M) (Battaglin et al. 2014).

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 (Krüger et al. 2014). In a review of 8 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 (Niemann et al. 2015).

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 (De Roos et al. 2005; Eriksson et al. 2008; Fritschi et al. 2015; Karunanayake et al. 2012). The exact mechanism by which glyphosate may increase NHL risk, though, is still unclear. Bolognesi et al. demonstrated slightly increased levels of micronuclei derived from chromosome breakage events (MN) in exposed workers from Colombia (Bolognesi et al. 2009). 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 (Kier and Kirkland 2013). 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, while the full Roundup formulation was far more potent (Bolognesi et al. 1997). A followup study using radioactive P-32 postlabelling 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 (Peluso et al. 1998). 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 (Mesnage et al. 2013). 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 (Richard et al. 2005). Guilherme et al. showed increases in 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 (Guilherme et al. 2010). Çavaş and Könen showed dose dependent increases in the same criteria in goldfish, but starting at the higher dose of 5ppm (Çavaş and Könen 2007). 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 (Guilherme et al. 2014). 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(Mañas et al. 2009). 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 2-hit mouse model (Fritschi et al. 2015). At the same time, many other studies demonstrate no direct increase in risk of carcinogenesis (Williams et al. 2016). 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.

Endocrine Disruption and Nonmonotonic 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 (NOAEL or LOAEL), between which a gradation of effects is usually assumed (Council 1983). 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 (Jenkins et al. 2011). The nonmonotonic dose-reponse relationships of these compounds mean that effects may be present below a previously set NOAEL (Lagarde et al. 2015; Vandenberg et al. 2012).

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 (Romano et al. 2010). In estrogen receptor-reporter transfected HepG2

cells, glyphosate alone had a nonlinear effect at under 0.05% concentration, while the full Roundup formulation reduced androgen receptor-induced transcription linearly at lower doses (Gasnier et al. 2009). Testosterone-producing Leydig cells provide another model for endocrine disruption effects both *in* and *ex vivo* (Akingbemi et al. 2004). Walsh 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 (Walsh et al. 2000). Full Roundup formulation significantly changed progression of puberty and decreased serum testosterone in prepubertal Wistar rats exposed from 5mg/kg once per day.

Thongprakaisang et al. noted the nonmonotonicity of glyphosate alone on human hormone-dependent breast cancer cell line proliferation, observing a greater effect at concentrations of 10^{-8} -10^{-9} rather than 10^{-6} M. This effect was mediated by the estrogen response element (ERE) and could be blocked by the addition of an estrogen receptor antagonist (Thongprakaisang et al. 2013).

Jin et al. recently observed a nonmonotonic 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 (Jin et al. 2018). Armiliato et. al detected significantly increased expression of steroidogenic factor-1 and oocyte growth in zebrafish exposed to water concentrations as low as 65 μg/L (Armiliato et al. 2014). In other fish, like 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} – 10^{-4}, nor did it increase levels of plasma vitellogenin in young rainbow trout themselves at 0.11 mg/L (Petit et al. 1997; Xie et al. 2005). In larval amphibians, environmentally relevant aqueous concentrations of glyphosate were associated with a greater perturbation of behaviors, such as movement frequency, than that associated higher concentrations. If a nonmonotonic mechanism underlies the response, the results suggest that subtle effects on the nervous system may be possible at very low doses (Gandhi and Cecala 2016). Despite these recent observations, the effects of glyphosate at very low concentrations may remain 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 to a “second genome” due to its far-reaching effects on nearly every aspect of human health. Determination of the species inhabiting the human GI 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 (Havenaar and Huis 1992).

Probiotic, or beneficial, bacteria, benefit human health via a number of mechanisms from the GI 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 (Isolauri et al. 2001). 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 (Mulder et al. 1997). Lactic acid bacteria (LAB), although they represent a very small portion of the total microbiome, have been demonstrated to be of particular importance with regards to gut homeostasis (Bezkorovainy 2001; Hsieh et al. 2013). 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 (Hamer et al. 2008; Russell et al. 2013). 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 (Puertollano et al. 2014). For example, butyrate generation can directly ameliorate pro-inflammatory signaling by inhibiting histone deacetylase activity in local T-cell populations (Nepelska et al. 2012). 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 *E. coli.* SCFAs are generally derived from the ability of LABs and other beneficial bacteria to metabolize otherwise indigestible dietary fiber (Brüssow and Parkinson 2014). Deficiency in these strains is often associated with inflammatory states such as Celiac Disease (Di Cagno et al. 2009; Di Cagno et al. 2011). 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 (Jiang et al. 2015). The "leaky gut" dysbiotic phenotype has been linked to negative effects ranging from inflammatory bowel disease to depression (Mass et al. 2008; Sheehan et al. 2015). All chronic inflammatory diseases of the gut have been strongly linked to increased risk of cancer (Balkwill et al. 2005; Tsuei et al. 2014). 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 (Yamamoto and Schiestl 2014), while the stomach pathogen *H. pylori* is especially well linked to stomach inflammation and gastric cancers (Group 1993; Smoot et al. 2000). 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 (Westbrook et al. 2009). 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 (Trivedi and Jena 2012). Much individual NHL risk is associated with initiating germline mutations, so inflammation induced 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 (Hill et al. 2006; Lee et al. 2001; Rodríguez-Malavé and Rao 2015). 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 – 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 EPSP (Amrhein et al. 1980; Schönbrunn et al. 2001). 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 that inhibit growth of different pathogenic and beneficial gut bacteria. Shehata et al. found over 50 times greater tolerance to glyphosate in disease-causing *Clostridium* species than that in *Bifidobacteria,* species considered to be largely beneficial (Shehata et al. 2013). The potential effects of glyphosate are more disruptive than simple differential growth inhibition, as well. 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 (Krüger et al. 2013). At levels over regulatory limits, but beneath application concentrations, glyphosate also induced antibiotic resistance mechanisms in *Salmonella* species (Kurenbach et al. 2015). *Staphylococcus aureus's* EPSP is insensitive to glyphosate inhibition, which might enable a disproportionate growth of this species compared to others, including more beneficial species (Priestman et al. 2005). 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. Another mechanism by which this could occur is the inhibition of microbially-derived SCFAs decribed previously. For example, Nielsen et al. report that a brief two-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 short-chain fatty acid production by beneficial strains (Nielsen et al. 2018). 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 (Dosselaere and Vanderleyden 2001). Probiotic bacteria are a major source of folate, producing the vitamin on site in the gut (Rossi et al. 2011). Folate deficiency in humans has been directly linked to genotoxicity, carcinogenicity, and chromosome breakage events (Ames 2001; Blount et al. 1997). Deficiency increases the frequency of IR-induced DNA strand breakage in human lymphocytes (Courtemanche et al. 2004). 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 LAB *Lactococcis cremoris* at concentrations of 200ppm, while glyphosate alone does not (Clair et al. 2012). Emulsifiers and surfactants alone have been shown to induce colitis in a mouse model, a condition associated with increased rates of colon carcinogenesis (Chassaing et al. 2015; Viennois et al. 2016). Interestingly, one of the mechanisms by which emulsifiers like Tween 80 appear to cause dysbiosis is by creating an environment favorable to flagellin-expressing pathogenic bacteria, rather than directly harming beneficial strains (Chassaing et al. 2017b). Dietary grade emulsifier exposure in this model led to population increases in bacteria directly associated with chronic, low-grade inflammation (Chassaing et

al. 2017a). 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 gut, a far more complex environment than growth media. An external push from even a small factor, like 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 (David et al. 2014). 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 (Ackermann et al. 2015).

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 clearly 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 MIC 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 in order 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, and especially developmental, effects of glyphosate on the human body, we suggest the United States invoke the Food Quality Protection Act's enforcement of a tenfold safety margin for pesticides or herbicides without reliable data showing no risk to children (Code 1996). Current glyphosate cRfD 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 E.U. and closer to that recommended by multiple research groups (Antoniou et al. 2012; Myers et al. 2016). The vast majority of human urine samples collected from herbicide workers still fall well below this level, so enforcement to this standard is not unreasonable (Niemann et al. 2015). Debate over the E.U.'s re-approval of glyphosate, and whether to change the ADI 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 (Mesnage et al. 2014). 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 (Mesnage et al. 2013).
5. If glyphosate is a human carcinogen, the mechanisms by which it acts are likely obfuscated behind such complex mechanisms as nonmonotonic 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 OECD and the US EPA (Cimino 2006). 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 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 (Brennan and Schiestl 2004; Carls and Schiestl 1994).

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 (Hsu and Stedeford 2010). While this may be warranted in some situations, these criteria could cause low-dose, nonmonotonic 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 nonmonotonic dose response (Lagarde et al. 2015; Vandenberg et al. 2012; vom Saal et al. 2010). 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 completely disregarding studies that do not meet current criteria for standardized carcinogenicity testing for reasons such as sample size (Zoeller and Vandenberg 2015).

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 towards 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 glyphosate-based herbicides 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 pre-harvest 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 likely 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 nonmonotonic 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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Table 1 – EPA Allowable Glyphosate Residue for Selected Crops (EPA 1980)

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

Table 2 - Studies Demonstrating Inhibitory Concentrations of Glyphosate on Gut Microbiota

Study	Bacterial Strains	Role	Glyphosate Tolerance (MIC)	Notes
Shehata 2013 (Shehata et al. 2013)	*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 1985 (Schulz et al. 1985)	*Pseudomonas aeruginosa*	pathogen	high, ~ 1 mg/mL	
Ackermann 2015 (Ackermann et al. 2015)	*Clostridium botulinum*	pathogen	high, >1 mg/mL	Botulism toxin production increased at this level
	Ruminococcus sp.	ruminant fermenter	low , 0.01 mg/mL	
Krüger 2013 (Krüger et al. 2013)	*Enterococcus* sp.	commensal/beneficial	low, 0.1 mg/mL	Capacity to inhibit *C. botulinum* toxin production decreased at this level
	Clostridium botulinum	pathogen	High, >1 mg/mL	