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Is our environment ready for Roundup?

Investigation of Genetically Modified Organisms (GMOs) and their indirect effects on the environment

By

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Abstract

The introduction of genetically modified organisms (GMOs) has caused unforeseen effects on humans, animals, and the ecosystem. Although GMO crops may not be a direct hazard to health, their popularity has led to an increase in the application of the herbicide, Roundup. This research serves to understand the details of GMOs and Roundup in order to evaluate their safety. To understand what GMOs are, this paper focuses on *Zea mays* L., or corn, which has been modified to be herbicide tolerant and insect resistant. There have been studies showing little to no negative effects of genetically modified (GM) crops on animals or humans. However, indirect effects such as increase in herbicides must also be accounted for. Roundup is a nonspecific herbicide that will kill any plants that are not specifically resistant. Since the popularity of GM crops has increased, there has been a 90% decrease in milkweed and a correlated 81% decrease in monarch butterfly populations. Roundup has also been shown to be toxic to frogs and cause morphological changes in tadpoles. Our ecosystem is suffering from Roundup because the EPA/FDA regulations focus solely on the active principle in Roundup which is glyphosate. Mesnage (2011) and Richard (2015) conducted studies that show major discrepancies between glyphosate and Roundup safety. Data analysis has shown Roundup is 250 times more toxic than glyphosate alone. Although GMOs safety is often controversial, the larger issue is the effects of the chemical formulations that we are applying to the GM crops.

1. Introduction

This research was inspired by the controversial views on genetically modified organisms (GMOs). They have recently increased in popularity and there have been multiple documentaries filmed to both prove and disprove GMO safety. This research paper looks into the dangers of GMO food and their indirect effects. I found that there were no concrete studies concerning safety issues and GMO crops. In general, once the food is metabolized it is broken down and poses no threat to humans or animals. The other aspect to consider is the indirect effects that come along with the use of GMOs. Many GMO crops have been modified to be resistant to glyphosate. Glyphosate is the active ingredient in the herbicide, Roundup. The Environmental Protection Agency (EPA) vetted over 100 studies on glyphosate to conclude there were no meaningful risks to human health (EPA 2016). The Acceptable Daily Intake (ADI) of glyphosate is used as the measurement used to judge the safety of the Roundup herbicide.

However, Roundup contains added chemicals such as adjuvants, to keep the glyphosate from degrading, and surfactants, to help the glyphosate enter through cell membranes (Brausche 2007). Around 70% of genetically modified crops are glyphosate resistant (EPA 2016), and the use of Roundup ready containing glyphosate has increased 15 fold (Benbrook 2016). Studies on the effect of Roundup on human cells, amphibians, and monarch butterflies are used to understand Roundup's toxicity. I have concluded that due to the fact that Roundup is much more toxic than glyphosate alone, increasing the amount of GMO crops will increase Roundup use, and therefore GMO crops do have indirect effects on our environment.

Zea mays L. in the most abundant crop cultivated in the United States, accounting for 90 million acres across the country. According to the US Department of Agriculture, in 2012, it was calculated that 88% of corn grown in the US was genetically modified (Heinemann 2014). The genetically modified traits incorporated into corn include herbicide tolerance, Lepidopteran insect resistance, and antibiotic resistance. MON810 uses the target gene Cry1Ab from *Bacillus thuringiensis*, which codes for a protein that functions as a toxin to Lepidopteran insects. To understand the implications of inserting foreign bacteria DNA into our crops, it is important to understand what the bacteria is. While there are many explanations to Cry proteins mechanism, the most common is that the Cry protein will bind to a midgut receptor, start a cascading signal, and initiate a form of programmed cell death (Ibrahim 2010). However, Bt accumulation does naturally occur in soils (EPA 2011). There are many strands of Bt and each strand has specific parameters to which insects it is toxic to. Different Cry proteins are specifically utilized based on the target pest: MON810 utilizes Cry1Ab to target Lepidopteran insects. The Cry1Ab toxin binds to the Bt-R1 receptor, which causes the formation of a pre-pore oligomeric structure. Once the Cry1Ab is bound it begins to form pores in the midgut of the target insects (Bravo 2007). The pores are formed in the midgut epithelial cells and cause a disruption of ionic gradients which will cause lysis of the epithelial cells, leading to cell death (Wang 2015). Nevertheless, since Bt naturally occurs and only targets insects, its safety is not a large concern.

A common concern for organic farmers and critics of GMO crops, is the possibility of GMO corn pollen dispersal. If GMO corn pollen travels to an organic farm, it could fertilize non-GMO corn. This could result in an unintended hybrid or tainting the organic endorsement of the crops. In contrast to a self-pollinating crop, corn is an out-crossing crop. Jemison and Vayda (2001) conducted a study to observe airborne cross pollination from GMO pollen via wind. They found minimal dispersal and significant declines after 40m (Jemison 2001).

Since GMOs and GMO pollen spreading is not cause many issues, it is time to look at indirect reasons that stem from the chemicals used. Glyphosate is a non-discriminatory herbicide that was discovered in 1970 by Dr. John Franze. Glyphosate is the main active ingredient in Monsanto Company's top selling product -- Roundup herbicide. Popularity of Roundup gained momentum around 1995 and now, 70% of maize is modified to be glyphosate resistant. Approximately 15 million acres are treated with about 18 million pounds of glyphosate annually (EPA 2016). Its calculated that since the invention of glyphosate resistant crops, the use of glyphosate has gone up 15-fold (Benbrook 2016). Glyphosate functions by targeting the shikimate pathway in plants cells, and works as an inhibitor to prevent synthesis of necessary bio matter. The bio matter produced through the shikimate pathway makes up more than 35% of the plants dry weight. By inhibiting the production of amino acids, the plants do not produce a significant portion of their weight and cannot survive (Alibhai 2001).

There have been conflicting publications on the safety of glyphosate done by the World Health Organization: International Agency for Research on Cancer (WHO IARC), and the

Environmental Protection Agency (EPA). However, the most recent EPA release in December 2017 states that glyphosate's safety was measured by the EPA and concluded that there are no meaningful risks to human health when the product is use according to the label. They did find potential ecological risks for birds, mammals, terrestrial, and aquatic plants. Ultimately, since the introduction of glyphosate, its safety has been frequently reevaluated, which provokes uncertainty among the researchers (EPA 2017).

According to the EPA, glyphosate is not expected to sink more than six inches into the soil because it adsorbs to the soil. Glyphosate breaks down into aminomethylphosphonic acid (AMPA) and glyoxylic acid, which will further break down into CO_2 (Roberts 1998). However, if glyphosate attaches to soil particles in runoff, it could potentially pollute the surface water. If glyphosate got into the water cycle, it would not be broken down by water or sunlight (EPA 2016). This affects the length of time glyphosate remains in the soil. The degradation depends on the contents of the soils as well as how much is applied. These factors could influence the degree to which glyphosate is found in nearby streams, lakes, ponds, or any body of water. Another potential flaw when using the same herbicide repeatedly, is the frequently reapplication could result in resistance by weeds. In 1996, 24 glyphosate resistant species were discovered worldwide. 14 of the 24 were found in the United States (Hanson 2013). Eventually, this could result in a tolerance, needing to increase the amount of Roundup necessary to achieve the same results. If glyphosate remains the most used herbicide and weeds become resistant, it could cause an even larger problem in the future.

Roundup herbicide is the major herbicide produced by Monsanto Company and glyphosate is the active principle (AP) in the herbicide, Roundup. The U.S EPA classifies glyphosate as having evidence of non-carcinogenicity in humans based on the long term studies to determine the acceptable daily intake (ADI) of glyphosate (NPIC 2011). However, Roundup contains more than just glyphosate, it also has adjuvants, surfactants, and unidentified chemicals. Surfactants aid in breaking through the outer membrane of cells to allow the other chemicals to infiltrate the cell body. Adjuvants improve herbicide activity in the formulation and serve as cell penetrants. Although these factors change the toxicity levels of Roundup, they are not studied because they are considered inactive chemicals.

Mesnage (2011) compared the effects of Roundup and glyphosate on human cells. He found that glyphosate-based herbicides were 250 times more toxic than the glyphosate alone when tested on human cells. (Mesnage 2011). Richard et al., (2005) also looked at the effects of glyphosate and Roundup. He applied the chemicals on human placental cells from healthy, full-term pregnant women and analyzed the change in excretion of hormones. In both Roundup versus glyphosate experiments, the Roundup proved to be much more toxic than the glyphosate alone. Yet Roundup is not being regulated before being released into the market. Clearly, testing just the active ingredient is not an accurate representation of the entire product. Increasing GM crops is directly correlated with more Roundup use, of which the toxicity is in question.

Amphibian species have been declining since the 1990s. POEA was added to Roundup to act as a surfactant so it could dissolve the exterior cuticle on the leaves. POEA is able to dissolve in water and cut through barriers which is why it was thought to be a potential threat to aquatic animals (Brausche 2007). After monitoring the populations for three weeks, 96-100% of the larvae amphibians died. A similar study with juveniles killed 68-86% after one day. Raylea suspects that POEA is the reason for the high toxicity results (Raylea 2012).

A large concern regarding GMOs and Roundup use has been the effect on *Danaus plexippus*, monarch butterfly, populations. In the last decade, there has been a noted decline in the monarch population and increase in herbicide use. Monarchs undergo overwintering - an annual migration pattern that spans the United States and Mexico. Pleasants (2012) looked at the quantity of milkweed that has been lost in agricultural fields in the Midwest in the past decade. Specifically, Iowa is primarily (75%) cropland and produces about 78 times more monarchs than the non-agricultural habitats Harzler (2010) analyzed the common milkweed infestations in different habitats in Iowa. He found a 90% decrease in milkweed from 1999 to 2009. Hartzler concluded that the increase in GM crops and simultaneous applications of glyphosate are the primary reasons for the decrease in milkweed populations (Hartzler 2010).

This research initially wanted to understand the negative stigma surrounding GMOs but found no toxicity issues. However, there were studies showing the dangers of Roundup using experiments on human cells, placental cells, amphibians, and monarch butterflies. The combination of glyphosate and other chemicals created a dangerously toxic mix that we are spraying on our GMO crops. Thus, there is evidence that increase in GMO cause increase in Roundup, which could have toxic effects on our environment.

1.1 Introduction of GMOs

Genetically modified organisms (GMOs) have been a topic of controversy since their introduction into the public market in the 1990s. The Food and Drug Administration (FDA) and United States Department of Agriculture (USDA) worked together to promote the use of GMOs in the United States. In 1994, the FDA created a simple approval technique for genetically-engineered foods. Modified foods were easily integrated into the public market due to their defined method of being produced through the *process* of genetic engineering rather than being a *product* of genetic engineering (Lynch 2001). If it were a product of genetic engineering it infers that the food was completely artificially created. By stating it went through the process of genetic engineering, the food sounds like it went through a smaller change. In 1997, the USDA Animal and Plant Health Inspection Service (APHIS) further simplified the procedures for about 80% of GMOs, so they could be more easily transported over state lines. Furthermore, the FDA determined that labels identifying foods with GMO content was not required (Lynch 2001). A series of strikes, laws, and boycotts started because of the introductions of unidentified GMOs in grocery stores. However, much of the controversy is due to lack of thorough knowledge and understanding of GMO mechanisms and effects. GMOs are officially defined as organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and/or natural recombination (WHO 2014).

Foods to be genetically engineered undergo a process in which genes from selected species are inserted into the organism of interest. There are two ways of inserting the target gene into the specified plant targets: biolistic and Agrobacterium-mediated transformations (Raska 2015). Biolistic, or the Gene-Gun delivery, shoots the genes into the target organism. The target gene is coated onto colloidal gold micro projectiles that are shot toward the tissue surface of the species. Due to the intensity of the gas-accelerated shot, the particles enter deep into the cytosol and cell nucleus (Raska 2015). Since this process has some disadvantages, such as the expensive machinery and the low average success rate, other methods have been implemented (Sanford 1990). Agrobacterium-mediated transformations are often used with mass production crops, such as corn. The DNA that expresses the gene of interest (aka the transfer DNA), is isolated from its species and multiplied through polymerase chain reaction (PCR). Once the desired gene is multiplied, it is used to create a construct, which will contain the desired transfer DNA (T-DNA). The construct is then entered into the agrobacterium, allowing the desired gene to be multiplied in the bacterium (Beardmore 2003). The agrobacterium containing the T-DNA is then inserted into the host plant (Gelvin 2003). The plants that successfully express the transformed genes are used, bred, and sold.

1.2 MON810 corn

Zea mays L. in the most abundant crop cultivated in the United States, accounting for 90 million acres across the country. According to the US Department of Agriculture, in 2012, it was calculated that 88% of corn grown in the US was genetically modified (Heinemann 2014). One of the largest distributers of genetically modified crops is the corporation, Monsanto. According to the International Service for the Acquisition of Agri-Biotech Applications (ISAAA): MON810 is a corn strain developed by Monsanto Company. The genetically modified traits incorporated into corn include herbicide tolerance, Lepidopteran insect resistance, and antibiotic resistance. MON810 uses the target gene Cry1Ab from *Bacillus thuringiensis,* which codes for a protein that functions as a toxin to Lepidopteran insects. The protein Cry1Ab is expressed directly in plant cells and tissues to help protect the plant against insects. When consumed, it will exterminate the insects by selectively destroying their midgut lining (ISAAA 2018). MON810 is also genetically modified to be glyphosate resistant. Glyphosate resistant crops have a gene that will produce a glyphosate insensitive enzyme from *Agrobacterium* strain CP4. Glyphosate binds to the gene CP4 EPSP synthase, which allows for the shikimate pathway to not be inhibited by glyphosate (Funke 2006). Furthermore, there has been studies that found higher amounts of aminomethylphosphonic acid (AMPA), a glyphosate metabolite, in glyphosate resistant crops.

Deducing that if the crop came in contact with glyphosate, it would be able to break it down (Duke 2011). Since GMO crops are essentially resistant to the Roundup herbicide, they can be sprayed with high amounts of the herbicide without fatality (ISAAA 2018). MON810 is one of the genetically modified (GM) strains of GM crop, but it is well understood and helps us understand the implications of using GM crops.

The bacterial DNA that expresses the Cry1Ab protein is *Bacillus thuringiensis* (Bt). To understand the implications of inserting foreign bacteria DNA into our crops, it is important to understand what the bacteria is. It is a large, gram positive rod-shaped bacterium that is motile via multiple flagella (Ibrahim 2010). Bt is capable of forming long term endospores which aid in handling long term environmental stress, which allows survival through harsh conditions (Hutchison 2014). It is a species within the genus Bacillus with three defining characteristics: it is able to produce parasporal crystalline proteins that can become toxic once ingested, it can live independently from other gram-positive spore-forming bacilli, and lastly can survive in the midgut and the hemocoel (body cavity) of insects. The ability to produce Cry proteins is what allows the Bt to act as an insecticidal toxin. While there are many explanations to Cry proteins mechanism, the most common is that the Cry protein will bind to a midgut receptor, start a cascading signal, and initiate a form of programmed cell death (Ibrahim 2010).

However, Bt accumulation does naturally occur in soils (EPA 2011). There are many strands of Bt and each strand has specific parameters to which insects it is toxic to. For example, the naturally occurring Bt survives and spreads because is it found in some species of caterpillars who are unaffected by the bacteria (Rodríguez-Sánchez 2006). Different Cry proteins are specifically utilized based on the target pest: MON810 utilizes Cry1Ab to target Lepidopteran insects. Since Bt naturally occurs, its safety is not a large concern.

To understand the effects and safety concerns of Cry1Ab, it is vital to understand the effects of the protein. Bt cry toxins are water-soluble, pore-forming toxins (PFTs), which can be inserted into cell membranes after undergoing conformational changes. Cry toxins are an alpha helix toxin: the helix region forms a trans-membrane pore (Bravo 2007). The Cry1Ab toxin binds to the Bt-R1 receptor, which causes the formation of a pre-pore oligomeric structure. Oligomeric structure are a collection of monomers that have more than one polypeptide chain. The oligomer is soluble in the cell membrane and therefore inserts into the membrane easier than the monomer. The oligomeric structure allows the Cry1Ab binding affinity to increase 100-200 fold. Once the Cry1Ab is bound it begins to form pores in the midgut of the target insects (Bravo 2007). The pores are formed in the midgut epithelial cells and cause a disruption of ionic gradients which will cause lysis of the epithelial cells, leading to cell death (Wang 2015). Looking into various experiments on the protein, there has been little evidence of the effect of Cry1Ab on animals or human cells (Mesnage 2013, Osamu 2007). Although the safety of GMO corn has been disputed, theories revolving around the spread of GMO pollen's effects are still a cause for controversy. In fact, there was theories that the spread of GMO could be causing declines in the monarch population. To respond to these claims, studies were done to examine the chances of GMO corn pollen accidentally spreading into unplanned areas.

A common concern for organic farmers and critics of GMO crops, is the possibility of GMO corn pollen dispersal. If GMO corn pollen travels to an organic farm, it could fertilize non-GMO corn. This could result in an unintended hybrid or tainting the organic endorsement of the crops. In contrast to a self-pollinating crop, corn is an out-crossing crop. Pollen is produced in the tassel and the seed-bearing organ is in the shoot. In order for reproduction to occur, the pollen must travel to the shoot by means of air transport (Poehlmanv 1995). Jemison and Vayda

(2001) conducted a study to observe airborne cross pollination from GMO pollen via wind. They found minimal dispersal and significant declines after 40m (Jemison 2001). Similar results were found when the case was reevaluated in 2015. The pollen distribution decreased sharply as the distance increased between the GMO and non-GMO corn source (Figure 1) (Baltazar 2015).

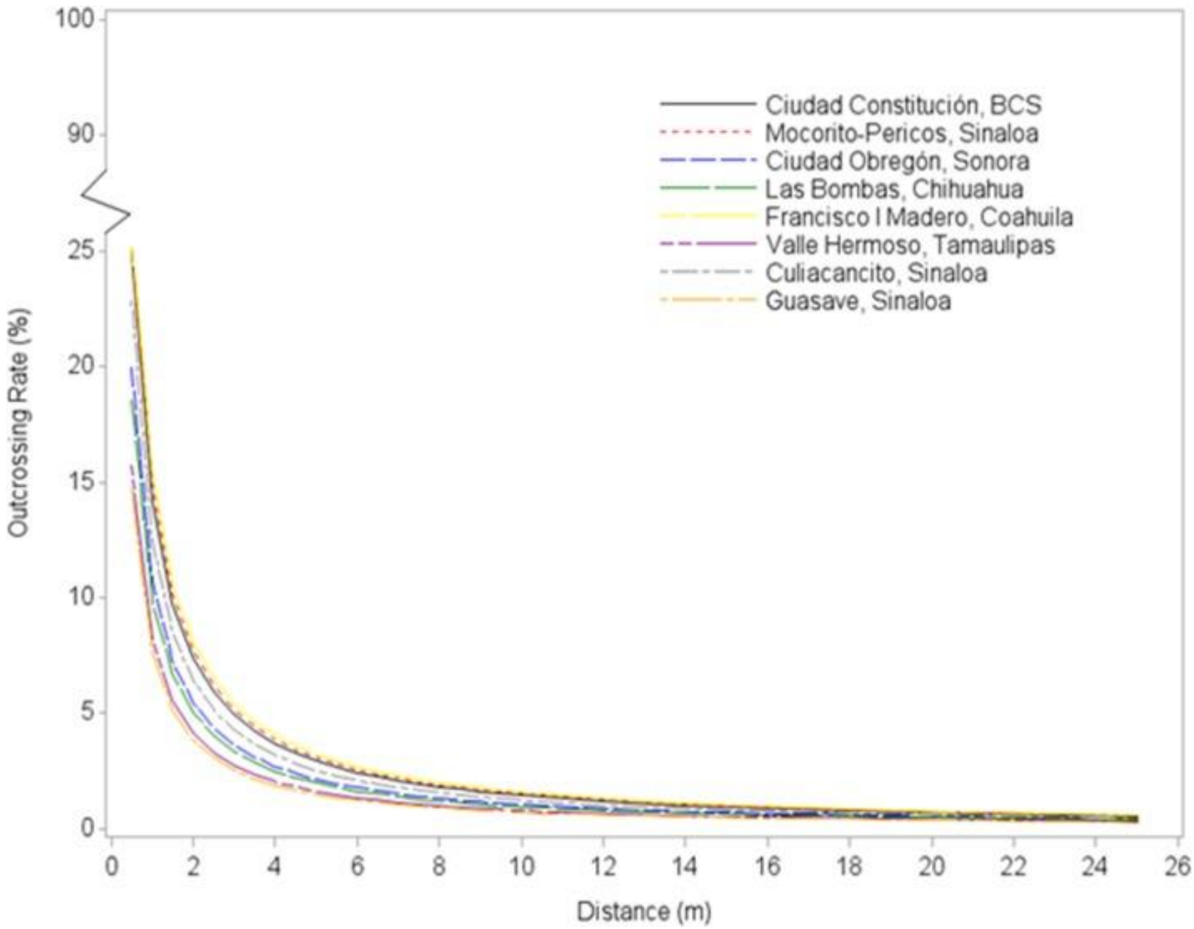


Figure 1. The amount of pollen naturally distributed up to 26m. The dispersal rate decreased after 4 meters (Baltzar 2015).

Roundup Ready corn was planted at a farm in Maine in May 1999 and 2000. There were two field plots of non-GMO corn located at 30m and 350m away (Figure 2). During that summer, dominant winds were southwestern. The closer field (30m) had a higher likelihood of receiving the Roundup Ready pollen through air transportation. To measure the amount of pollen that traveled, measurements were taken in 1999 and 2000, after the pollination and growth seasons. At the end of the season, each field was sprayed with glyphosate to quantify how many corn survived on each patch (Jemison 2001).

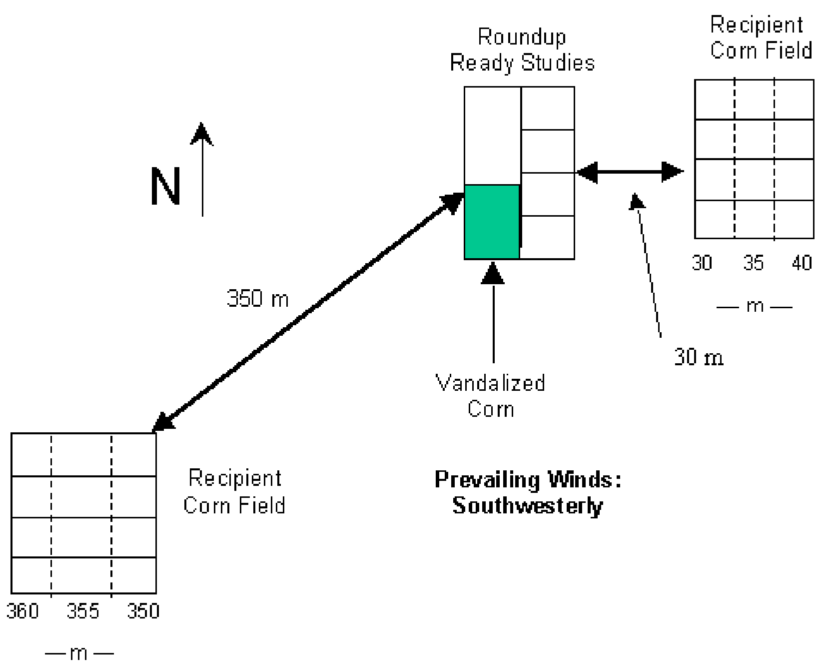


Figure 2. The aerial view of the corn plot distances and prevailing winds (Jemison 2001).

Table 1. The survival of corn in 30, 35, and 40m organic fields after being sprayed with glyphosate

Distance (m)	**Mean germination**	**Survival (%)**
30	691	1.04
35	701	0.11
40	716	0.03

Jemison and Vayda (2001), observed there were minimal amounts of cross-pollination between the GMO corn and the non-GMO corns. The 30, 35, and 40 m E plots showed some percentage of survival – indicating that there were low levels of pollen transfer between GMO and non-GMO fields (Table 1). In fact, the highest survival rate was 1% of GMO corn which was 30m E downwind from the GMO pollen origin (Jemison 2001). In Baltzar's study (2015), there were similar results showing the degree of GMO corn dropped significantly as distance increased (Figure 1). Although the percentage of pollen dispersal is minimal, it could still cause concern when growing an organic certified crop. The farmers would have to be cautious to separate their field plots from any GMO plots.

2. Glyphosate - (N-(phosphonomethyl)glycine)

2.1 Glyphosate

Glyphosate is a non-discriminatory herbicide that was discovered in 1970 by Dr. John Franze. Glyphosate is the main active ingredient in Monsanto Company's top selling product -- Roundup herbicide. Monsanto is responsible for utilizing biotechnology and creating crops that are resistant to glyphosate. They created an herbicide that will cause no harmful effects on the desired GM crops, only inflicting damage on unwanted weeds (Benbrook 2016). Once glyphosate caught on in the market, it became the most widely used herbicide by volume. Popularity of Roundup gained momentum around 1995 and now, 70% of maize is modified to be glyphosate resistant. Approximately 15 million acres are treated with about 18 million pounds of glyphosate annually (EPA 2016). Its calculated that since the invention of glyphosate resistant crops, the use of glyphosate has gone up 15-fold (Benbrook 2016). This dramatic increase in herbicide is causing unintended effects on our ecosystem. More GM crops grown means more Roundup will be applied; thus, its safety should be evaluated accurately to understand the implications of using it in our ecosystem.

Glyphosate functions by targeting the shikimate pathway in plants cells, and works as an inhibitor to prevent synthesis of necessary bio matter. 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is the target of glyphosate. EPSPS is an important step in aromatic amino acid biosynthesis – a large percentage of biomass in plants. EPSPS is the enzyme that catalyzes the transfer of a particular section (enolpyruvyl moiety) of phosphoenolpyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P). That pathway produces 5-enolpyruvylshikimate-3-phosphate (EPSP) and inorganic phosphate (Alibhai 2001). Glyphosate works as an inhibitor of EPSPS by competing with PEP for the active site (Figure 3). They have similar binding interactions, which allows them to bind to the same active site. Glyphosate is a relatively simple yet highly specific competitive inhibitor for EPSPS; this permits glyphosate to function outstandingly well as an inhibitor and thus, an herbicide (Alibhai 2001).

By inhibiting the synthesis of EPSP and inorganic phosphate, it inhibits the synthesis of aromatic amino acids: phenylalanine, tyrosine, and tryptophan and other metabolites: tetrahydrofolate, ubiquinone, and vitamin K. The shikimate pathway is present in both microorganisms and plants. The bio matter produced through the shikimate pathway makes up more than 35% of the plants dry weight. By inhibiting the production of amino acids, the plants do not produce a significant portion of their weight and cannot survive. Chorismate

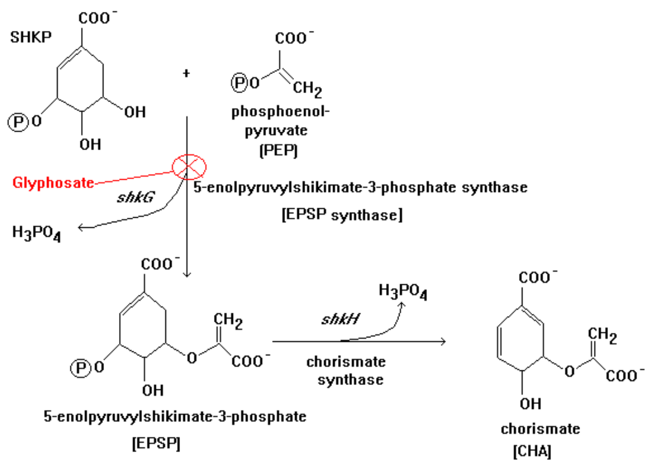


Figure 3. The alteration of the Shikimate pathway with the addition of glyphosate (Rhodes 2009).

(CHA) is the precursor for amino acids and is inhibited when glyphosate is introduced to the plant (Figure 3). Although I have not found much research on microorganisms and glyphosate; bacteria utilize the shikimate pathway therefore, microorganism could also be affected. Nevertheless, the shikimate pathway is not present in any insects, reptiles, birds, fish, or mammals, essentially glyphosate should have no effect in those systems. However, there are still unintended effects such as harming microorganisms in the soil and accidentally killing non-targeted flora (Alibhai 2001).

2.2 The legality of glyphosate in the US

There has been conflicting publications on the safety of glyphosate done by the World Health Organization (WHO): International Agency for Research on Cancer (IARC) and the

Environmental Protection Agency (EPA). Glyphosate has undergone multiple tests and has been reevaluated many times by the EPA. The EPA classified glyphosate as possibly carcinogenic to humans in 1985 after tests on mice were released demonstrating tumor formation. However, the EPA requested more tests to be run with a more accurate study design and consequently, reevaluated glyphosate as being not classifiable as a human carcinogen (EPA 2016).

Furthermore, the IARC used the evidence gathered by the USA EPA to reevaluate, study, and classify glyphosate as having sufficient evidence of carcinogenicity in animals. The IARC also found it to cause DNA and chromosomal damage in human cells (WHO 2014).

In 2016, an EPA document stated that glyphosate is currently under Registration Review. Glyphosate is required to undergo a series of tests including epidemiological, animal carcinogenicity, and genotoxicity studies. The studies were individually assessed for quality and correctly analyzed data. From the available data the EPA collected, they determined there are no carcinogenic properties in glyphosate. However, they have not dismissed the proposed link to Non-Hodgkin's Lymphoma. The limitations and conflicting results of available experiments prevent the EPA from drawing a definitive conclusion about Non-Hodgkin's Lymphoma (EPA 2016).

The most recent EPA release in December 2017 states that glyphosate's safety was measured by the EPA and concluded that there are no meaningful risks to human health when the product is use according to the label. They did find potential ecological risks for birds, mammals, terrestrial, and aquatic plants. Ultimately, since the introduction of glyphosate, its safety has been frequently reevaluated, which provokes uncertainty among the researchers (EPA 2017).

2.3 Glyphosate in the environment

Glyphosate is a zwitterion with amphoteric characteristics. This means that the molecule has both positive and negative ends – creating an overall neutral charge. Thus glyphosate has strong adsorption qualities to the soil (Adsorb: when a thin layer of liquid/gas forms on the surface of a solid) (Szekas 2012). According to the EPA, glyphosate is not expected to sink more than six inches into the soil because it adsorbs to the soil. Glyphosate breaks down into aminomethylphosphonic acid (AMPA) and glyoxylic acid, which will further break down into CO_2 (Roberts 1998). However, if glyphosate attaches to soil particles in runoff, it could potentially pollute the surface water. If glyphosate got into the water cycle, it would not be broken down by water or sunlight (EPA 2016). This affects the length of time glyphosate remains in the soil. There have been discrepancies in lab versus field soil studies regarding glyphosate's half-life (NPIC 2011). Essentially, the effect of glyphosate on our ecosystem varies depending on the location and amount distributed (EPA 2016).

To fully understand the implications of glyphosate degradation, using numerical values gives a better idea of the levels of glyphosate in the soil. The half-life is defined as the given amount of days it takes for 50% of the glyphosate to breakdown in the soil. The half-life of glyphosate in water varies from 3 to 91 days. In soil it ranges from 2 to 197 days. The average half-life is 47 days which means after around 8 months there would be 3% of glyphosate remaining in the soil (NPIC 2011). However, those studies were conducted in laboratory settings. Studies done in 2005 have shown that degradation of the parent compound could last from a few days up to a year in the soil. If there are high amounts of cations in the soil, it would increase glyphosate adsorption. Therefore, the degradation process is heavily dependent on the contents of soil (Szekas 2012).

The degradation depends on the contents of the soils as well as how much is applied. These factors could influence the degree to which glyphosate is found in nearby streams, lakes, ponds, or any body of water. The amount of glyphosate sprayed on a field depends on the growth of the weeds. Monsanto's Roundup Ready Plus crop management solutions recommends 32 fl oz/acre when weeds are four inches or less. Studies have been done to estimate concentration levels of Roundup in runoff water after being sprayed on fields. Battaglin et al., did a study measuring the water samples of 51 different streams in 9 different Midwestern states (Battaglin 2002). Three measurements were taken: after applying pre-emergent herbicides, after post-emergent herbicides, and during the harvest season. Glyphosate was measured using gas chromatography and high performance liquid chromatography. The results show that glyphosate was not found in concentrations exceeding the EPA's maximum contamination level (700 μg/l) (Battaglin 2002).

Another potential flaw when using the same herbicide over and over again, is the frequently reapplication could result in resistance by weeds. Tillage is a common procedure used by farmers to uproot and decrease infestation of unwanted weeds. The use of Roundup eliminates/reduces tillage since the herbicide will replace the job of diminishing unwanted weeds. However, this means glyphosate could remain in the soil each season, collect, and potentially spread. This raises the concern of glyphosate residue in soils and the possibility of weed resistance. If a weed has a DNA mutation to be resistant to the glyphosate, it could reproduce rapidly and spread the resistant DNA. In 1996, 24 glyphosate resistant species were discovered worldwide. 14 of the 24 were found in the United States. The most common form of resistance to glyphosate is the development of a point mutation in the EPSPS gene. This mutation reduces affinity of glyphosate to the EPSPS active site. This allows the enzyme to become less sensitive to the effect of glyphosate on the shikimate pathway. Plants containing the mutation will have higher reproductive success (Hanson 2013). Eventually, this could result in a tolerance, needing to increase the amount of Roundup necessary to achieve the same results. If glyphosate remains the most used herbicide and weeds become resistant, it could cause an even larger problem in the future.

3. Roundup

3.1 Roundup Herbicide

Roundup herbicide is the major herbicide produced by Monsanto Company. Before Roundup, companies had trouble producing herbicides that could penetrate through the plants protective outer layer. Plants have a waxy, outer cuticle to provide protection. In order to overcome this challenge, surfactants are often added to serve as a detergent and assist in releasing surface tension and breaking through the cuticle. Polyethoxylated tallow amines (POEA) are surfactants that were added to the Roundup formula to break through the waxy layer and grant other chemicals entrance (Brausche 2007). Since POEA aids in allowing glyphosate through cell membranes, the mixture of active glyphosate and POEA poses a hazardous combination.

Due to patents there is not much other information on the ingredients in the formulation. There have been limited studies done on the effects of Roundup on humans, animals, and the environment. In the EPA document they state that they focused on the effects of glyphosate only. The EPA admits that there are relatively few research projects that directly compare glyphosate and the formulations with identical experimental design (EPA 2016). This is a major issues since more GMO crops that are glyphosate (and therefore, Roundup) resistant are being planted.

Inevitably, this caused an increase in the use of Roundup which can affect our environment. The following case studies on human cells, amphibians, and monarchs show the indirect effects of GMO crops.

3.2 Impact of glyphosate and Roundup on human cells

The U.S EPA classifies glyphosate as having evidence of non-carcinogenicity in humans. There have been various long term studies to determine the acceptable daily intake (ADI) of glyphosate (NPIC 2011). This is important since glyphosate is the active principle (AP) in the herbicide, Roundup. However, Roundup contains more than just glyphosate, it also has adjuvants, surfactants, and unidentified chemicals. Surfactants aid in breaking through the outer membrane of cells to allow the other chemicals to infiltrate the cell body. Adjuvants improve herbicide activity in the formulation and serve as cell penetrants. Although these factors change the toxicity levels of Roundup, they are not studied because they are considered inactive chemicals. Mesnage (2011) found that glyphosate-based herbicides were 250 times more toxic than the glyphosate alone when tested on human cells. These tests were performed on three different human cell lines: embryonic (HEK293), placental (JEG3), and hepatic (HepG2). Each of these cell lines have been validated as useful models to test toxicities of pesticides, characterized as being as or less sensitive than average cells (Mesnage 2011).

Focusing on Roundup, the two ingredients tested were the active principle glyphosate (N-phosphonomethyl glycine, G, CAS: 1071-83-6) and the accompanying formulation Roundup GT+. These were both applied to the embryonic, placental, and hepatic cell lines and the experiments were repeated three separate times. Succinate dehydrogenase (SDH) activity was determined after treatments to measure cellular mitochondrial respiration. Apoptotic cell death was evaluated using Caspase-Glo 3/7 assay. The data showed there was significant differences for necrosis and apoptosis when comparing the cells exposed to Roundup and those to glyphosate (Mesnage 2011).

The results show that Roundup formulation is much more toxic than its AP, glyphosate (Figure 4). The study found that Roundup was the most toxic herbicide tested. Roundup has a lethal concentration (LC_{50}) of 63 parts per million. LC_{50} is the lethal concentration of chemicals that will cause the death of 50% of the subjects tested (CCHOS 2018).

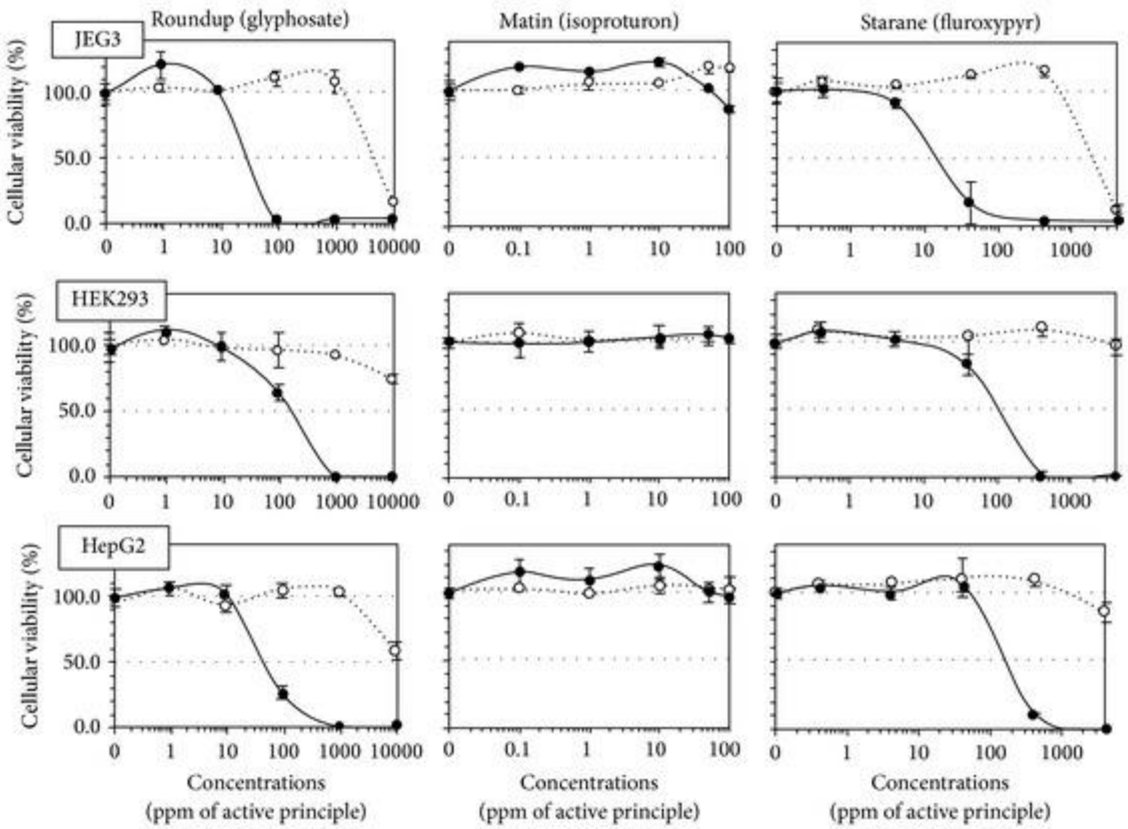


Figure 4. Cytotoxic effects of the herbicides formulations and their active principles. The AP is represented by the dotted line. The adjuvant with formulation (Roundup) is represented by the bold line. The cellular viability percentage is based on the results from the SD test of the mitochondrial respiration inhibition of the cells (Mesnage 2011).

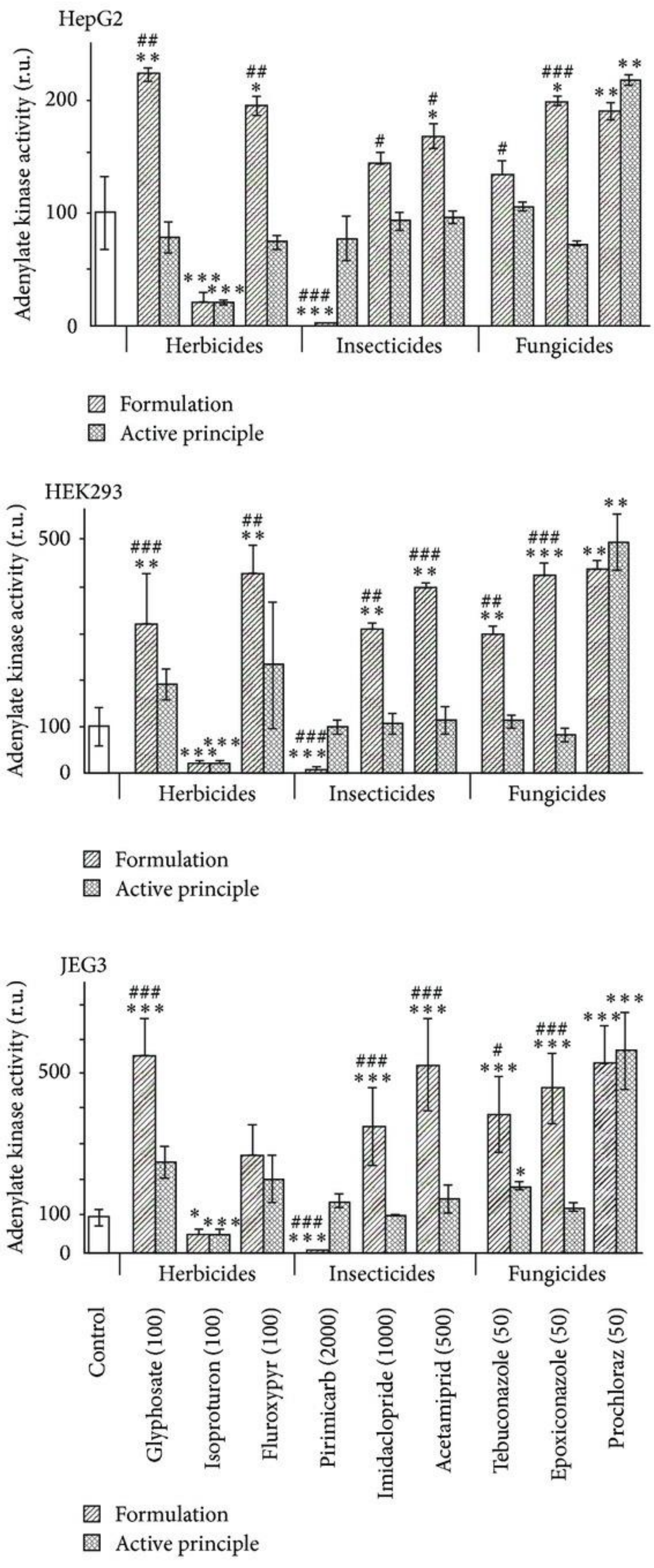


Figure 5. Shows the necrotic effects of formulations and their APs. The bars on the left are the formulation and the right are the AP's. The majority had abnormally high levels of Adenylate kinase activity in the formulation than the AP (Mesnage 2011).

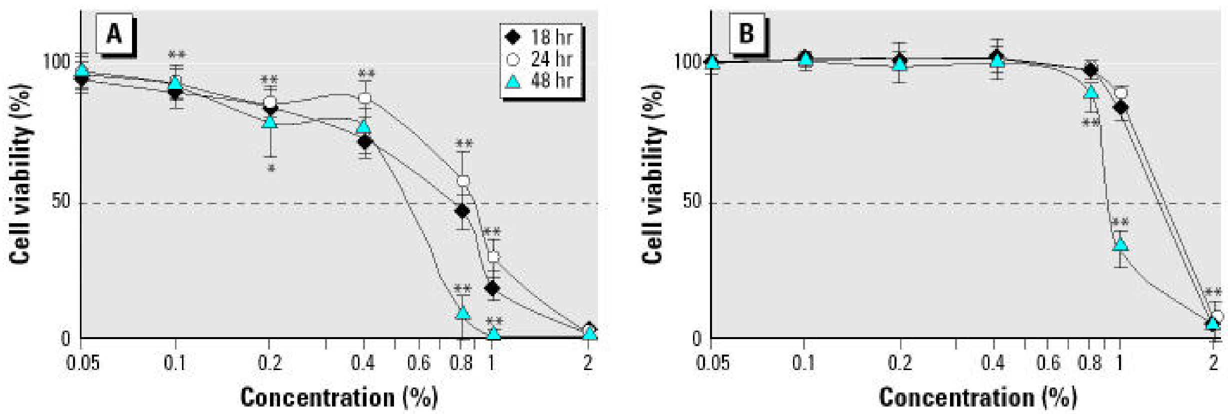
The adenylate kinase activity was measured by MTT assay to understand the energy production in the cells. Overactive adenylate kinase could cause unintended issues in the cells. It assists in signaling and overproduction of the kinase could cause negative effects on metabolic and energetic signaling pathways (Figure 5) (Dzeja P 2009).

The data on Roundup and glyphosate proves the cytotoxicity of herbicide formulations. This 24 hour study was compared to a similar 72 hour study. The longer study showed similar results, the Roundup was 5 times more toxic than glyphosate. It also shows that long term exposure to the chemicals could increase the chances of toxicity on human cells.

In conclusion, adjuvants are declared as inerts (chemically inactive) and are not required to undergo long-term regulatory experiments (Mesnage 2011). According to the EPA, inert ingredients are substances other than the active ingredient (the active ingredient is deliberately chosen to be included in the herbicide) which are used to increase the success of the product. However, the term inert does not indicate that the chemical is nontoxic (EPA 2016). Adjuvants are utilized to increase the solubility and protect the degradation of the active principles. They can intensify serious side effects and heighten herbicide activity by assisting in cell penetration and increasing their half-life. Although Roundup is advertised as a safe chemical, it was – by far – the most toxic herbicide tested. Moreover, Roundup was found to be 125 times more toxic than glyphosate. Basically, the companies claiming that glyphosate is safe in Roundup are only evaluating one aspect of the chemical formulation. When the same cell lines are exposed to Roundup and glyphosate, the Roundup is much more toxic (Mesnage 2011).

3.3 Glyphosate versus Roundup

Richard et al., (2005) determined the effects of glyphosate and Roundup on human placental JEG3 cells and their participation in disrupting hormonal balances (Figure 6). Furthermore, they observed the aromatase activity in the cells, to determine the effect on estrogen production. Aromatase assist in the production of androgens to estrogen; if affected, the amount of estrogen being synthesized could change.



Roundup is recommended at 1-2% so the concentrations used were measured up to 2% to test on the placental cells viability. Equal amounts of glyphosate and Roundup were tested using the same conditions. Figure 6 shows the decrease of cell viability when the glyphosate and Roundup were incubated with the cells. They were measured using an MTT assay. The cell

Figure 6. Graph A represents Round up. Graph B represents glyphosate alone. These are the effects of both on JEG3 cells viability. Assessed by MTT assay (Richard 2015).

viability in the Roundup incubation dropped once the Roundup reached 0.4% concentration. In glyphosate, the viability doesn’t dramatically decrease until around 0.8% concentration. Thus, the toxicity lethal dose was 1.8 times lower for Roundup than for glyphosate (Richard 2005).

Pertaining to the aromatase activity, there were no significant effects from the glyphosate on the JEG3 cells. However, with Roundup there was a 40% improvement of estrogen synthesis. It is hypothesized that the adjuvants could assist in increasing membrane fluidity and change the availability of the hormone. Then, once the Roundup entered the cells, there was a decrease in the aromatase activity, concluding that the Roundup effected the function of the aromatase. The added chemicals in Roundup are what allow this change in enzyme activity. The inhibition of aromatase activity by Roundup and glyphosate was tested and confirmed in both humans and equine cells. It was found that Roundup's inhibition effect was 4 times higher than glyphosate. Figure 7 shows the discrepancies between the effects of glyphosate and roundup on aromatase activity (Richard 2005).

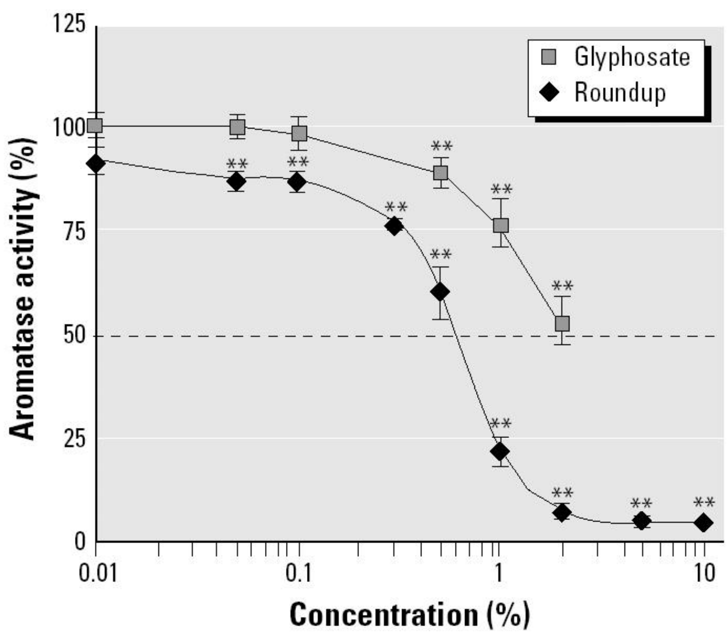


Figure 7. Shows the effects of Roundup or glyphosate on human placental microsomes. The aromatase activity was measured for 15m.
**p<0.01
(Richard 2005)

In both Roundup versus glyphosate experiments, the Roundup proved to be much more toxic than the glyphosate alone. Yet Roundup is not being regulated before being released into the market. Clearly, testing just the active ingredient is not an accurate representation of the entire product. Increasing GM crops is directly correlated with more Roundup use, of which the toxicity is in question. If GM crops are going to continue to flourish, these types of indirect effects need to be thoroughly analyzed.

3.4 Effects on Amphibians

Amphibian species have been declining since the 1990s. Various factors including global warming, exploitation, and habitat loss, are thought to be large contributors (Hayes 2010). Polluting their ecosystems would clearly exacerbate the issue, therefore studies have been done to understand the effect of Roundup moving from the soil into aquatic ecosystems.

POEA was added to Roundup to act as a surfactant so it could dissolve the exterior cuticle on the leaves. POEA is able to dissolve in water and cut through barriers which is why it was thought to be a potential threat to aquatic animals (Brausche 2007). According to Raylea et al. (2012), Monsanto conducted tests with amphibians exposed to Roundup and found no negative effects. However, replication studies found the effects of Roundup were highly toxic to amphibians. Once this study was released, Monsanto released a press statement that claimed Roundup caused no harmful effects and that their study was wrong. In fact, a Monsanto spokesperson even called Raylea to tell them him was POEA surfactant was not in the Roundup formulation. However, he would not divulge which surfactants were added, declaring it was a trade secret. Nevertheless, the group continued studies which reaffirmed their findings.

Supportive experiments were conducted using pond mesocosms (outdoor experiment systems) containing soil that absorbs the herbicide. After monitoring the populations for three weeks, 96-100% of the larvae amphibians died. A similar study with juveniles killed 68-86% after one day. Raylea suspects that POEA is the reason for the high toxicity results (Raylea 2012). This study focuses on aquatic life in our ecosystem, and reinforces the fact that there can be indirect consequences of a switching to a primarily GMO crop food source.

Raylea et al. (2012), also looked into the effects of Roundup on tadpoles in two species: wood frog and leopard frogs. Raylea et al. (2012), used outdoor mesacosms to create wetland communities that were exposed to different concentrations of herbicide. First, they found that when predators were present, Roundup was less lethal. Roundup tends to remain near the surface of the water but when the predators were present, the tadpoles remained at lower depths to avoided predation. Secondly, it was found that application of Roundup cause morphological changes by prompting deeper tails in the tadpoles. This is the same change that normally occur when a predator is present. These two discoveries imply that the presence of the herbicide causes abnormal development in the tadpoles. From what Raylea has found, the effects of Roundup are definitely questionable and need to be meticulously evaluated (Raylea 2012).

3.5 Effects on Monarch Populations

A large concern regarding GMOs and Roundup use has been the effect on *Danaus plexippus*, monarch butterfly, populations. In the last decade, there has been a noted decline in the monarch population and increase in herbicide use. From 2000 to 2010 Roundup Ready herbicide use increased by 26.7 million kg. Roundup is a non-selective herbicide and kills any weeds/plants that are not glyphosate resistant; therefore, GMO's are indirectly causing a decline in Monarch's populations (Hartzler 2010). The common milkweed, *Asclepias syriaca,* is a victim of the indiscriminate targets. Pleasants and Oberhauser (2012), found that approximately 92% of the butterfly populations used common milkweed as their mating and laying ground for eggs.

Monarchs undergo overwintering - an annual migration pattern that spans the United States and Mexico. A theorized reason for their decline is the decrease in milkweed. Milkweed serves as food for the monarch's larvae; the loss of area for eggs to be laid and larvae to receive nutrients would cause a significant decline in the population (Pleasants 2012). In the United States, the Midwest is the most common area for monarchs to travel when overwintering between Mexico and United States: Pleasants (2012) looked at the quantity of milkweed that has been lost in agricultural fields in the Midwest in the past decade. Specifically, Iowa is primarily (75%) cropland and produces about 78 times more monarchs than the non-agricultural habitats Harzler (2010) analyzed the common milkweed infestations in different habitats in Iowa. He found a 90% decrease in milkweed from 1999 to 2009. Hartzler concluded that the increase in GM crops and simultaneous applications of glyphosate are the primary reasons for the decrease in milkweed populations (Hartzler 2010).

Overall, it is estimated that there has been a 58% reduction in milkweed in the Midwest. From 1999-2009, farms in Iowa recorded that the milkweed population has dropped from 51% to 8% (Pleasants 2012). Furthermore, by surveying farmers in the area, Hartlzer (2010) found that 75% of crop rotations in Iowa consisted of glyphosate resistant crops.

Pleasants (2012) monitored the population of monarchs by counting larvae on milkweed leaves in various locations. The monarch eggs were observed in agricultural and non-agricultural habitats. The analysis showed a 14.2% decrease each year. The estimate of milkweed lost from 1999 to 2010 was 72%, with the majority (92%) from agricultural and (8%) non-agricultural.

This decrease in available milkweed may have contributed to the 81% decline in monarch egg production in the Midwest. Discrepancies between studies could be a result of using egg density as the method of tracking population size, the amount the Midwest contributed to the population each year, and the mortality rate in the fall. Environmental factors could also influence the monarchs population loss, but it cannot account for the entire decrease in percentage. Although the monarch's milkweed decreased, the monarchs did not search elsewhere for a food source for their larvae. One possible hypothesis to explain this is that monarch females would have to exert more energy in order to find other milkweed patches. In nature, the patches are less dense than in agricultural fields, so the monarchs do not search for more milkweed outside of the agricultural fields (Pleasants 2012).

Clearly, there is an inverse correlation between monarch population and GMO crops and Roundup application. This strongly supports that increased GMO and Roundup use is a main contributing factor (Hartzler 2010). The increased usage of GM crops and Roundup herbicide has presumably caused a substantial decline in the monarch population, since the herbicide has destroyed a large portion of their habitat. GM crops may not directly hurt us or our animals, but the use of nondiscriminatory herbicide is another factor that needs to be taken in account when looking at the safety of GM crops.

4. Discussion

When I originally began this research, I was very skeptical of the safety of GMO food. The idea of engineering foreign bacterium and genes into our crops seemed suspicious. From the data I have found, actual GMO crops do not largely contribute to health issues. The case studies on pollen dispersal show little reason to worry about GMO seed invasion in other farm plots.

Still, there are indirect effects of GMOs on our environment. The largest impact stems from the increased use of herbicides on our farmland. This is perfectly exemplified by the study on monarch butterflies. The herbicide is nondiscriminatory and will kill any plants that are not glyphosate resistant. Roundup herbicide is doing exactly what it was made for but in doing so it is destroying thousands of monarch's homes and breeding grounds.

Secondly, the development of glyphosate resistant crops has caused a sizable increase in glyphosate-based herbicide use. Major organizations (EPA and WHO) do not seem to come to a concrete overall conclusion on the safety of glyphosate. The majority of the evidence pertaining to glyphosate toxicity is dismissible, but the lack of concrete knowledge on Non-Hodgkin's Lymphoma is concerning.

While that is unsettling, I've concluded that the predominant issue comes from the formulations that contain glyphosate. Roundup is a concoction of secret chemicals. This mix will not be disclosed to the public since Monsanto deemed it as a trade secret of the company. Therefore, to understand Roundup's effects, researchers started conducting experiments. Tests on human cells using Roundup showed considerably higher toxicity than testing only glyphosate. A similar study showed toxicity toward amphibians. To add suspicion, Monsanto refused to accept any data found by Raylea. Monsanto will not disclose all of the ingredients, and attempts to eradicate any evidence against its product. These studies bring up safety concerns that absolutely require further investigation.

In truth, it is inexcusable that these chemicals have slipped through the safety precautions put in place for so long. Putting our farmers, our animals, and our ecosystem at risk is a matter that should be treated with high priority.

5. Future studies

Interesting research has been done on how micro RNA (miRNA) from plants can potentially be absorbed into the human blood stream upon consumption. Micro RNA is an important factor and can control the expression of messenger RNA. There have been studies on mice showing that *Brassica oleracea* (Wild cabbage) micro RNAs survive digestion and make it into the blood stream. The possibility of obtaining miRNA from our vegetables creates concern. If a vegetable was genetically engineered and its miRNA was altered, it could potentially enter the blood stream (Pastrello 2016). More studies are needed on common GMO crops and their relationship to miRNA uptake. There also should be comparisons conducted on the variances between miRNA between regular crops and GMO crops.

In general, the need for more studies on Roundup herbicide is imperative to understanding safety disputes. Since glyphosate is not the only ingredient in Roundup, we cannot assess the entirety of Roundup's safety based on the one ingredient. The added anonymous components should be tested both individually and as a whole by scientists outside of Monsanto's supervision. If Monsanto refuses to due to patents, it is absolutely necessary to conduct equal experimentations on glyphosate alone compared to the entire Roundup formulation. Once juxtaposed, the hazardous potential would be easier to evaluate. These types of indirect consequences of GM crops need to be evaluated properly to understand and prevent any dangerous they pose to our ecosystem.

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