

GENETICALLY MODIFIED CORN – LITERATURE REVIEW

by

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## Abstract

Corn, also known as maize, is one of the most important cereal crops and a staple food in many countries around the world. The United States is the largest producer and exporter of corn, and its use includes human consumption, animal feed, and industrial products. According to USDA, in 2021, about 92% of the corn planted in the USA was genetically modified corn (GM corn). GM corn has been genetically modified to resist pests, herbicides, and to better combat environmental stressors such as droughts. It has been the subject of intense debate and scrutiny, with proponents claiming that GM corn has the potential to increase yields and reduce the use of harmful pesticides, while opponents argue that it poses unknown risks to the environment and human health. The intense debate is not only between scientists or people, but also between whole countries. While in the USA GM corn is very popular and widespread, its cultivation in Mexico is banned, and recently Mexico announced that they plan to ban import of GM corn. After the announcement, USA entered into trade consultation, and promised retaliatory U.S. tariffs against Mexico if no resolution is reached. It is surprising that the US would sanction a country that is considered the birthplace of corn, has rich historical and cultural relationship with corn, just because they want to continue in their tradition and do not want to use GM corn. The literature review focuses on whether the US is right to push aggressively other countries to buy their “superior” seeds and products, or if there is a reason why countries like Mexico should be cautious about GM corn.

# Chapter 1 - Corn

## 1.1 History of Corn

In ancient America, corn (*Zea mays* L.) was by far the most important cultivated plant and is considered the great American crop. Corn was domesticated from teosinte (*Zea mays* L. spp Mexicana) in the Western Hemisphere about 7,000 to 10,000 years ago (Carena, 2009). It was used as a staple food by several ancient American civilizations, including the Mayan, the Aztec, and the Incan. Corn is one of the most efficient major crops for converting sun energy into food (Aldrich et al., 1986), and continues to be a staple food in many countries worldwide, like for example in Mexico and South Africa (Ruan et al., 2021)

Archeologists first thought that domestication of corn began about 2000 years ago, but radiocarbon dating revealed that the oldest known remains of fragmentary ears and grains of corn date to be more than 4500 years old (Aldrich et al., 1986). They have been found 150 feet under Mexico City, formerly known as Tenochtitlan. Interestingly, the ancestor city of Tenochtitlan is believed to be founded in 1325 A.D, several thousand years later than these corn remains are dated, and they doubtlessly belong to a period many centuries earlier than the beginning of anything that we can designate as agriculture (Weatherwax et al., 1954).

According to Sprague (1988), in 1492 the story of corn began for western civilizations, only a few days after discovery of the New World. Today it is still uncertain when corn first went to Europe, but according to some historians Columbus took it to

Spain in the return from his first voyage to America. It appeared under a variety of names, for example, *Triticum*, *Fruementum*, *Indian maize*, *Turkish maize*, and many others. The word corn, used currently, was converted by Spanish writers from the word maiz used by Caribbean Indians (Sprague,1988). The term corn is used interchangeably with maize because all grains were called corn under early British and American trade, and the name was retained for maize because it was the most common grain in commerce (Ranum et al., 2014). A corn plant was placed in an herbarium in Italy as early as 1532 and the first published picture of the plant was in *Fuchs' herbal of 1542* (Weatherwax et al., 1923). European settlers in America depended on corn as a food source and traded with and learned agricultural techniques from Indigenous populations. The progress of Spanish conquerors was often measured by the availability of corn to feed the expeditions (Sprague,1988). Even though significant changes have been made to corn over the centuries through selective breeding and other traditional forms of plant breeding, corn continues to be staple food for many countries around the world (Bennetzen, 2009).

## 1.2 Types of Corn

Many types of corn have been developed over the centuries for specific purposes and regions. One important difference between corn kernels is not only sweetness, but also appearance (**Picture 1**). Corn kernels can be different colors ranging from white to black but most of the corn grown in United States is yellow. White corn is preferred in Southern and Eastern Africa, Central America, and Mexico, and has a lower content of  $\beta$ -carotene and other vitamins and minerals as opposed to yellow corn. This preference

of white corn is mostly caused by tradition and social status perception. In these countries, yellow corn is perceived as consumed only by poor people or animals. The manufacture of animal feed uses mostly yellow corn (Ranum et al., 2014).

Another classification of corn is based on the size and the composition of the endosperm. These artificial kernel definitions are sweet corn, pod corn, Indian corn, flower corn, waxy corn, flint corn, and dent corn. In this review the focus is on sweet corn, one of the most popular vegetables on Earth. Sweet corn is grown specifically for its sweetness and tenderness and is used both in animal feed and food production. It is the most important vegetables in the United States and Canada (Huet et al., 2021). It stores poorly, and therefore it must be eaten fresh, be canned, or frozen, before kernels age and become small, tough, and starchy (Ranum et al., 2014). Increased sugar content depends on disruption of endosperm starch synthesis on various altered alleles (usually loss of function), altering polysaccharide composition and causing a sugar content increase (Revilla, 2021). These characteristics distinguish sweet corn from other types of corn, like for example pod corn (*Tunicate maize*). Pod corn is a morphological corn mutant where the mature kernels are not naked, as it is with other corn varieties, but covered by long glumes. Because kernels are entirely enclosed in long glumes they resemble most grasses, pod corn is widely regarded as a primitive form of cultivated maize (Mangelsdorf, 1986). This paper focuses only sweet corn as it is the type of corn that was genetically modified and is the most cultured variant in the US.

### 1.3 Corn Production and Utilization in USA

In 2020, the United States of America produced 357 million metric tons of corn, ranking to be the world's biggest producer, followed by China with 260 million tons. These two countries are considered super-producers of corn. In contrast, the European Union, in 4<sup>th</sup> place, produced “only” 67 million tons (USDA Foreign Agricultural Service, 2022). The top ten world corn producers are shown in **Table 1**.

Corn grown in U.S. contains about 72% starch, 10% protein, and 4% fat, with a combined energy density of 365Kcal/100g. It also provides many essential minerals and B vitamins, although it lacks some other nutrients like vitamin B12 and vitamin C. The use of maize in U.S. for the last 30 years was primarily for animal feeding, followed by human consumption and ethanol production (Ranum et al., 2014). Today in the US approximately 60% of all maize is used as livestock feed, and one can find more than 4,200 products containing maize in a typical supermarket. The more common products containing maize include soda, alcohol, paint, ink, batteries, toothpaste, aspirin, crayons, marshmallows, glue, and many others (Shultz et al., 2008).



## Chapter 2 – GM Corn

### 2.1 From Corn to GM Corn

Breeding of corn started with a various mixture of Indian varieties in which corn was adapted by mass hybridization and selection to be viable in a variety of climates, soils, and for specific uses. The next chapter in the story of corn began by the end of nineteenth century when details about meiosis, fertilization and angiosperm life cycle of the plant were revealed (Sprague,1988).

In 1908, the lack of rain and the need to grow corn in unfavorable soils led to an interesting discovery about corn cultivation. Strains that had been carefully cross-fertilized by hand yielded less grain than crosses between two unselected self-fertilized strains. It was simply because these strains first approached the pure homozygous state because of self-fertilization. As a result of this observation, it was concluded that a new method of separating and recombining definite pure lines may give results quite worth striving for (Shull, 1909).

Since the time corn has been domesticated it has become significantly different from wild corn to the extent that its survival is now dependent on humans. For example, in a field in which corn had been left to its own (corn was not deliberately re-planted), the neighboring soybeans took over the field the following year. The race for higher yield made the domesticated corn to lose its ability to scatter its seeds and therefore is

dependent on human planting. The wild corn plant either did not have this handicap or had some compensation for it (Weatherwax, 1954).

In the early 1980s scientists first began experimenting with genetic modification techniques, however, it was not until the 1990s that GM crops became commercially available (Zhang et al., 2016). GM corn was first achieved by a team of researchers led by Mary-Dell Chilton who introduced foreign genes into the corn using *Agrobacterium tumefaciens*. This process, described in 1991 on tobacco plant, uses Cre/lox recombination system as previously described by Dale (Dale, 1991) (**Figure 1**). The use of recombinase-based applications improved over time, and new advances and concepts emerged. For instance, recombinase can not only delete just the marker, but the recombinase gene itself, which is a very practical thing for plants that propagate asexually, and back-crossing might be a problem (Ow et al., 2007).

The first commercial GM corn was introduced in 1996 by biotech company Monsanto, and the corn was engineered to be resistant to the herbicide glyphosate and was seen as a breakthrough in agricultural technology (Coupe et al., 2016). At the time GM corn was met with raised concern from environmental and consumer groups who were concerned about possible negative effects on environment and human health. Despite the negative reactions from the public, GM corn became quickly popular with American farmers (**Figure 2**). Since the first commercially planted GM corn in 1996 steady increase in GM crops use in US was observed. In 2012, eighty-five percent of all corn grown in U.S was GM (Carzoli et al., 2018). USDA charts from 2021 (USDA.gov) state that up to 92% of all U.S. corn is genetically engineered (GE). In the U.S. corn yields increased substantially, due, in part to the introduction of GM varieties. In 1930

one hectare produced 1.26 metric tons of corn, in 1985 it grew to 7.41 tons, and in 2009 it was 10.29 tons. It is due both to plant breeding and improved field management practices which includes increase in plant density. GM corn performs better in unfavorable growing conditions and gives higher yield than non-GM hybrids (Chavas et al., 2014).

## 2.2 GM Corn Breeding Techniques

Because the most economically important traits in corn breeding are inherited quantitatively, the limitation of traditional methods of corn breeding lies in determination of the genetic worth of lines in hybrid combinations. Today corn breeding relies on understanding the molecular genetics of corn and identifying the molecular basis of desirable traits. Approaches to molecular breeding have included Quantitative Trait Locus (QTL) experiments (Carena et al., 2009).

QTL analysis is a statistical method that links phenotypic and genotypic data (**Figure 3**). To conduct QTL, a researcher needs at least two strains of an organism that differ genetically in the trait of interest, such as, the sweetness of the kernel. As it is with all statistical analyses, the sample size plays a critical factor and larger is always better. Genetic markers are needed in order to distinguish between different parental strains. Types of markers include SNPs (single nucleotide polymorphism), SSRs (simple sequence repeats), RFLPs (restriction fragment length polymorphism), and transposable element positions. During the QTL analysis parental strains are crossed, producing heterozygous (F1) individuals that are further crossed to produce offspring

(F2) with different phenotypic and genotypic traits. These traits are then scored resulting in trait of interest being segregated more frequently with trait values (e.g., kernel sweetness), and unlinked markers will not show significant association with the desired phenotype. Presently the phenotype could be for example the amount of RNA transcripts or amount of protein produced from a specific gene (Silver et al., 2001).

Techniques like QTL allow for identification of the genetic basis of naturally occurring traits for GM (genetically modified) corn containing agriculturally desirable traits. Other technological advances, such as microarrays (**Figure 4**) and qRT-PCR (**Figure 5**) enabled monitoring of transcription on a genome wide level (Dolezal et al., 2014). Microarrays are microscope slides with thousands of tiny spots in defined positions containing a known DNA sequence, sometimes referred to as DNA chips. Sample mRNA and reference mRNA are converted into florescent labeled cDNA's, mixed, and applied to the microarray slide. After the hybridization the microarray slide is scanned to measure expression of each gene. Samples where the reference sample has a higher expression than the experimental sample appears in different color and vice versa. Quantitative real time PCR (qRT-PCR) is often used to validate the microarray data (Luo et al., 2011).

One of the major public concerns with GM corn is that it could lead to unwanted inputs into non-GM corn via cross-fertilization, seed impurities, but also during seed mixing and sowing, which could lead to unpredictable changes in corn plant (Sanvido et al., 2007). These issues were partially solved by above-described techniques. For instance, these techniques were used for transformation of corn by Ow et al., where researchers incorporated a *cordapA* gene responsible for production of a seed-specific

enzyme dihydrodipicolinate synthase. To facilitate the transformation, the kanamycin resistance marker was used (npt11), and was subsequently removed by recombination (Ow et al., 2007). Removal of these markers is important step in GM corn creation because it is not introducing “garbage” DNA into the wildlife. Today the predominant traits in GM corn are herbicide tolerant (HT) traits, but recently there is a significant rise of stacked traits that combine multiple herbicide and insecticidal traits in a single corn plant (ISAAA, 2022). The long-term controversy and debate about GM crops are promoting technical innovation and the use of novel safety assessment approaches, such as profiling technologies to reduce potential risks of GM crops, for example seed impurities, but it has unintended effects which are described in this literature review.

### **Chapter 3 Corn and its Pesticides**

Traditional corn farming used moderate doses of pesticides, but with the introduction of GM corn the use of pesticides changed dramatically. Many negative effects on human health can be directly linked to pesticides and GM corn was designed to mimic or withstand pesticides. More than two decades after the introduction of GM corn virtually all large scale planted GM corn has been engineered to tolerate herbicides, thus accumulating herbicides to produce their own insecticide toxin (Bonny S. et al., 2015). In recent years GM herbicide and insect resistant corn variants have become the major type of corn grown in 26 countries, including the US, Brazil, and Argentina (USDA.gov, 2022). Interestingly, development of GM corn resistant to molds

has proven to be difficult because of lack of resistance phenotyping markers and insufficient understanding of host resistance mechanism (Ow, 2007).

Various pathologies in humans can develop after acute intoxication or repeated exposure to pesticides (including herbicides). These include cancer (Kachuri et al., 2021), infertility (Bretveld et al., 2008), diabetes (Juntarawijit et al., 2018), and neurodegenerative disfunctions such as Parkinson's disease (Van Maele-Farby et al., 2012). This literature review focuses mostly on Glyphosate because it is the most used herbicide for GM corn cultivation. Its popularity risen dramatically after the introduction of GM corn resistant to glyphosate, but recently it is combined with bellow mentioned herbicides due to newly developed glyphosate resistance in weed.

Pesticides, e.g., insecticides and herbicides, are often used in combination with other pesticides, and it makes the mixture far more toxic than when using only one type on its own. Tsatsakis et al., (2017) simulated real-life exposures and found out that the diseases can be markedly enhanced when different pesticides are combined (Tsatsakis et al., 2017). To provide a deeper understanding of toxicity, scientists need to take advantage of "omics" (metabolomics, proteomics, transcriptomics), because standard blood biochemistry is not sufficient to accurately indicate negative health impacts, especially in studies with short treatment period (< 90 days) or relatively low doses of pesticides (Mesnage et al., 2021). Another factor that can add to pesticides causing a serious disease is environment. There are many unaccounted environmental factors, including chemical pollutants that can negatively alter gene expression patterns. Pesticide compounds are poorly tested, and the gap exists between the introduction of new pesticides and the detection of its toxic effects on human population, which can be

undiscoverable until the effect is detected and inevitably embedded in future generations (Mesnage et al., 2021). GM corn could not thrive without pesticides, therefore further investigation is warranted into how pesticides affect the cellular makeup of the GM corn itself and how it can affect human health.

### **3.1 Insecticides**

Farmers rapidly adopted transgenic corn producing insecticidal toxins derived from *Bacillus thuringiensis* (Bt) discovered in the 1990s. Bt corn targets both lepidopteran (caterpillars) and coleopteran (beetles) insect pests (Naranjo, 2014). The main benefits of planting Bt corn is reduction of conventional pesticides and suppression of key insect pests, however the rapid adoption of Bt crops led to several cases of insect pests evolving Bt resistance, and therefore diminishing the benefits of Bt corn (Tabashnik & Carrière, 2017).

The first GM corn producing Bt toxin (Cry3bb1) was registered by US Environmental Protection Agency in 2003. Shortly thereafter three additional Bt toxins have been approved (mCry3A, eCry3.1Ab and Cry34/35Ab1) to manage Western corn rootworm (Gassmann et al., 2019). Western corn rootworm is a key pest of maize in the mid-western United States (Gray et al., 2009). This pest is univoltine, having only one generation per year. Over the winter the eggs in diapause are protected by the soil, ready to be hatched during the late spring to start feeding on corn roots (Meinke et al., 2009). Injury to the corn plant can be severe as larvae feeds on multiple nodes,

damaging 8 to 15 roots. This results in an average yield loss of 16% (Tinsley et al., 2013; Dun et al., 2010).

In 2017 Gassmann described a field that evolved resistance to CRY34/34Ab1 and other Bt traits by Western corn rootworm in transgenic corn. The study showed that corn pyramided with Cry34 and Cry35Ab1 sustained high levels of injury caused by Western corn rootworm. Additionally, these rootworm populations were found to be resistant to all other commercially available Bt toxins. These results suggest that resistance to Cry34/35Ab1 is becoming more prevalent in the agricultural landscape and is associated with resistance to all other commercially available Bt products. Furthermore, in one of the field populations a complete resistance to Cry34/35Ab1 was observed (Gassmann, 2017). Apart from eCry3.1Ab these three above mentioned traits were brought to market singly but were later pyramided to delay the evolution of resistance. Interestingly by the time the insects evolved a complete resistance to CryBb1 in the state of Iowa, the first rootworm pyramid (Cry3bb1 with Cry34/35Ab1) was brought to market (Zukoff et al., 2016; Schrader et al., 2016). This suggest that companies like Monsanto knew about the negative effects of their product and were prepared to introduce superior product when it was needed. These issues are common for all GM crops. Chen et al., (2013) noted an occurrence in China , where after a wide scale adoption of GM cotton (*Bacillus thuringiensis*), reduction in pesticide use caused an increase in population size of mirid bugs (*Heteroptera miridae*) to such a level that it gained a status of a pest in cotton. This shows that not only pest can develop resistance, but also that a secondary pests can quickly fill a gap after the elimination of major pests (Chen et al.,2013).



To delay the Bt resistance farmers are using the refuge strategy by planting non Bt host corn in conjunction with Bt corn. This is promoting survival of Bt susceptible insect that can mate with Bt resistant individuals producing heterozygous offspring. This strategy is expected to delay Bt resistance, especially in fields that are planted with corn producing high doses of Bt toxin (Tabashnik & Carrière, 2017). Another approach, known as pyramid strategy, utilizes multiple toxic traits in corn that target the same insect pest. As result, the individuals harboring resistance alleles for one toxin get killed by a second toxin in the pyramid (Tabashnik & Carrière, 2017; Gassmann & Owen, 2016). Also, field crop rotation was shown to be associated with decreased levels of Cry3Bb1 resistance compared to the field where corn was planted every year. It is because of dilution of resistance alleles from extinction and recolonization of fields by rootworm after crop rotation (Gassmann, 2017). The next generation of transgenic maize for corn rootworm management will use gene silencing in hope to solve the problem. This will be achieved by exposing pests to double-stranded RNA (Tabashnik & Carrière, 2017). The effects on the environment of this new technology will probably be seen after many years of use, but the previous experience shows us that nature always finds a way to sustain life, and even if the targeted pest is eliminated, another species quickly fills the gap.

## 3.2 Herbicides

### 3.2.1 Glyphosate

Glyphosate is a broad-spectrum herbicide capable of controlling various kinds of weeds (annual and perennial) after they emerge in the field (Duke et al., 1980). Glyphosate is the most used herbicide component globally, with more than 700 commercial formulations, and one of the most controversial pesticides for its toxicological capability (Mesnage & Antoniou, 2021). Herbicide products like glyphosate are commonly produced in combination with additives, inert ingredients, and surfactants, and the commercial products containing glyphosate ranging in concentrations up to 71% (Agency for toxic substances and disease registry, 2023). Glyphosate based herbicides increased in use approximately 100-fold since the 1970s, pushing other herbicides (such as alachlor and metolachlor) out of the market (Benbrook, 2016). Glyphosate was first discovered as a weed killer by Monsanto in 1970s and was available to purchase in 1974 (Duke et al., 2020).

Shortly after GM crops (including corn) were engineered to tolerate glyphosate, other herbicides lost their market share and further herbicide research was put on hold (Coupe et al., 2016). That changed rapidly as farmers overused this technology with little or no change to weed management, allowing widespread emergence of glyphosate resistant weeds (**Figure 6**). The farming biotech industry responded to this issue by creating GM crops that are tolerant for multiple herbicides which can lead to the evolution of generalized resistance in weeds (Comont et al., 2020). Glyphosate works

by inhibiting enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). This synthase in the Shikimate pathway is necessary for biosynthesis of aromatic amino acids in plants (**Figure 7**) and the plant is killed when this synthase is inhibited (Steinrücken et al., 1980, Funke et al., 2006). For a corn plant to be able to withstand this pesticide different genes have been introduced to obtain glyphosate resistance. According to ISAAA there are currently 148 glyphosate-tolerant corn genetic changes that have been approved for cultivation use, and direct food and feed processing (ISAAA, 2022). For instance, these genes are AM79 aroA (Ren et al., 2015), G2-EPSPS (Liu et al., 2015), gat4621 (Green et al., 2009), mepsps (Hernández et al., 2004), 2mepsps (Matthews et al., 2017), cp4epsps (Mesnage et al., 2016), and G10-EPSPS (Liu et al., 2018).

Toxicology of glyphosate remained out of focus for 30 years until a series of studies suggesting glyphosate is involved in carcinogenesis led to a public scandal (Mesnage et al., 2021). It is classified as a probable carcinogen by International Agency for Research on Cancer (IARC). Glyphosate carcinogenic properties in humans are still debated, but a large body of evidence exists to show it is causing cancer when high doses are administered to lab animals (Portier, 2020). Animals' bioassays significances are nonetheless doubted due to a large number of tests implying detecting carcinogenic effects by chance (Crump et al., 2020). A glyphosate dose of 10mg/kg body weight/day is negatively impacting mitochondrial activity and causing oxidative damage leading to chronic toxic effects on liver biochemistry in rats. In a dose of 50 mg/Kg body weight/day, maternal toxic exposure causes developmental effects in rabbits (Mesnage et al., 2015). Neurotic effects (Astiz et al., 2009), sex hormone disturbances (Avdatek et

al., 2018), and liver function alternation (Pandey et al., 2019) can be also caused by oxidative stress inducing properties of glyphosate. Although glyphosate is known to negatively impact mitochondria since 1970s (Olorunsogo et al., 1979), the exact mechanism is still not agreed upon. Some studies claim that glyphosate modulates complexes of mitochondria respiratory chain (Pereira et al., 2018; Olorunsogo, 1990), others made a link between increased production of oxidative reactive species and mitochondrial dysfunction caused by glyphosate (Bailey et al., 2018; Gomes & Juneau, 2016). Another notion that is not supported by evidence at the time is that glyphosate replaces glycine in protein polypeptide chain that leads to protein misfolding and impaired mitochondrial function (Mesnage & Antoniou, 2017). Some studies suggested that the oxidative stress is concomitant with DNA damage (Honk et al., 2018; Wozniak et al., 2018), and that administering antioxidant mitigates DNA damage caused glyphosate exposure (Santo et al., 2018).

Kwiatkowska et al. conducted a study in 2017 showing that glyphosate is altering patterns of gene expression by decreasing DNA methylation, which is correlated with increased TP53 (Tumor suppressor gene) promoter methylation, and suppression of TP53, and therefore constituting to an oncogenic step. In a follow-up study they additionally found that there are more changes of genes involved in the regulation of the cell cycle and apoptosis. Glyphosate is (via altered methylation) decreasing the expression of P16 and PT53 tumor suppressors while increasing the expression of BCL2, CCND1 and P21 proto-oncogenes. They also observed an increase in expression of CCND1, but its promoter DNA methylation status was not altered (Woźniak et al., 2020).

In a study conducted by Duforestel et al., (2019) a link between glyphosate-induced DNA methylation and oncogenesis has been described. Human non-neoplastic mammary epithelial cells (MCF10A) were cultured in presence of an extremely low concentration of glyphosate (10 pM) for 21 days, and as noted before in this literature review, the levels of DNA methylation decreased. This decrease seems to be mediated by TET3 activity. After transplanting cells treated with glyphosate, and breast cancer associated miR182-5 into a mouse, 50% of mice developed tumor, whereas none of the mouse without miR182-5 developed tumor, suggesting that epigenetic changes induced by glyphosate in MCF10A cells primed them for oncogenesis (Duforestel et al., 2019). Very high doses (350mg/kg bw/d) of glyphosate also reduce gene expression in mammary gland and increase expression in the uterus of Wistar rats (Gomez et al., 2019). DNA methylation in sperm is also altered by glyphosate, leading to transgenerational epigenetic effects. This was demonstrated by Kubsad et al., (2019) in mice, where F0 generations seemed unaffected, but F2 and F3 generations showed a significant increase in pathologies. For F2, researchers observed higher death rates for late stage pregnant F2 females and their pups, and for the F3 transgenerational offspring prostate, ovarian, kidney disease, and obesity dramatically increased (Kubsad et al., 2019).

Glyphosate can also be metabolized by gut microbiomes. Microorganisms degrade glyphosate by cleaving the C-P bond to phosphate and sarcosine, and/or to AMPA and glyoxylate. Some Proteobacteria in the human gut degrades glyphosate by the carbon-phosphorus lyase pathway (Mesnage & Antoniou, 2020). The above-mentioned metabolites are not known to have toxicological properties; however, it was

reported that in higher doses (magnitudes higher than today's exposure levels) glyphosate degradation to glyoxylate increase liver triglyceride and cholesterol ester levels (Ford et al., 2017).

The Shikimate pathway, the mechanism of action of glyphosate, is not present directly in mammalian cells, but it is present in microorganism inhabiting human digestive track, and most bacteria found in human gut is predicted to possess EPSPS enzymes which can be inhibited by glyphosate (Mesnage et al., 2021). Regulating immune function and fermentation of indigestible substances is one of the important roles of human gut microbiota. Alteration of gut microbiome composition and/or function have been linked to development of diseases affecting digestive tract, but also affecting distant organs such as the brain (Martin et al.,2018). For rat gut microbiomes EPSPS gets inhibited in supposedly safe glyphosate dose (0.5 mg/kg bw/d) causing accumulation of metabolites upstream of EPSPS catalyzed reaction of the Shikimate pathway and increase the level of dipeptides involved in regulation of redox reaction that in turn produces reactive oxygen species (Mesnage et al.,2020).

Since the introduction of transgenic corn expressing glyphosate tolerant genes, significant pressure was placed on weeds by frequent and heavy use glyphosate and the lack of weed management diversification. Due to this practice, there are currently 56 known species of glyphosate-resistant weeds around the globe. They include *Amaranthus palmeri*, *Conyza bonariensis*, *Cynodon hirusutus*, *Digitaria insularis*, *Echinochloa colona*, *Elueisine indica*, and many others (Heap, 2022). Therefore, the GM corn tolerant to glyphosate may cause irreversible changes to the surrounding

biosystems, alternations in gut microbiome, and gene expression changes in many organs.

### 3.2.2 Glufosinate

Because of the developed weed resistance on glyphosate other herbicides must be used to effectively kill the weeds. Glufosinate-ammonium (GLA), commonly known as Basta, is a non-selective herbicide. The name comes from bacterial species *Streptomyces hygroscopicus* that reside in soil produces amino acid called Glufosinate. Since its discovery in the 1960s it was used as herbicide active ingredient commonly known as ammonium salt. GM corn tolerant to glufosinate was first commercialized in 1997 (Mesnage et al., 2021). It works by inhibiting catalyzing enzyme glutamine synthetase (GS). GS is responsible for catalyzing glutamine synthesis from glutamate and ammonia, leading to a fast augmentation of ammonia and subsequently to the plant death (Darrow et. Al, 1980) (Liu et al., 2019).

Two genes are used in the development of transgenic corn tolerant to glufosinate. It is *bar* from *Streptomyces hygroscopicus* (Thompson et al. 1987) and *pat* from *Streptomyces viridochromogenes* (Wohlleben et al., 1988). Both genes encode for phosphinotricin acetyltransferase (PAT) which is responsible for detoxifying phosphinotricin (active ingredient in glufosinate) (Milcamps et al., 2009). Ammonia metabolism in plants is shattered by glufosinate (structural analogue of glutamate) by disrupting glutamine synthase (GS) and therefore glutamate synthesis (Lacuesta et al., 1989). Fortunately for mammals, GS in brain operates in excess capacity (far from its

limiting turnover) in a brain, and even with a dose of glufosinate that inhibited the activity of GS in rat brain by 40%, the ammonia concentrations remained unchanged (Hack et al.,1994).

Even though glufosinate does not alter mammalian ammonia homeostasis, it can disrupt both inhibitory and excitatory neurotransmission (Hack et al.,1994).

Neurogenesis disruption was also reported by Calas et al., where they intraperitoneally gave mice 2.5 – 10 mg/kg of glufosinate 3x a week, for 10 weeks, and discovered dose-dependent structural changes in the somatosensorial cortex and in the hippocampus (Calas et al., 2008). Glufosinate, a structural analogue of glutamate, binds to NMDA excitatory receptors that in turn increases the production of nitric oxide (Lantz et al.,2014). Disturbances associated with autistic symptoms were revealed by Herzine et al. where brain transcriptomic data showed altered expression of mice genes regulating cell proliferation and migration after administering sub regulatory doses (0.2 – 1 mg/kg bw/d) of glufosinate-ammonium (Herzine et al.,2016). Reduced serum retinol levels, typical in children with autistic spectrum disorder, were inhibited in glufosinate-exposed animals through an alternation of gut microbial metabolism (Iyer & Vaishnava, 2019).

Because glufosinate was not used as extensively as glyphosate for corn weed control, although it was used for over 30 years, no reports exist of weeds evolving resistance to glutamine synthase (Yu, 2023). Glufosinate use is on the rise, and therefore we should expect to see weeds evolving resistance to it, as it was with glyphosate. Unfortunately, we may not be able to uncover negative effects of glufosinate any time soon, because human brain structural changes are hard to uncover.



### 3.2.3 Dicamba

Dicamba, a benzoic acid, is a selective post emergent broad leaf herbicide, and is often mixed with 2,4-D. Dicamba resistant GM corn is carrying the *dmo* genes from soil bacteria that are encoding for mono-oxygenase enzyme responsible for converting dicamba (3,6-dichloro-o-anisic acid) into 3,6-dichloro-salicyc acid (Green & Owen, 2011). Dicamba acts by interfering with plant phytohormone response by mimicking natural auxin indole-3-acetic acid (IAA) action, which in turn inhibits plant growth due to an increase in ethylene and abscisic acid levels (Cernato et al., 2015). IAA is not known to have effect on the auxin pathway in mammalian cells, but through a different mechanism (tryptophan metabolism making IAA into uremic toxin) it damages the cell membrane, disrupts acetyl coenzyme A metabolism, and uncouples oxidative phosphorylation (Bradberry et al., 2004). Even though dicamba was not found to be carcinogenic or neurotic (Mullins et al., 2022), transgenic corn was modified to demethylate dicamba and therefore produce DCGA (3,6-dichlorosalicylic acid), a metabolite that may be more toxic than dicamba (Mesnage et al., 2021). Dicamba use decreased after the introduction of glyphosate tolerant GM corn, but its use is on the rise since 2010 after the spread of glyphosate resistant weeds (Mesnage et al. 2021). Because dicamba was not extensively used it steered from scientific focus, but hopefully more research is going to be conducted on its transgenic corn effects on human health.

### 3.2.4 2,4-Dichlorophenoxyacetic acid (2,4-D)

Like dicamba, 2,4-D is a synthetic auxin mimicking IAA activity (inhibits the plant growth), but it can also alter the actin structure in plant cells, influencing corn growth and development (Takahashi et al., 2017). 2,4-D was introduced by the American Chemical Paint Company in 1945 and today there are more than 600 products on the market (Loomis et al., 2017). Its status became controversial after its use by Americans in the Vietnam war where it caused serious health and birth effects when mixed with picloram (Agent White) and 2,4,5-trichlorophenoxyacetic acid (Agent Orange) (Holt et al., 2010).

In a rat chronic toxicity study, that administered a dose of 0.005 mg/kg bw/d, it showed decreased T4, glucose, cholesterol and triglycerides, and decreased body weight in females associated with decreased food consumption (Kobal et al., 2000). 2,4-D disrupts activity of growth hormones in plants, and its potential disruption of endocrine and reproductive toxicity in non-target organisms still has not been answered due to mixed outcomes of conducted studies (Mesnage et al., 2021). However, a study among 35,150 male and female pesticide applicators showed thyroid disruptive effects including increased hypothyroidism (Shrestha et al., 2018).

There is also a strong mechanistical evidence of 2,4-D to induce oxidative stress which was demonstrated by Leero et al. where they found high 2,4-D in urine of Iowa farmers which was associated with elevated 8-hydroxy-2'-deoxyguanosine (a marker of oxidative damage to DNA) (Leero et al., 2017). In gut microbes 2,4-D mimics the above-mentioned IAA, but also decreases acylcarnitine in plasma and changes composition of

function and composition of gut microbiome in mice (Tu et al., 2019). Even though the health effects of 2,4-D are still debated, the fact it was used in biological weapons is enough to cause concern. It was not right to spray this chemical on people, and maybe it's time to learn not to spray it on crops used for food and feeds.

### **3.2.5 Sulfonylurea**

Sulfonylureas, like nicosulfuron and rimsulfuron, have been extensively used since 1970 on corn, and their main target is acetolactase synthase (ALS). ALS is an enzyme used for the biosynthesis of branched-chain amino acids in plants, and because of its presence in some microorganisms it gives sulfonylureas potent antifungal activity (Lee et al., 2013). Sulfonylureas are deemed as easily biodegradable; however, they commonly contaminate not only food but also water. They are considered somewhat poorly toxic due to ALS enzyme nonexistence in mammals (EFSA, 2008). However, research concluded that exposure to sulfonylureas increases the risk of rheumatoid arthritis (Meyer et al., 2017), increased risk of bladder cancer (Koutros et al., 2015, Vineis & Piratsis, 1998), and higher frequency of first-trimester miscarriages (Garry et al., 2002). Unfortunately, ALS-inhibition sensitive bacterial communities exist in human and animal gastrointestinal tracts, and recent discoveries of health impacts of gut microbiome suggests that the true effects of ALS-inhibiting herbicides are not fully understood (Mesnage et al., 2021).

## Chapter 4 GM Corn Today

As mentioned above, weed and pest resistance caused by GM corn is a significant problem for farmers. This problem was already “solved” by international biotech companies like Monsanto by stacking genes in GM corn. This makes GM corn resistant to multiple pesticides at once. For example, Monsanto developed corn tolerant to 4 herbicides by expressing *pat*, *dmo*, *ft\_t*, and *cp4 epsp genes*. The new corn variety MON87429 contains a single insert consisting of one copy of the *pat*, *dmo*, *ft\_t* and *cp4 epsps* expression cassette. In 2022 EFSA (The European Food Safety Authority) released an assessment of genetically modified corn MON 87429, which was used to gather the following data. MON 87429 was developed by *Agrobacterium tumefaciens*, and confers the tolerance to dicamba, glufosinate, glyphosate, quizalofop and 2,4-D by adding four genes (*pat*, *dmo*, *ft\_t* and *cp4*). The four cassettes include:

- A) The *pat* expression cassette consists of 5' UTR and intron sequence from ubiquitin gene (Ubq) from *Erianthus ravennae*, the *pat* coding sequence of the phosphinothricin N-acetyltransferase and the 3'UTR sequence of the fructose-biphosphate aldolase (Fba) gene of *Setaria italica*.
- B) The *dmo* expression cassette consists of 5'UTR and intron sequence of ubiquitin gene (Ubq) from *Coix lacryma-jobi*, the codon optimized sequence of the chloroplast transit peptide of the Albino and pale green 6 (Apg6) gene of *Arabidopsis thaliana*, codon optimized coding sequence of the dicamba

- monooxygenase (DMO) gene of *Stenotrophomonas maltophilia*, 3' untranslated sequence of the OsMt gene of *Oryza sativa*.
- C) The ft\_t expression cassette consists of the promoter, 5' untranslated and intron sequences of ubiquitin gene (Ubq) of *Arundo donax*, transit peptide containing sequence of Mdh gene of *Arabidopsis thaliana*, the R-2,4-dichlorophenopropionate dioxygenase (RdpA) coding sequence of *Sphingobium herbicidovorans* that expresses the dioxygenase protein (FT\_T) than confers tolerance to both FOP's (aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase inhibitors) and 2,4-D herbicides, and 3' untranslated region of the apical meristem (Nam) gene of *Oryza sativa*.
- D) The cp4 epsps expression cassette consists of a promoter and leader sequences of the 35 S RNA of cauliflower mosaic virus (CaMV), 5'UTR sequence of the chlorophyll a/b-binding (CAB) gene of *Triticum aestivum*, intron and flanking UTR sequence of the act1 gene of *Oryza sativa*, the chloroplast transit peptide containing sequence of the ShkG gene of *Arabidopsis thaliana*, the codon optimized codon sequence of the aroA gene of the *Agrobacterium s.* strain CP4 that encodes for EPSPS protein, modified partial 3' UTR sequence of *Zea mays* cDNA (EU974548) that contains male tissue specific siRNA target sequence and the 3' UTR sequence of glycine-rich RNA binding protein (*Grp3*) gene of *Oryza sativa*.

The EU safety panel identified a significant difference for levels of phytic acid in grain (0.58 for non-GMO, 0.63 for GMO). Phytic acid is the primary storage form of phosphorus in the seeds protecting it from oxidative stress, and as much as 75% of total

phosphorus in corn is bound to phytic acid (D'Ordine et al.,2003). But because conventional food and feeds do not contain enzymes with high sequence homology to *dmo* proteins, and the *dmo* protein amino acid sequence differs from the wild type *S. mathophilia*, it was not possible for the panel to confirm a documented history of safe consumption of the *dmo* proteins. I must note that not having enough data did not stop this panel from approving the product, and it may be one of the reasons why Mexico is considering the ban on US GM corn imports.

The following study conducted by Monsanto was enough for the panel to assess the safety of the new GM corn. A dose toxicity study was conducted on 40 mice for 28 days. Animals were singly housed, and feed with a substance containing about 96% *dmo* protein. Even though two male mice died, the panel did not consider these deaths to be related to the treatment with *dmo* protein. Other significant findings that were considered safe are increased body weight in males, decrease in body weight in females, reduced food consumption for all mice, basophil count reduced by 50%, and increased cholesterol. All these were deemed as being no adverse effect of the treatment. The panel also stated that humans can be exposed to the newly expressed proteins via pollen (with the exception of CP4 EPSPS), but the exposure could not be estimated because of no data for presence of expressed proteins were available. The panel also concluded that the accidental release of viable GM corn into the environment possess no safety concerns, and there is a very low likelihood of environmental effects. Overall, the panel concluded that GM corn is safe and beneficial and that it will not alter the surrounding environment but given the experience with glyphosate resistant corn many scientists may disagree with that conclusion.

## Chapter 5 GM Corn is Beneficial

Whether the farmer uses GM corn with stacked traits or not, the important factor that makes GM corn beneficial for farmers is bigger yield, or lower yield risk. Advances in seed breeding and genetic modification of corn traits hold the conventional wisdom of lowering yield risk, but some recent research argues that the converse is true (Goodwin & Piggott, 2020). It is a controversial topic because yield risk depends on many variables and the opposing sides often use different approaches to identify the risks.

For instance, Aglasan (2022) assessed the yield risk effects of GM corn with rootworm resistant traits (GM-RW) in 2021. Yield risk was measured using a loss cost ratio (LCR) (a variable taken from crop insurance), and Cluster-Lasso procedure to address the high-dimensional nature of the weather. The LCR is used by insurance companies to access premiums for farmers. Cluster-Lasso procedure was used because the researchers were unsure about the exact set of control variables (weather). The results were consistent with other research papers (Shi et al., 2013; Goodwin & Piggott, 2020) and showed that GM-RW corn reduces yield risk by 1% which is considered a significant risk reducing effect (Aglasan, 2022). Also, Goodwin & Piggott, (2020) compared two major US droughts that occurred in 1988 (non-GM corn) and 2012 (91% GM corn). Weather conditions were comparable in terms of total precipitation, and average and maximum temperatures. The results showed that the yield losses were significantly lower in 2012 as opposed to 1988 (Goodwin & Piggott, 2020). The paper, however, did not account for difference in increased corn planting density, or for enhancements in irrigation and farming technology.

Completely opposite opinion on corn yield risk was presented by Lobell et al., (2014) where they used single dataset from randomly sampled unit level crop insurance records for Indiana, Iowa, and Illinois and concluded that due to increase in planting density of GM corn, corn yields have become more sensitive to environmental stresses (Lobell et al., 2014). The yield risk is important for farmers that plant GM corn because they are having lower insurance premiums as opposed to farmers that use traditional corn. Difference in insurance premiums for farmers growing GM corn is about 20% as opposed to the farmer that grows non-GM corn (Goodwin & Piggott, 2020). It is surprising that for 1% lower yield risk farmers get 20% discount on insurance premiums. This can partially explain the relatively quick development of GM corn in the USA and why it was so rapidly adopted by US farmers.

Another factor that farmers use to see the benefits of GM corn is increased profits. Ala-Kokko et al., (2021) published a study about economic and ecosystem impacts of GM maize in South Africa. The typically produced corn for direct human consumption is white GM corn in South Africa as opposed to yellow GM corn in US. As described above, it is mostly due to social stigma around yellow corn in Africa. In 2001, three years after the commercial adoption of Bt yellow corn, Bt white corn made South Africa the first GM subsistence producer in the world. This was followed by adoption of Ht white corn in 2004 and stacked traits white corn on 2008 (Gouse et al., 2016). Ala-Kokko estimates that adoption of white GM corn in South Africa resulted in welfare benefits of \$694.7 million in total between 2001 and 2018 and improved local and regional food security. To further show the benefits of GM corn Ala-Kokko uses is the use of pesticides, where NON-GM corn uses seven different herbicides, while GM corn



uses only glyphosate. That was a surprising fact because in 2012 (this study is from 2021) it was starting to be apparent that using only glyphosate in fields leads to weed resistance and it is sad to see that in South Africa they are repeating the same mistake as did US farmers. It is because the total cost difference of production 1 ha of conventional corn is \$9.11 vs \$8.77 for GM corn (Ala-Kokko, 2021). This data was calculated by using environmental impact scores, with numbers that were not fully explained by the author. For example, the data supposedly explaining the total cost difference, the Ozone layer depletion for 1 ha is valued at \$0.0019 for NON-GM and \$0.0011 for GM corn, but no explanation is given on how this was calculated. It seems very strange that the author put a price on ozone layer depletion and includes no explanation on how this was calculated.

In summary it is obvious that GM corn is more profitable than traditional corn, but it is hard to estimate how much increasing planting density contributed to the higher profits. Ala-Kokko also mentioned the use of a single herbicide as another factor that increases profits, but some scientists now claim it is not the case anymore. Due to the evolution of weed resistance to glyphosate, caused by intensive and prolonged use of glyphosate in farm weed management, significant economic benefits are diminished in GM corn tolerant to glyphosate (Yu et al., 2023).

In 2014 U.S. Government published a study about "Perspectives on transgenic herbicide-resistant crops in the United States almost 20 years after introduction" conducted by USDA researchers under Stephen O Duke. Duke et al. analyzed the state and potential future of glyphosate resistant crops (GRC's). This technology has increased yield due to improved weed management, but also decreased cost of weed

management. Another benefit of GRC's is according to Duke the time saving that allowed some small farmers to have a second job. Unfortunately, continuous extensive use of glyphosate in GM fields facilitated the evolution of glyphosate resistant weeds. This is reducing the environmental benefits of GM crops (including GM corn) due to increased tillage and use of other herbicides mixed with glyphosate (Duke, 2014). "It was too good to last" is the quick description Duke used to describe the state of the GRC technology. Complete and simplified weed control was so beneficial to farmers that warning of impending resistance led to little or no effect on their weed management practices (Duke, 2014). Duke, representing the government, is trying to get rid of responsibility and instead blame farmers for not listening to the warnings. Duke is staying away from the fact that it was the government who approved this technology for field use, and instead blames poor stewardship for the technology clear and painful failure. For the negative impact of GRC technology other than weed resistance Duke states there are "relatively few" papers that reported reduction in crop yields, increased crop disease, altered soil microbiota, and altered mineral nutrition in CRCs, and that USDA Economic Research Service does not support any of these claims (Duke et al., 2014). Continuous extensive use of glyphosate in GM corn fields facilitated the evolution of glyphosate resistant weeds. This is reducing the environmental benefits of GM corn due to increased tillage and use of other herbicides mixed with glyphosate (Duke, 2014). It is in my opinion tragic that in 2021 South Africa was still using only glyphosate and did not switch to multiple traits for their white corn. By using multiple traits, they would prevent, or delay glyphosate weed resistance.

## Chapter 6 GM Corn is Healthy

Apart from pesticides and yield benefits, GM corn when consumed is beneficial for human health, or is it not? Can biotech companies be trusted to thoroughly test the health effects of GM corn? Answer may lie in analysis of three animal feeding studies of GM corn varieties conducted by Monsanto. Studies were analyzed by Vendômois et al., (2009) and revealed sex and dose dependent side effects linked to the consumption of GM corn.

Three main commercialized GM maize; NK603, MON 810, and MON 863 were evaluated due to their presence in food and feeds around the world. Mon 810 and MON 863 were designed to synthesize two different BT toxins used as insecticides, while NK603 has been modified to contain residues of the formulation of Roundup (to be resistant to this broad-spectrum herbicide). Four hundred 4 to 6 week old male and female rats were used as a model animal, and approximately 60 different biochemical parameters were measured and compared to a control group after 5 and 14 weeks of feeding food containing 11% and 33% of GM crops. Because only two periods and doses were used it is difficult to evaluate dose and cumulative effects. Also, due unnecessary sources of variability due to non-GM maize type used, the number of rats fed by non-GM diet was 320 vs 80 fed with GM diet per transformation event, showing considerable unbalances in the experimental design.

In addition, there is no data showing that the controls and reference groups feed was free of GM crops. Vendômois repeated the statistical analysis conducted by Monsanto and then employed their statistical methods (Benjamini and Yekutiely) which

showed that the interpretation and the level of precision of the main effects are highly different. For rats fed by NK 603 the main difference was observed in male kidney parameters that showed 11% weight increase and an increase of ion concentration in urine. Variations in females was far less frequent without any significant differences (except for urine phosphorus and blood potassium). Rats fed by MON 810 showed significant effects in females, highlighting the sex-detrimental effects. While significantly disrupted parameters in males are concentrated in liver function, females are affected in blood cells, adrenal gland, spleen, and kidney weight, and also increase of nitrogen in blood. MON 863 showed a male-specific effect in kidney function, and caused serum glucose, triglyceride increase in females. Almost all GM-fed groups showed tendency for physiological disturbance with physio-pathological differences according to dose or sex. In summary statistically significant effects and signs of toxicity were observed for all three GM maize varieties, and most numerous effects are observed in organs involved in detoxification.

Proof of toxicity was not obtained for following reasons. The statistical power of the tests conducted by Monsanto is low (30%) because of the experimental design. Only one mammalian species was used, and the feeding trials were conducted only once with the length of feeding to be only 3 months, thus only acute and medium-term effects could be observed. Long-term feeding experiments up to two years are needed and clearly justified due to a little chance that above describe effects were random or chance of occurrence. The conducted studies also did not include cancer, hormonal, or hepatic function markers like for example oncogene expression, sex steroid hormones, cytochrome p450 levels, and others). The lack of availability of this data type may

benefit the doubt of observed toxicity. Vendômois recommends that for each GM crop additional long term multi-generational studies should be performed in at least three different animal species to obtain scientifically valid data on the acute and chronic effects of GM crops. Unfortunately, analytical methods for herbicide toxicology are not sensitive enough to detect the ability of ingredients to promote chronic diseases and current animal model use is not suitable to detect hormonal or metabolic disturbances (Patisaul et al., 2018). Therefore, this literature review has to conclude that GM corn is as healthy as is the traditional corn, but if we let the companies that create the GM corn also evaluate its health risks, we may never find anything alarming.

## Conclusion

In the US, government agencies agree with biotech companies' conclusions and push their product to the market without the approval of the public (**Picture 2**). It may be the standard approach in many countries, but there are more countries like Mexico who stand strong backing its citizens opinions. In 2010 Aerni published a study about Swiss consumers and their freedom of choice. In this study he described how in 2005 the Swiss expressed their negative attitude towards GM crops which resulted in national referendum that banned GMOs from Swiss agriculture for 5 years together with other laws on genetic engineering in agriculture, which are widely regarded as being one of the most restrictive in the world (Einsele, 2007). Aerni surveyed the Swiss consumers and discovered that they treat GM foods just like other novel food, and that it was the lack of transparency a freedom of choice that consumers worried in connection of GM

food. The manufacturers don't want to clearly label their products as GM food because some consumers are willing to pay premium to avoid GM products, which creates the above-mentioned lack of transparency. The GM products for sale in Switzerland had no negative reactions when transparent, offered freedom of choice and the product was not associated with industrial food production (Aerni, 2011). The study offers an opinion that it might not be mistrust in GM products as much as mistrust for international food companies creating a product that would be benefiting the consumer rather than the company. Interestingly the US did not pressure Switzerland to accept GM corn as they pressured Mexico, as the Swiss corn market is probably not as important for the US.

Another problem with GM corn can be its verifiability. In other words, how can one be sure that the product they buy as non-GM is truly free of GM material, or that the GM product one buys contains only approved target genetic element. Fortunately, the GMOMETHODS database offers assays for detection of GMO or target genetic element. It was created under European Reference Laboratories for Genetically Modified Food and Feed (EURL GMFF) who is responsible for "providing national reference laboratories with details and guidance on the methods of laboratory analysis, testing or diagnosis, including reference methods". The GMOMETHODS web application offers *in-silico* e-PCR predictors of the GM events making legislative and inspection control more efficient, and it will be a useful tool in the future for GM corn control. This will in turn increase the transparency of GM corn and make it more appealing to consumers.

However, it will not tell the consumer if GM corn is good or bad and how the use of pesticides may alter human health. This is a very complex question that depends on

various factors, including environmental factors, economic factors, and also the perspective from which it is viewed. Some people view GM corn as positive development that can help humanity combat global warming, others see it as threat to biodiversity and natural environment. The scientific literature overall found GM corn to be safe for human consumption and the environment, but many of the studies are founded by biotech companies and the results may be trimmed to desired outcomes.

GM corn has increased resistance to pests and herbicides, has higher yields and can withstand more weather extremes than traditional corn, having a positive impact on agriculture and food security. However, in recent years there is a rising number of studies that voice concern about potential negative long-term impacts of GM corn, such as herbicide weed resistance, impacts on non-target species, gene flow to wild relatives, and long-term consequences of relying on limited number of varieties that are created by a handful of international companies. The GM corn pesticide resistance is often portrayed as positive, but the overuse of pesticides in GM corn can have serious impacts on environment and human health. There may be a link between the pesticides used in GM corn fields and the rising health issues like cancer, autism, and diabetes.

To answer the question whether the US is right to push aggressively other countries to buy their “superior” seeds and products, or if there is a reason why countries like Mexico should be cautious about GM corn, this literature review must answer that the recently acquired resistance of pests and weeds is valid a reason to be cautious. Nowadays GM corn manufacturers must alter multiple genetic traits in GM corn because single ones are not efficient anymore. Because this is a novel approach there are no studies that would research the combined effects of above-mentioned

pesticides on human health. Due to newly acquired weed and pests' resistances to pesticides, it is possible that in the future GM corn farmers will need to buy new seeds every year to combat the acquired resistance caused by seeds they both previous years. It is also almost impossible for farmers that cultivate GM corn to switch back to traditional corn, because of the newly acquired weed and pest resistances. This may lead to an end of a traditional farming and complete dominance of handful of international companies (like Monsanto) over the agriculture, something Mexico is right to oppose in order to keep its agriculture from dependence on technologically advanced nations.



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## Figures, Pictures, and Tables

**Table 1 Corn Production in 2020, Top 10 Producers**

Country/region	Maize production in 2020 (million MT/year)
World	1,129.29
United States	358.45
China	260.67
Brazil	87
EU	67.44
Argentina	52
Ukraine	30.3
India	31.65
Mexico	27.35
South Africa	16.65
Russia	13.87

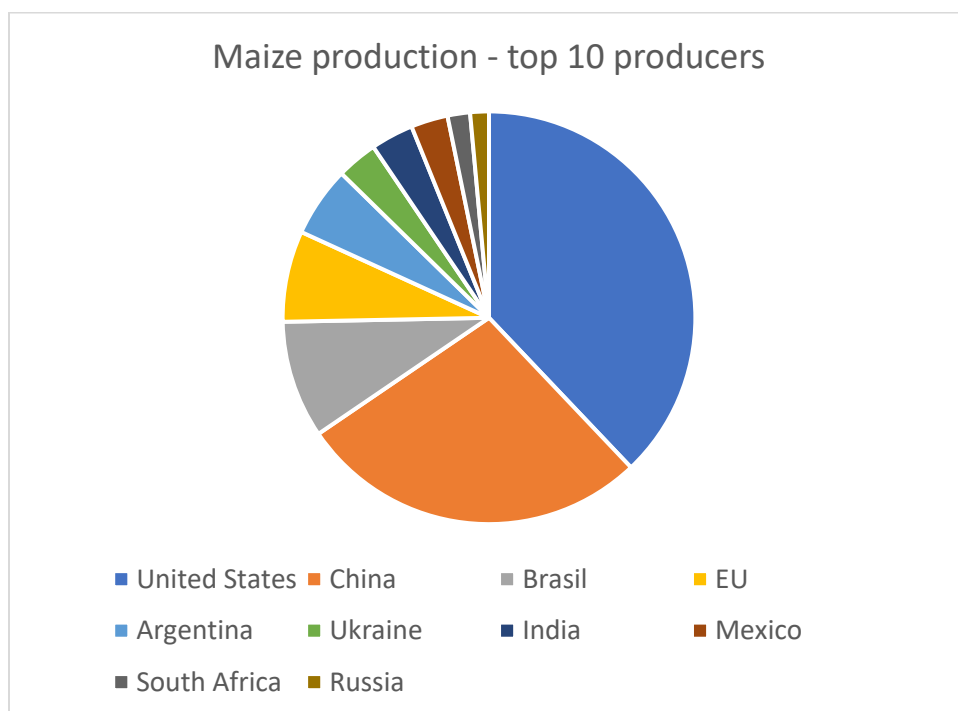


Table 1 shows top 10 world corn producers with two super producers leading the chart.

USDA Foreign Agricultural Service. (n.d.). Retrieved November 22, 2022, from <https://www.fas.usda.gov/data>

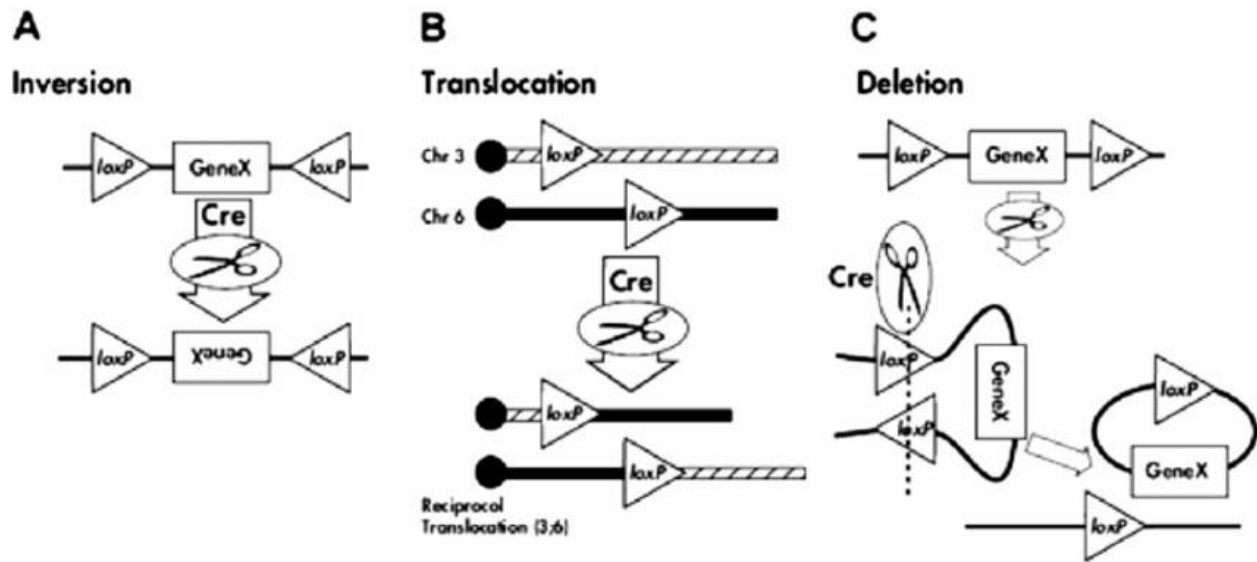
## Picture 1 – Corn Varieties



Picture 1 – Corn varieties showing phenotypic differences of non-commercial varieties. The maize grown in USA is mainly yellow.

Library, P. M. P. (n.d.). *Maize varieties - stock image - H110/3220*. Science Photo Library. Retrieved November 13, 2022, from <https://www.sciencephoto.com/media/218877/view/maize-varieties>

**Figure 1 – Cre-lox recombination**



A) If the sites are oriented in opposite directions, Cre recombinase mediates the inversion of the floxed segment.

B) If the sites are located on different chromosomes (trans arrangement), Cre recombinase mediates a chromosomal translocation.

C) If the sites are oriented in the same direction on a chromosome segment (cis arrangement), Cre recombinase mediates a deletion of the floxed segment

*The outcome of a CRE-LOX recombination is determined by the orientation ...* (n.d.). Retrieved April 10, 2023, from [https://www.researchgate.net/figure/The-outcome-of-a-Cre-lox-recombination-is-determined-by-the-orientation-and-location-of\\_fig1\\_50907191](https://www.researchgate.net/figure/The-outcome-of-a-Cre-lox-recombination-is-determined-by-the-orientation-and-location-of_fig1_50907191)

Figure 2 – Genetically Engineered Crops in the USA

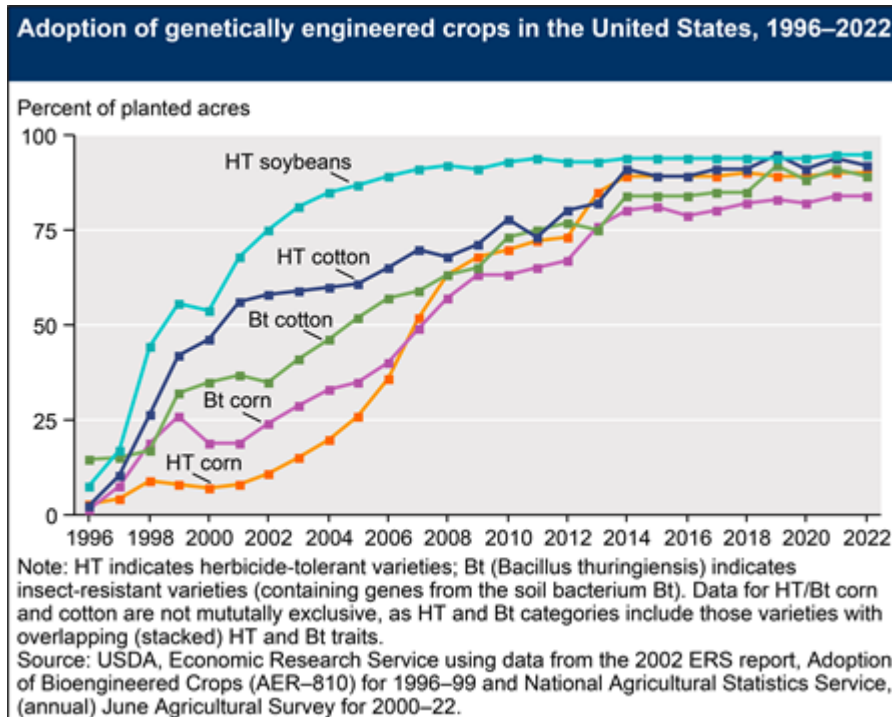
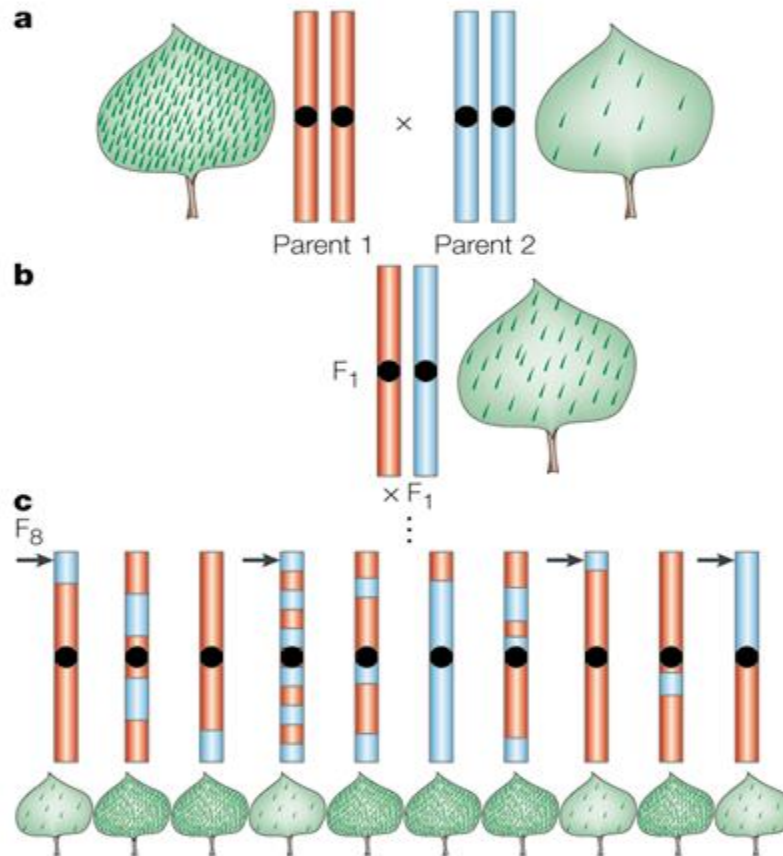


Figure 2 is showing increase of use of genetically modified crops in USA over time. Today over 92% of maize produced in USA is genetically engineered.

*Recent trends in GE Adoption.* USDA ERS - Recent Trends in GE Adoption. (n.d.). Retrieved November 13, 2022, from <https://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-u-s/recent-trends-in-ge-adoption/>



**Figure 3 - Principles of mapping quantitative trait loci**



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Figure 3 illustrates the basic strategy behind mapping quantitative trait loci (QTL).

- The difference in density of hairs that occur on a plant leaf.
- An F<sub>1</sub> individual is self-fertilized to form a population of F<sub>2</sub> individuals.
- Each F<sub>2</sub> is self-fertilized for six additional generations, ultimately forming several recombinant inbred lines, and is scored for several genetic markers. The leaves of all individuals that have inherited desired section of chromosome are marked with an arrow.

Mauricio, R. (n.d.). *Mapping quantitative trait loci in plants: Uses and caveats for Evolutionary Biology*. Nature News. Retrieved April 10, 2023, from <https://www.nature.com/articles/35072085>

**Figure 4 – Microarray analysis process**

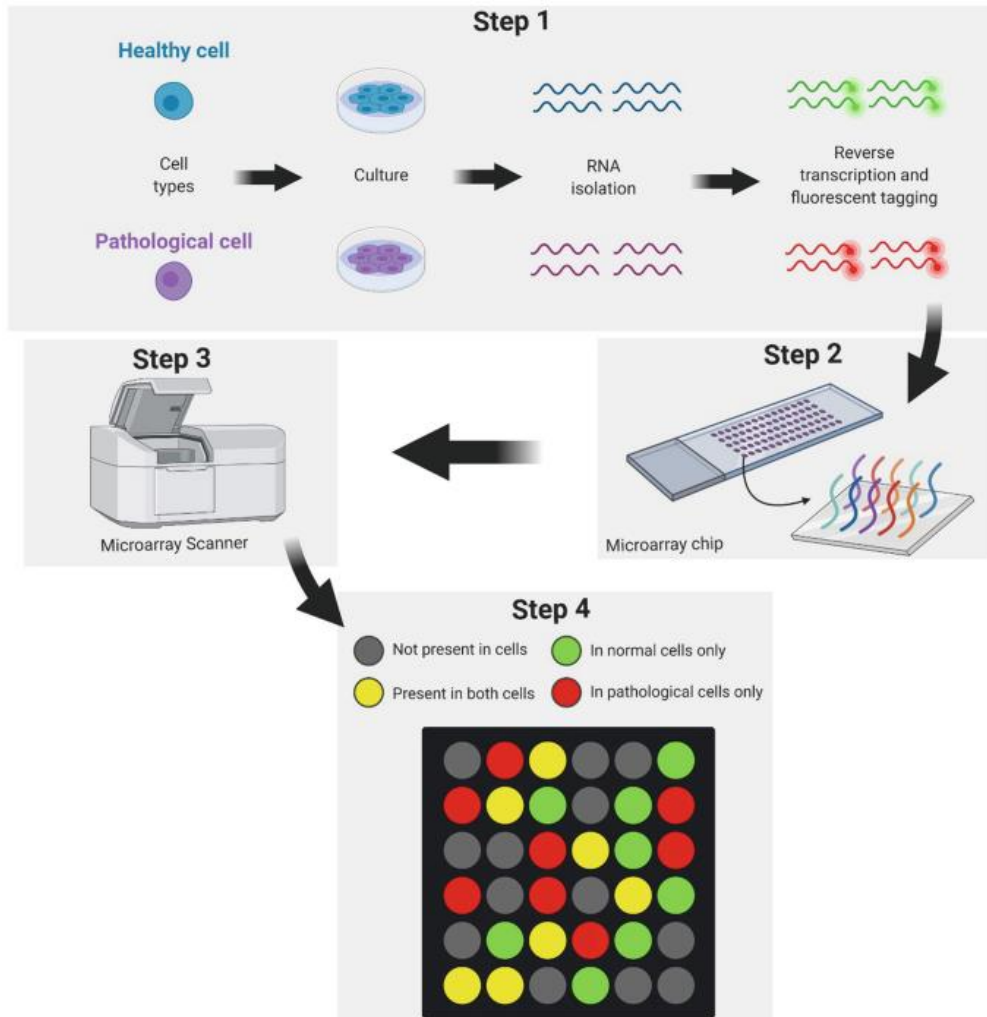


Figure 4 shows analysis processes used in microarrays.

Step 1) cell culture, RNA extraction, RT-PCR of the RNA, and cDNA fluorescence tagging.

Step 2) Adding the cDNAs into the microarray chip. The cDNA will hybridize in the matching single-strand DNA attached to the chip.

Step 3) The microarray chip is taken into a microarray scanner to read the fluorescent tags in each spot.

Step 4) Data analysis. Each color in the array corresponds to the cDNA from a specific organism.

de Travençolo, R. F., Ferreira, V. G., Federici, M. T., de Lemos, E. G. M., & Carrilho, E. (1970, January 1). *Microarrays application in life sciences: The beginning of the revolution*. SpringerLink. Retrieved April 10, 2023, from [https://link.springer.com/chapter/10.1007/978-3-030-82381-8\\_25](https://link.springer.com/chapter/10.1007/978-3-030-82381-8_25)

**Figure 5 – Principle of qRT-PCR**

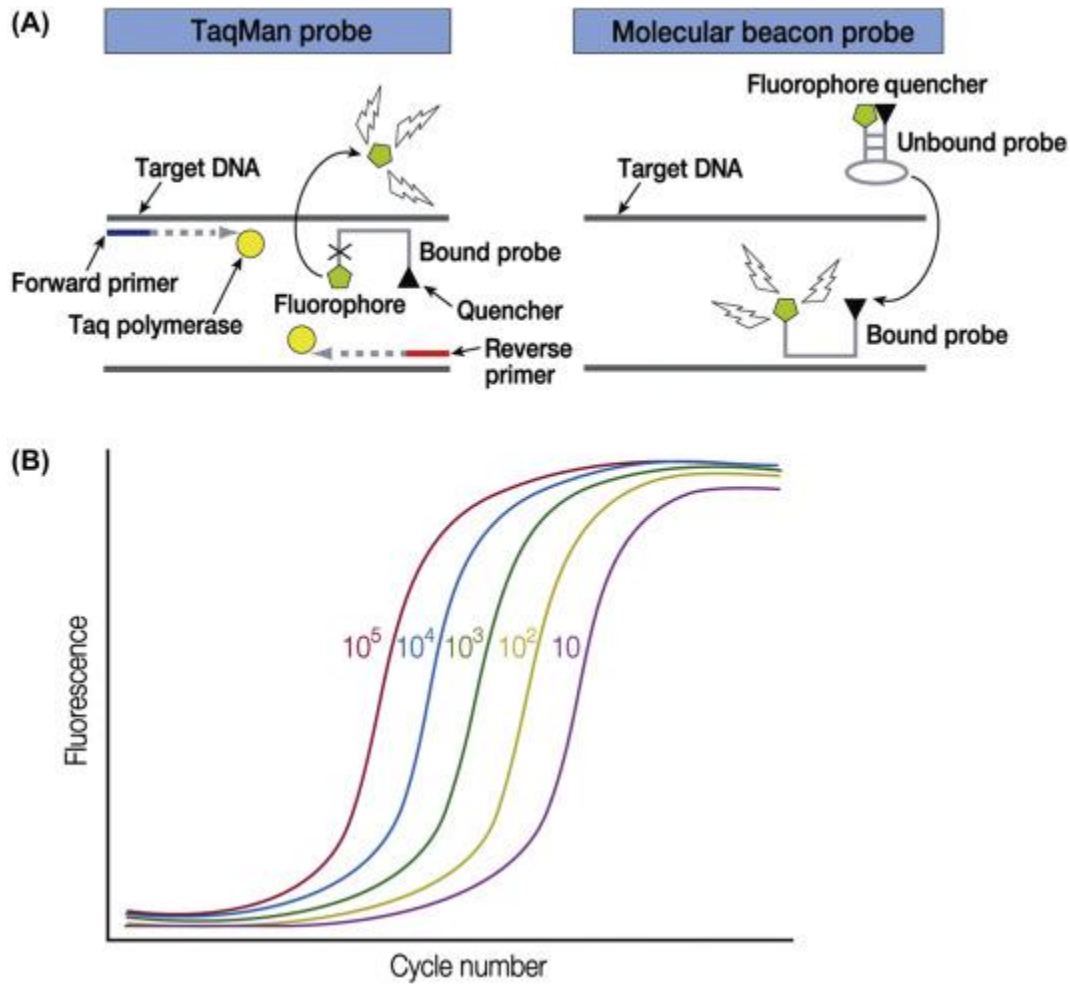


Figure 5 shows the principle of qRT-PCR

A) TaqMan probe. Fluorescence emitted from Fluorophore is quenched before it is removed by Taq polymerase.

B) A diagram showing the relationship between the amount of DNA measured by copy number and the amplification cycle.

*Real time polymerase chain reaction.* Real Time Polymerase Chain Reaction - an overview | ScienceDirect Topics. (n.d.). Retrieved April 10, 2023, from <https://www.sciencedirect.com/topics/medicine-and-dentistry/real-time-polymerase-chain-reaction>

**Figure 6 – Percentage of US Acres Sprayed with Glyphosate**

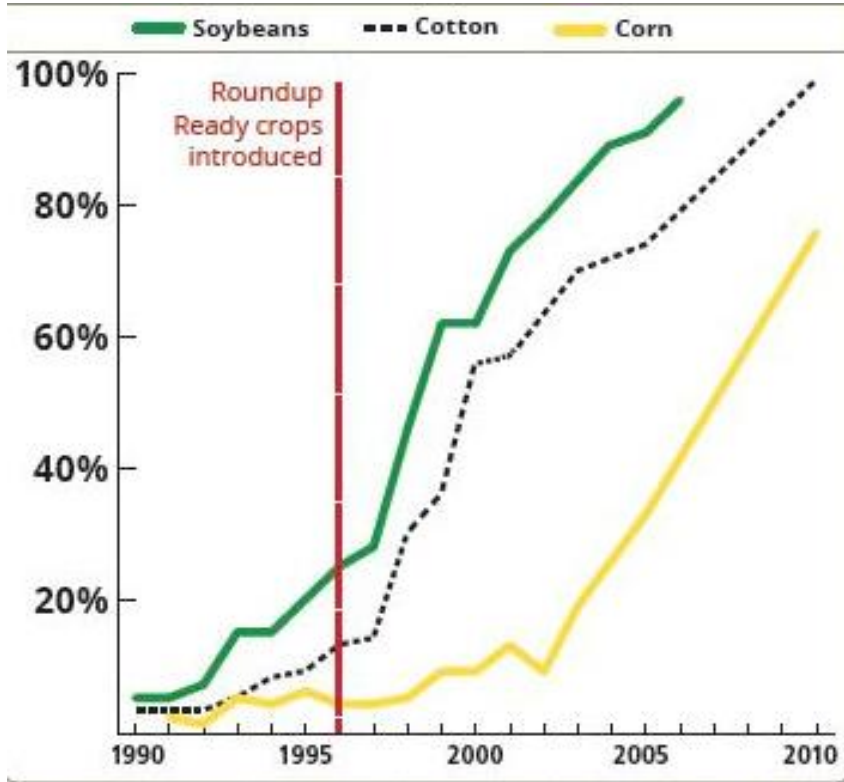


Figure 6 shows the increase in use of glyphosate-based herbicides in the US after the adoption of GM corn.

Hoffman, B. (2013, July 2). *GMO crops mean more herbicide, not less*. Forbes. Retrieved April 13, 2023, from <https://www.forbes.com/sites/bethhoffman/2013/07/02/gmo-crops-mean-more-herbicide-not-less/?sh=404e22c33cd5>

**Figure 7 – EPSP Synthase Action in Glyphosate Based Herbicides.**

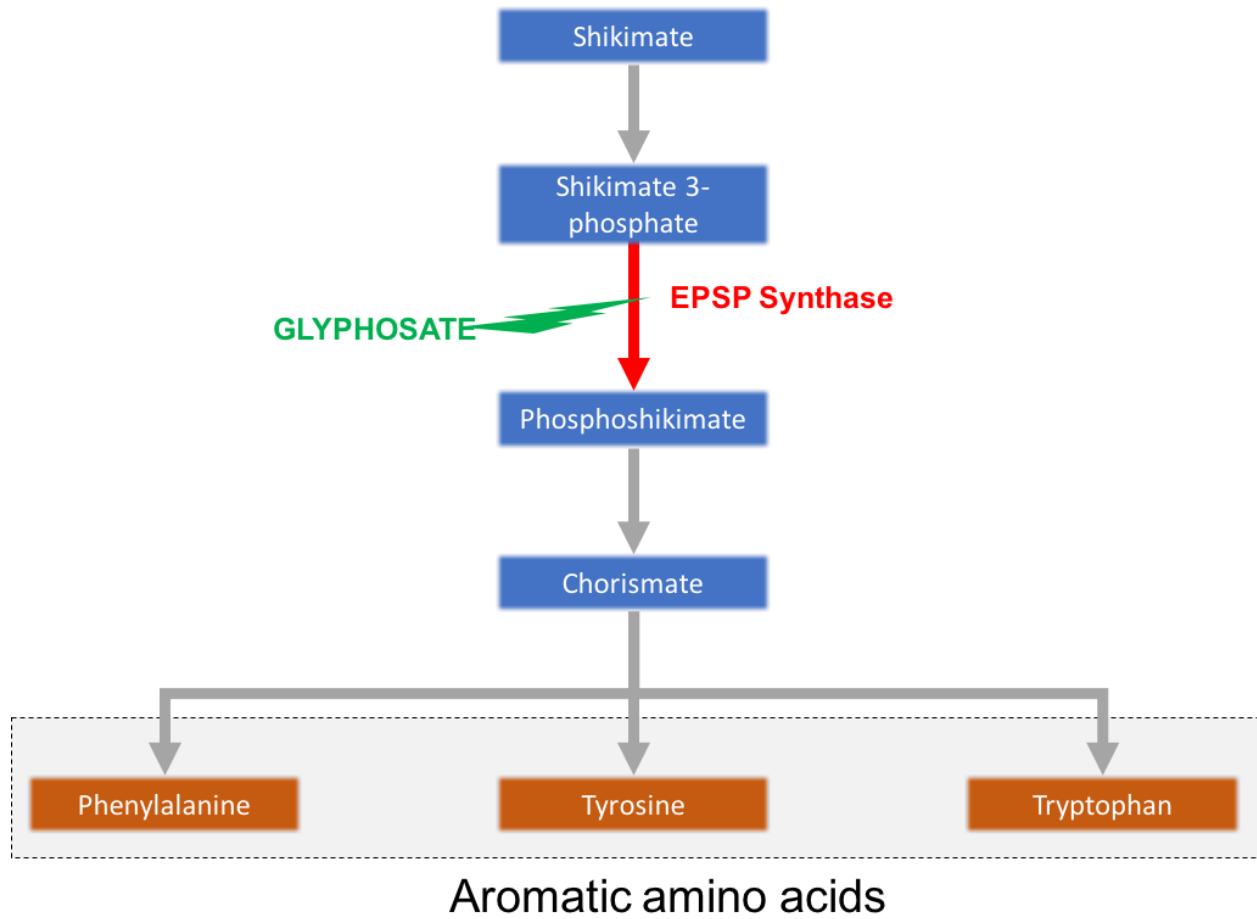


Figure 7 shows the disruption of aromatic amino acids biosynthesis by EPSP Synthase.

EPSPS class. (n.d.). Retrieved April 10, 2023, from <https://ppuigbo.me/programs/EPSPSClass/>

Picture 2 – Example of Negative Public Opinion

# **BAYER-MONSANTO'S MOST DANGEROUS GMO YET**

Corn genetically engineered to tolerate 5 toxic herbicides:  
2,4-D, dicamba, glufosinate, glyphosate, and quizalofop



endocrine disruption  
reproductive harms  
kidney damage  
liver damage  
neurotoxicity  
birth defects  
cancer  
autism



Hundreds of millions of pounds of the genotoxic carcinogen glyphosate is sprayed across America because of Roundup Ready crops. Now, Bayer wants chemical farmers to spray hundreds of millions of pounds of four additional toxins. **Bayer gets richer, while Americans get sicker.**

**TELL THE USDA TO STOP THE MADNESS:**  
**WWW.TINYURL.COM/FIVEWAYSTODIE**

Picture 2 shows negative public opinion on MON87429 variety described by this literature review.

*Tell the USDA: Stop the madness and reject Bayer-Monsanto's Mon-87429 Gmo Corn.* Retrieved April 11, 2023, from [https://gmofreeusa.salsalabs.org/stopgmo87429corn\\_4](https://gmofreeusa.salsalabs.org/stopgmo87429corn_4)