SCIENTIFIC DOSSIER ON GENETICALLY MODIFIED CORN AND ITS EFFECTS

Effects of GM corn on human health, the environment and biodiversity, including the biocultural richness of native corn in Mexico

Content

Executive summary

When the first GMOs appeared in different countries around the world, there was little or no scientific evidence demonstrating that they had no negative effects on human health, the environment and biodiversity. At the same time, the scientific community has for decades been warning about the potential risks and, more recently, the harm inherent to the consumption and cultivation of these organisms.

Today there is no scientific consensus on the safety of human or animal consumption and the releasing into the environment of GM crops. What there is, however, is a corpus of scientific research that has shown that transgenesis is an imprecise technology with unexpected and undesired effects; in particular, it has demonstrated the risks and harm it entails.

Few countries around the world have accepted the planting of GM corn in their territory, and a few more have authorized the entry of this grain into their country for human or animal consumption or for industrial processing. In Mexico, the introduction of GM corn has led to the transgenic contamination of native corn varieties, with negative environmental, biocultural, social, economic and political consequences. This has been systematically demonstrated by various scientific investigations.

In addition, groups of scientists, free of conflicts of interest, have shown that the consumption of GM corn is harmful to the health of laboratory and farm animals, affecting in particular the organs of their reproductive and digestive systems, as well as causing exacerbated immunological and allergic reactions, increased mortality rates and the development of chronic degenerative diseases, especially cancer. Furthermore, since GM corn is inextricably associated with highly dangerous pesticides that are part of the technological package deployed to grow it, people and animals consuming food containing ingredients based on this GMO are exposed to its damaging effects. This is particularly the case with glyphosate, which has been shown, even at low doses, to have carcinogenic effects through different routes, acting as an endocrine disruptor, altering reproductive systems and causing various metabolic diseases and affectations in different organs and systems.

Mexican corn, on the other hand, has exceptional nutraceutical and nutritional qualities, in addition to a great genetic and biocultural richness, bestowing it with adaptive advantages under diverse climatic and ecological conditions. It is also used in a huge range of dishes and foods that foment human health and wellbeing.

1. Technical and scientific aspects essential for understanding the effects of GM corn on human health and the environment

1.1 Conceptual background: living organisms and their genetic material

All living organisms contain genetic information that allows them to self-replicate and perform all the metabolic functions necessary for development, growth and reproduction. This hereditary information is stored primarily as a sequence in the deoxyribonucleic acid (DNA) molecule and translates into certain functions using segments from this sequence, i.e. genes. This genetic material, together with the information a living being relies on to form and develop, is known as the genome.

The simplest theory explaining how the genome performs its hereditary functions involves a pathway in which each gene is transcribed onto Ribonucleic Acid (RNA) and this RNA is translated into a protein, which carries out the function primarily contained in the DNA.[1] This is known as the "central dogma of molecular biology",[2] a theory of great relevance for molecular studies of genetic sequences in the Twentieth Century. Today, however, it is outdated beyond any doubt.[3]

We now know that not all genetic material follows that simple informative pathway and that, instead, a large number of sequences regulate the expression activities of genetic information, as well as functional-informative RNA molecules.[4] Moreover, the expression of all these genes is not determined solely by genetic information, but depends considerably on the environmental conditions the species live under.[5] All these pathways and mediations, taken together, are known as epigenetic processes.[6]

The expression of genetic information is so complex that it relies on genes that translate into proteins, on regulatory genes that are neither transcribed nor translated, and on functional genes in the form of RNA, all embedded in a myriad of conditions mediated by cellular and extracellular metabolism and the environment.[7]

In the particular case of the *Zea mays* species, or corn, it is estimated to have approximately 42,000 to 56,000 genes, along with two billion DNA base pairs (bp).[8] It is quite large and complex compared to other genomes, such as that of humans – *Homo sapiens* – with between 20,000 and 25,000 genes and around three billion bp.[9]

In most living species, especially differentiated multicellular organisms, genetic information is transferred vertically only; in other words, from one generation to the next, from the progenitor or progenitors to their offspring. Exceptionally, in single-cell species, specifically bacteria and archaea, genetic information can flow both vertically and horizontally when genetic material is obtained from an organism other than the progenitor.[10] In order for a horizontal transfer to occur, there needs to be a donor and a recipient of genetic information.[11]

For example, bacteria can pass on their genetic material by different means in such a way that the recipient individual can incorporate DNA fragments and, eventually, new genes that will potentially translate into new genes and, potentially, new functions.[12] When this new material remains stable in the donor genome, the evolutionary dynamics of that particular gene change dramatically, thereby making this new function's impact on a foreign genome unpredictable.[13]

Such horizontally or vertically acquired genetic material is exposed to various mechanisms that alter the genetic makeup of individuals within a population. The main source of alteration of the genetic material's makeup is mutation, which involves random changes in the genetic material's sequence.[14] These changes can basically have three potential effects on carrier organisms: the mutation can confer some advantage to the individual carrier and increase the number of individuals carrying the change; the change can have a negative effect and the individual carrier is eliminated from the population or, at best, is less likely to reproduce; or the change can be neutral within the population, i.e., it provides no apparent advantage or disadvantage that could be manifested in several future generations.[15] This mechanism that maintains or eliminates these mutations is Darwin's natural selection.[16]

There are other mechanisms that can modify the genetic structure of a population, such as gene flow, which is the migration of genes from one population to another.[17]

1.2 Transgenesis and GM crops in Mexico and the world, with emphasis on transgenic events involving corn

Transgenesis and the scientific evidence ruling out its alleged advantages

Since the emergence of genetic manipulation techniques in the early 1980s, attempts have been made to replicate both the horizontal transfer process and the genetic information transposition mechanism by means of mobile genetic units, which are also exclusive to these groups of living beings.[18] From a technical point of view, the aim has been to introduce new functions that endow the organisms with particular characteristics. In the specific case of agriculturally important plants, the aim of this manipulation in theory has been to ensure better yields, although in real terms, as will be shown below, such an outcome has not been fully achieved in an efficient and sustainable manner.

The emergence of genetically modified organisms (GMOs) stems from the development of genetic engineering techniques used on living organisms. These techniques are different from the natural modifications that occur in species and from conventional or traditional human-mediated methods.[19] In theory, an organism can be genetically modified by simply changing, i.e. mutating a single DNA base pair, if this is done artificially. In addition, a certain function can be silenced or, on the contrary, exaggerated by altering the genetic material sequence.[20] In terms of evolutionary genetics, with this type of change we are altering the genetic material and generating a "new allele" which is embedded in a natural population and, therefore, subject to the same complex processes described above, as well as to the same evolutionary forces as other existing variants.[21]

There are also certain genetic manipulation techniques that, as in the case of bacteria and archaea, insert their genetic material (a multiplicity of genes) into the genome of a recipient organism.[22] When genes from the same species are inserted, the recipient organism is called a cisgenic genetically modified organism. However, if the inserted genes come from a different species, it is called a transgenic genetically modified organism. In this document the terms GMO and transgenic will be used interchangeably both for genetically modified (GM) and transgenic crops.

In terms of evolutionary genetics, with this type of insertion – specifically the transgenic type – we are altering the genetic material, because the insertion of a new gene or set of genes not only gives rise to new functions, but literally creates a new space inside the bacterial genome.[23] This is because each gene that is part of the whole genome of any organism has a space and therefore an order that has, in fact, been maintained for millions of years. These genomic spaces are known as locus in the singular and loci in the plural.

The development of a transgenic GMO requires that a certain DNA sequence of an organism (not just a gene), with known and desired characteristics, be isolated and implanted in the DNA of another organism whose modification is sought. Transgenic production techniques (transgenesis) in plants were first used in the early 1980s. The first tests were conducted on tobacco crops in France and the United States (USA) in 1986.[24] Then in 1994 the Flavr-Savr tomato or MacGregor tomato from the Calgene company – now part of Monsanto – became the first transgenic food for human consumption whose sale to the public was authorized. The properties that were modified were its appearance, flavor, ripening time and shelf life.[25]

Based on the scientific community's discoveries and understanding of the dynamics of genetic information translocation involving phylogenetically distant species, contemporary genetic engineers and biotechnologists highlighted the potential for "artificial improvement" of plants of interest in agriculture, promising better yields, reduced use of pesticides and an end to world hunger, which has not happened.

Transgenesis techniques in plants of agricultural interest: imprecise and inefficient

The transgenesis techniques used to produce the main commercial GM crops are based on the insertion of DNA sequences from different species into the genome of another phylogenetically distant species. This is achieved using two main techniques: biolistics or particle bombardment and the mediation of the bacterium *Agrobacterium tumefaciens*. As we will see below, these techniques are imprecise and inefficient, giving rise to undesired gene and epigenetic expressions.

A. tumefaciens is a species of bacteria that interacts as a parasite with dicotyledonous plant species, specifically leguminous plants. This species infects the roots by entering the plant tissue, where the bacteria inject several genes into the nucleus of the cells in a plasmid; in other words, in a unit of genetic material with the ability to move outside the bacterial cell. Once this plasmid is inside the plant cell nucleus, the genes in the plasmid enter the plant genome in such a way that they are subsequently expressed by the other species, in this case producing certain proteins and metabolic processes. The function of these proteins is to produce tumors which are called galls, as well as to induce the plant to produce large quantities of opines, which are part of the bacteria's food. This is a predatory interaction in which the bacteria leverages these genes to obtain the plant's resources thereby undermining growth and reproduction in the host species.[26]

The essential notion behind the production of transgenic organisms is to use this scientific basis to modify the plasmids, i.e. the mobile units of information transfer, in such a way that their genes are disabled and the information is therefore eliminated to the detriment of the plant.[27] The DNA sequences of interest are inserted using their attenuated plasmid. This plasmid is introduced into a transforming bacterium which, in the vast majority of cases, is the *Escherichia coli* species. The introduced genes begin to express themselves upon entering the bacterial cell, although this does not take place in a precise manner and the desired transformation often does not happen due to a large number of factors involved in this process.[28]

The other technique most commonly used today to insert genes from one species into another involves using a "gene gun" for biolistics.[29] This tool was initially designed and developed by engineers at Cornell University in Ithaca, New York, in the mid-1980s. The term was coined to refer to a gun that fires DNA-coated microparticles into the recipient cells, aiming to lodge the genetic material first in the cell, then in the cell nucleus and, finally, inserting it inside the genome of the plants or, in general, the organisms to be modified. These gene guns fire DNA-coated micro-pellets into plant cells[30] and were first tested in 1983 with onion epithelium cells. After the shot, some onion cells were still viable and a smaller proportion of those received and expressed the transferred gene. The engineers obtained a patent and opened a company to sell these guns, but in 1989 they sold the business to DuPont, who marketed the tool on a larger scale.[31]

The technique of gene transfer from one species to another begins when the particles cross membranes and become trapped in the nucleus, in any part of the recipient organism's DNA. As a result, this genetic material is integrated at a very low frequency into unknown regions of the chromosomes through reshuffling, which is a cell-creating process.[32] If this occurs, transformation is considered stable and the genetic material can be expressed in the transgenic cells. It should be noted that this type of transformation is extremely ineffective, especially because the cell is literally bombarded with genes and it is impossible to predict which part of the plant genome these genes will be inserted into.[33] This transformation takes place at a very low frequency, so an *in vitro* selection system needs to be used to distinguish between transformed and non-transformed cells.[34]

In addition to the above, it should be noted that transgenesis in plants of commercial agricultural interest is not achieved with the insertion of a single gene. In reality, a transgenic sequence consists of several functional elements to ensure that the genes of interest are expressed in GM plants, with this expression occurring through the complex processes of translation and transcription, as mentioned before. The simplest explanation of the genetic information transcription process indicates that it starts at sites with particular DNA sequences, where RNA polymerase binds to genetic material to start the process. Once the gene has been fully transcribed, the synthesis is stopped by a third coding sequence that halts the process.[35]

So, in order for transgenesis to occur, at least three types of functional sequences are initially required: a sequence that promotes the start of transcription, called the promoter region; the sequence of the gene to be expressed in the transgenic organism; and a termination sequence that codes the halting of the transcription synthesis process.[36]

The simplest transgenic constructs have just one functional gene accompanied by others that allow its expression; for example, the construction of a transgenic sequence inserted in corn (MON87427), mediated by the Ti plasmid of *A. tumefaciens* from the Monsanto Company is 3,269 bp in size and has: a 620 bp promoter; an 803 bp intron; a small 207 bp chloroplast peptide; the 1,367 bp functional gene and a 252 bp terminator.[37] In this construct, the promoter-intron combination is used to drive the constitutive gene's selective tissue expression, resulting in production of the protein in female vegetative and reproductive tissue and generating tolerance to glyphosate within these tissues.

A transgenic event is a transgenic construct that is inserted into the cells of an organism and expresses a desired trait. In the case of corn, each genetic modification is a transgenic event, including combinations of such modifications, conferred to different parental lines of conventional hybrids registered under a specific name and code and also protected under a patent or plant breeders' rights with a specific trade name. The expression obtained (such as glyphosate tolerance or insect resistance) is known as a transgenic trait.[38]

There are more complex constructs with more than two constitutive genes, as well as events that contain two or more transgene constructs which are inserted in the same sequence segment. These are known as stacked events.[39] For example, the DAS-ØØ15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR6Ø4-5 × MON-ØØ6Ø3-6 event from the firms Syngenta, Monsanto and Dow AgroSciences LLC is over 10,000 bpb in size, with 7 constitutive functional transgenes, each with its own promoter and terminator, and some of the genes have sequences that promote expressions in specific plant tissues.

In a transgenic event, the construction of the segment to be inserted comes from more than one species, be they bacterial, viral or some other plant species. For example, the promoter most commonly used to make the construct comes from the Cauliflower Mosaic Virus (CaMV), while the terminator comes from *A. tumefaciens* (from the nopaline synthase gene, T-NOS). At the same time, the inserted constitutive genes come from different bacterial species.[40,41] This means that a simple event with the glyphosate tolerance trait, which is to be transferred to the donor plant, consists of genetic material from three different species, so the transgenic plant will have transgenic material from three species – one viral and two bacterial – plus the genome of the species itself.

It has been shown that the long variants of the p35 promoter (CaMV) alone contain an open reading frame that, when expressed, can lead to undesirable phenotypic changes.[42] In complete transgenic constructs, pleiotropic effects (in which the same gene affects different and unrelated phenotypic characteristics) can cause component level changes that would then go undetected but nonetheless affect food safety.[43]

As we have seen in this section, the two main transgenesis techniques are imprecise (the loci in which the transgenic sequences will be inserted cannot be controlled or predicted, and nor can all the effects at the genetic and epigenetic level be predicted), not to mention that, in both cases, several genes are inserted.

As pointed out above, this simple operation expressed by the central dogma of molecular biology no longer stands: the imprecise insertion of transgenic sequences brings about undesired expressions. Unwanted effects are part and parcel of recombinant DNA technology, i.e. transgenesis, and since 2000, a group of experts convened by the UN Food and Agriculture Organization (FAO) and the World Health Organization (WHO) has been recommending appropriate assessments to identify the risks associated with such effects.[44]

Over thirty years of GM crops in the world: a history of unfulfilled benefits in the light of scientific evidence

There have been two main arguments for promoting biotechnology to produce GM crops: to increase crop yields and hence boost production of basic grains, especially corn; and to reduce the quantities of agrochemicals used, especially insecticides and herbicides.

More than 30 years after the first release of GM crops into the environment, statistical data obtained from the UN Food and Agriculture Organization (FAO)[45] reveal that corn production has increased, although this has been the trend since the 1950s with the use of hybrid seeds. Figure 1 shows, without any need for hypothesis testing, that this increased production is not actually associated with higher yields but with an increase in harvested land area; in other words, more crops are being produced because there is more land for growing them.

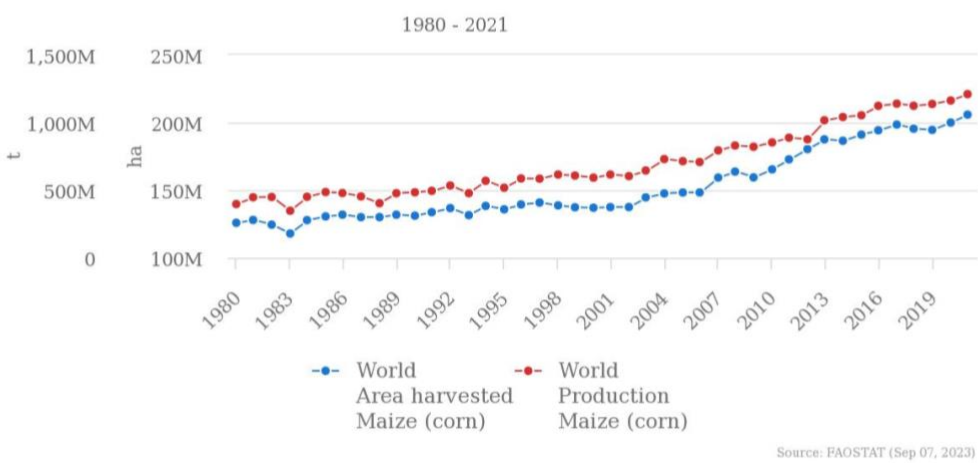

Figure 1. Corn production and amount of area harvested worldwide over the last 40 years. The graph shows that production has been increasing due to an increase in arable land.

Had the promise of higher corn production been fulfilled, we would see a near-zero rise in the arable land growth curve. In the case of the United States, the country with the largest area used for GM corn cultivation, the situation is similar to that of the rest of the world. Figure 2 shows that, in certain years, there is even an inverse relationship between production and arable area, a trend contrary to the arguments made by the companies offering these products.

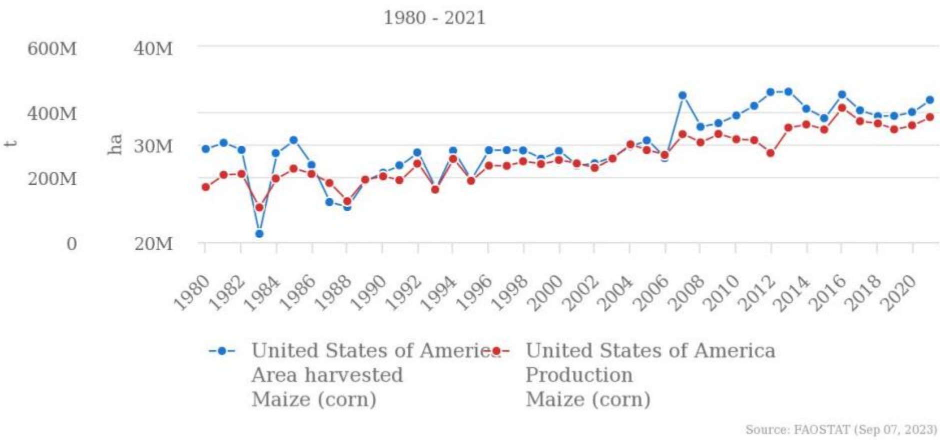

Figure 2. Corn production and amount of area harvested in the United States over the last 40 years. The graph shows that production has been increasing due to an increase in arable land.

A comparative study of production systems in the US (with GMOs since the 1990s) and Eastern Europe (no GMOs), characterized as highly productive under similar conditions in terms of production costs and agricultural subsidies, confirms that GMOs have not led to an increase in yields compared to non-GM crops. The comparison of corn yields in both production systems, from 1961 to 2009, reveals no significant differences; there is no higher yield in the US system even with the introduction of GM corn seeds in the 1990s.[46]

The National Academies of Science, Engineering and Medicine have analyzed potential and actual GM crop yields based on national data on GM corn, cotton and soybeans in the United States. The finding they report is that there is no significant increase in yield rates as a result of genetic engineering technology.[47] Corn yields in this country between 1996 and 2011, when GM corn began to be grown, increased by only 1% compared to the period from 1940 to 1995.[48] The introduction of GM corn has not led to a significant increase in yields compared to hybrid corn produced in Mexico.[49, 50, 51, 52]

Promoters of GMOs go so far as to make exaggerated claims about the benefits of these crops, particularly their yield. A detailed analysis of publications showing enhanced yield increases thanks to this technology reveals that these yields have only been obtained under controlled conditions (greenhouses) or with experiments with a few individuals (small-scale field trials), and they are not possible under real agrosystem conditions.[53]

In addition, field reports from the US Department of Agriculture (USDA) have stated textually that "Over the first 15 years of commercial use, GE seeds have not been shown to increase yield potentials of varieties. In fact, the yields of herbicide-tolerant or insect-resistant seeds may be occasionally lower than the yields of conventional varieties if the varieties used to carry the HT or Bt genes are not the highest yielding cultivars, as in the earlier years of adoption."[54]

Regarding the second promise of this agricultural biotechnology, FAO statistical information also shows (Figure 3) that the quantity of herbicides used on crops over these 27 years has not only not dropped but has actually risen more sharply from the end of the 1990s to date, during which time worldwide use of GMOs has increased (in countries opting for the transgenic model in the United States and Argentina).

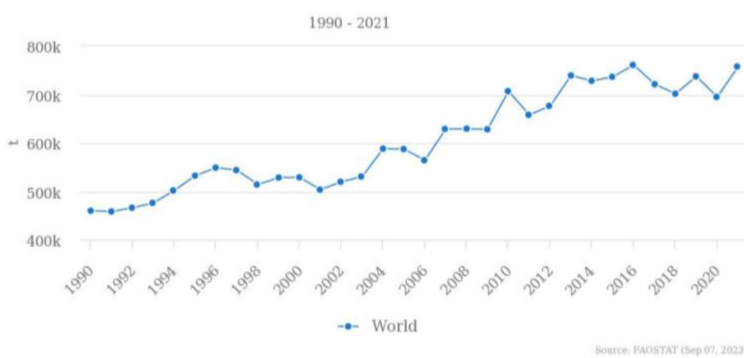

Figure 3. Worldwide herbicide production over the last 30 years. The graph shows that production has been increasing; from 2010 onwards the rise is even sharper than in previous years.

GMOs have also failed to reduce the quantity of insecticides, even with Bt *(Bacillus thuringiensis*) technology. In fact, the insecticide toxins produced by GM plants have led to the development of resistance among insect pests,[55,56] as will be discussed further below. The facts indicate that Bt technology is environmentally and agriculturally unsustainable. As for herbicides, the curve shows that there has even been an increase in their use over time following the introduction of herbicide-tolerant GM crops (mainly glyphosate and glufosinate-ammonium) (Figure 4).

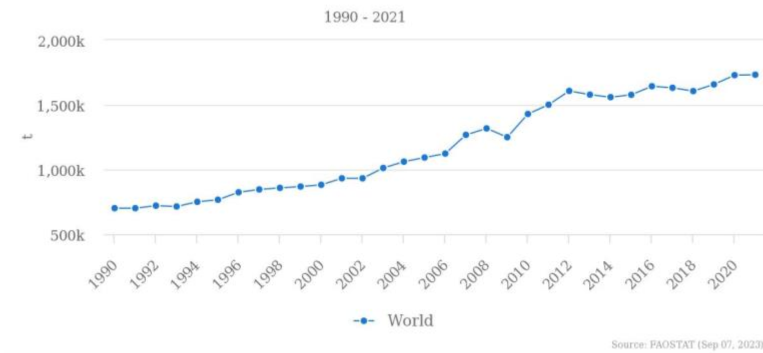

Figure 4. Worldwide insecticide production over the last 40 years. The graph shows that production has been increasing more sharply since 2005 than in previous years.

As we will see below, the planting of herbicide-tolerant and insect-resistant GMOs has led to the emergence of "superweeds" and "super pests", respectively, which has meant an increase in the quantity and types of pesticides used on agricultural land.

Increased GM crop production[57] by intensive agricultural systems is linked more to the production of raw materials to make large quantities of ultra-processed food, high in calories but nutritionally deficient, than to efforts to eradicate hunger.[58,59,60] This, in turn, underscores changes in the eating habits of populations in so-called "developed" countries. [61]

The number of calories consumed per capita by people living in the US, for example, has risen by more than 200% in just 10 years.[62] In so-called "developing" and "underdeveloped" countries, we are beginning to observe these same trends, where populations are adopting a Western-style diet characterized by high caloric content, low nutritional quality, excess refined sugars, highly processed foods and large amounts of additives.[63]

Far from seeking to provide the population with healthy food, one of the main aims of industrialized agriculture, which includes GMO crops, is to create inputs for the food industry to make fructose syrups and edible oils as ingredients in the production of foods of very low nutritional quality.[64] This type of food is closely related to GMOs, which are intended primarily for the production of livestock feed, ethanol and ultra-processed foods of low nutritional quality.[65] This point is explored further in later sections.

Most countries and farmers around the world do not plant or import GMOs

Of the 195 internationally recognized countries, 85% do not plant GMOs. By region, the 29 countries that do have GM crops in their territories are distributed as follows: 10 in Latin America, 2 in North America, 9 in Asia and the Pacific, 6 in Africa, and 2 in the European Union. While almost 80% of the world's countries do not import GMOs for any kind of use, only 43 (22%) import them for human food, animal feed or industrial uses.[66] Of the 165 countries in the world that planted and harvested corn in 2019,[67] plus the ones in the European Union, only 14 countries (8.5%) planted GM corn: the US, Brazil, Argentina, South Africa, Canada, Philippines, Paraguay, Uruguay, Spain, Vietnam, Colombia, Honduras, Chile and Portugal.[68]

These data reveal that there is no widespread or global preference for GM crops, especially GM corn, or for approving their importation for food, feed or industrial processing.

According to biotech industry data, in 2019 seventeen million farmers planted GMOs on a total of 190.4 million hectares of land, and more than 65 million people "benefited" from GM crops.[69] These are tiny figures when we consider that in 2019 approximately 1.23 billion people globally were employed in the world's agri-food systems and more than three times that number – almost half the world population – live in households linked to agri-food systems, according to the FAO. Of these 1.23 billion people, 857 million were working in primary agricultural production, while 375 million worked in the off-farm segments of agri-food systems.[70]

Insect resistance, by expression of Cry proteins, and tolerance to glyphosate and other herbicides: main traits of GM crops in the world, especially GM corn

Of the 472 transgenic events with country approvals, the most representative traits are herbicide tolerance (HT, depending on the use of herbicides for the expressed trait to be leveraged) and insect resistance (Bt, for the bacterium *Bacillus thuringiensis*, which is the genes' "donor" organism for expressing the production of insecticidal toxins from the Cry family).

HT crops have genetic modifications enabling the GM plant to withstand the use of a certain herbicide (e.g., glyphosate, glufosinate-ammonium, 3,6-dichloro-2-

methoxybenzoic acid or dicamba, and 2,4-dichlorophenoxyacetic acid or 2,4-D), so that it can be used as part of agricultural efforts to eradicate weeds. There are hundreds of records of glyphosate-tolerant transgenic events in different crops such as corn, cotton, soybean, canola, potato, alfalfa and wheat.[71] Internationally, 63% of herbicide-tolerant GM crops are glyphosate-tolerant.[72]

In terms of cropland area, the world's top five GM crops are: soybeans (91.9 million hectares), corn (60.9 million hectares), cotton (25.7 million hectares), canola (10.1 million hectares) and alfalfa (1.28 million hectares). Together, these five plants account for 99% of the world's GM croplands. The crop with the largest number of approvals is GM corn with 172 events (36.5%). The NK603 (HT) and MON810 (Bt) corn events have the largest number of approvals internationally.[73]

In the US, data from the National Agricultural Statistics Service (NASS) of the US Department of Agriculture (USDA) in the June 2020-2022 Agricultural Survey, reveal that 93% of US corn crops are GM. Sixty-five percent of GM corn events approved in that country are herbicide-tolerant and 42% are glyphosate-tolerant.[74]

Therefore, as far as the impacts to be considered for human health and the environment, due to GM corn consumption or planting, are concerned, it is essential to bear in mind the following, among other things:

1. The possible effects associated with Cry family proteins, which are expressed in Bt corn, form an inherent part of the chemical composition of their cobs, and can eventually find their way into foodstuffs made using this corn, and
2. The harm associated with the herbicides included in the technological package used on HT corn, given that they can remain as residues on the cobs and also end up in foodstuffs made with this corn; in particular, the herbicide that should be considered is glyphosate.

These same aspects should be considered as part of the impact on the environment and biodiversity, including the biocultural diversity of native corn, as a result of the releasing of GM corn into the environment. Nor should we overlook the unforeseen effects derived from the imprecision inherent in transgenesis, along with other aspects such as the inferior nutritional quality of GM corn and GM-based foods compared with Mexican corn.

Then there is the *Bacillus thuringiensis* bacterium, which has been used as an insecticide since the 1920s. This species forms resistance structures called endospores in a process that produces proteins in the form of crystals that have insecticidal properties (protoxins) when ingested by insects.[75] In the 1970s, the genes expressing these proteins, generically known as Cry, were isolated. These proteins act in the digestive tract of insects causing cell death.[76] Ultimately, these same toxins allow the bacteria's endospores to germinate inside the insect. The type and amount of different protoxins in the crystalline inclusions of *B. thuringiensis* determine the toxicity profile of a particular strain.[37]

Cry proteins are very diverse and some are specific to a certain group of insects, with results that can be lethal. They are most lethal against insects from the Lepidoptera (butterflies and moths), Diptera (flies and mosquitoes) and Coleoptera (beetles and weevils) orders;[77] others have been documented as highly virulent in Hymenoptera (wasps and bees)[78] and nematodes.[79]

As they pass through the digestive tract, Cry proteins enter primarily as a relatively inert protoxin and then form a cytotoxin:[80] the proteins must first of all be ingested by a susceptible larva. The midgut environment promotes crystal solubilization and subsequent protoxin release; protoxin cleavage sites are recognized and cleaved by host proteases to produce an active toxin that subsequently binds to specific receptors in the midgut epithelium. The toxin's subunits oligomerize to form pore structures capable of inserting themselves in the membrane; these pores allow ions and water to pass freely into the cells, resulting in swelling, lysis and ultimately death.[81]

The first GM plants expressing Cry proteins were planted in 1996. Since then, it has been known that these insecticide-producing proteins are not specific to pest insect species, but can eliminate various insects that feed on them.[82]

As mentioned above, other large-scale production GM constructs worldwide express herbicide tolerance (HT) traits, particularly against the glyphosate herbicide. Glyphosate or N-(phosphonomethyl)glycine is a systemic herbicide from the substituted glycine chemical group, with pre- and post-emergent application (this refers to whether glyphosate is used in the early stages of the plant life cycle, during germination for example, or in later stages). It is broad spectrum and non-selective (it is harmful to most known plants and can eventually kill them, including different species of herbaceous plants, shrubs and trees).[83] Glyphosate is used in agriculture to eradicate non-sown plant populations growing in and around the plot of agricultural land.

This herbicidal capability arises from the 5-enolpyruvylshikimate-3-phosphate synthase enzyme (EPSPS), which is found in the biochemical pathway of shikimic acid and is responsible for the production of three essential aromatic amino acids: phenylalanine, tyrosine and tryptophan, which are necessary for building plant proteins and perform vital functions in plants.[84] The EPSPS enzyme is not only found in plants but also in bacteria, including the bacteria comprising the intestinal microbiota in humans, and some beneficial soil fungi.

GM crops acquire tolerance to glyphosate through the insertion of the cp4 EPSPS gene from the *A. tumefaciens* bacterium into the genome of the parental hybrid.[85] In this case, the GM plants express a modified enzyme called S-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is tolerant to the effect of N-phosphonomethylglycine.[86] According to the Monsanto patent (MXPA05008725A), the construction of a modified EPSPS enzyme is described as follows: "Tolerance to glyphosate in plants can be achieved by the expression of a modified class I EPSPS that has less affinity for glyphosate, yet still retains its catalytic activity in the presence of glyphosate (US patents 4,535,060 and 6,040,497).

"Tolerant" or "tolerance" refers to the diminished effect of an agent on the growth and development of a plant, in particular tolerance to the phytotoxic effects of a herbicide, especially glyphosate. Enzymes such as class II EPSPS have been isolated from bacteria that are naturally resistant to glyphosate and when the enzyme is expressed as a transgene in plants, it provides the same tolerance to glyphosate (US patents 5,633,435 and 5,094,945). Enzymes that degrade glyphosate in plant tissues (US patent 5,463,175) can also confer plant tolerance to glyphosate.

DNA constructs containing the necessary genetic elements for expressing glyphosate-tolerant enzymes create chimeric transgenes in plants. These are used for producing glyphosate-tolerant GMOs that enable glyphosate-based herbicides (GBHs) to be used on croplands, thereby eliminating all but the genetically modified plants. An example of this is glyphosate tolerance in corn (US patent 5,554,798).

Transgenic events permitted and authorized in Mexico, with emphasis on GM corn traits

The release of GMOs into the environment in Mexico began in 1988 with the authorization of the trial planting of GM tomatoes by the then Ministry of Agriculture and Hydraulic Resources (Secretaría de Agricultura y Recursos Hidráulicos or SARH) for the company Sinalopasta (at that time owned by the US firm Campbell's). The

SARH consequently created an *ad hoc* working group to draw up regulations on the introduction of GM crops in the environment, as well as to monitor and evaluate field sowing trials. The following year this group became the Agricultural Biosafety Committee, comprised by highly qualified experts, including scientists and civil servants.[87]

Between 1988 and 2004, nearly 350 authorizations were granted to 38 companies, 3 research institutes and universities for 26 experimental GM crops in 48 sites in 17 Mexican states; 14% of the authorized trials were for GM corn.[88,89] In 1993, the Center of Research and Advanced Studies (Centro de Investigación y de Estudios Avanzados or Cinvestav) of the National Polytechnic Institute (Instituto Politécnico Nacional or IPN) requested the first field trial for GM corn, which was then followed by further trial requests from different institutions, all of them in areas of no more than one hectare and with strict control measures.[90]

According to data from the Inter-Ministerial Committee for the Biosafety of Genetically Modified Organisms (Comisión Intersecretarial para la Bioseguridad de los Organismos Genéticamente Modificados or Cibiogem), also in 1993, the multinational Pioneer Hi-Bred International Inc. requested authorization for the experimental planting of herbicide-tolerant and virus-resistant corn. In 1995, the International Corn and Wheat Improvement Center (Centro Internacional de Mejoramiento de Maíz y Trigo or CIMMYT) made its own application for the experimental release of insect-resistant corn, followed in 1996 by Asgrow Mexicana, who applied for permits to release herbicide-tolerant corn. In 1997, the multinational Monsanto began applying for permits to release different insect-resistant and herbicide-tolerant GM corn events. A total of 73 applications for the release of GM corn into the environment were registered between 1993 and 2003.[91]

In view of the rising number of requests between 1996 and 1998 for the authorization of GM corn planting trials, which were considered by the country's scientific and farming community a risk to the genetic richness of corn in its Center of Origin and Genetic Diversity (Centro de Origen y Diversidad Genética or CODG), the then Ministry of Agriculture, Livestock and Rural Development (SAGAR, currently SADER), imposed a *de facto* moratorium which remained in force from 1999 to 2005, based on the recommendations of the National Agricultural Biosafety Committee (Comité Nacional de Bioseguridad Agrícola or CNBA).[92] As we will show later on, the moratorium did not prevent transgenes from reaching native corn populations.

In 2005, the GMO Biosafety Act (LBOGM) was approved establishing three types of licenses for GMO-related activities:

1. Authorizations for importation for use or commercialization, for the purpose of human consumption (direct, as grains, or in processed food) or animal feed, or other purposes such as public health or bioremediation.
2. Permits for release into the environment (e.g. planting).
3. Notices of confined use (research and industrial uses).

Between 2005 and 2013, 651 permits were granted for the planting of GM crops in experimental, pilot and commercial stages, 53.6% of which were accounted for by GM cotton crops and 30.1% by GM corn crops. Seventy-six percent of the permits were for glyphosate-tolerant crops. [93] The issuing of environmental release permits was halted by court order in September 2013.

The main requesters of these permit applications have been the multinationals Monsanto (31.4%), Pioneer together with Dow AgroSciences (26.6%), Pioneer (24.8%) and Syngenta (13.0%), for planting in the states of Sinaloa, Sonora, Chihuahua, Tamaulipas, Coahuila, Durango, Nayarit and Baja California Sur.[94] Genetic modifications in the approved permit applications included lepidopteran insect resistance (54.5%), coleopteran resistance (24.1%) and tolerance to the herbicides glyphosate (56.3%) and glufosinate-ammonium (33.0%).[95] Half of these permit applications were made for stacked events.[96]

As for authorizations for transgenic events for importation, between 1995 and 2018, 181 permits were granted for an indefinite period. Almost half (49.7%) were for GM corn, with 67% for glyphosate-tolerant GMOs (83.4% are tolerant to various herbicides, including dicamba and 2,4-D). Of the GM corn crops, 90% were glyphosate-tolerant events.[97]

1.3 Scientific and statistical evidence on the undeniable relationship between GM corn along with other GM crops and glyphosate (and other pesticides)

Relevant conceptual background on glyphosate, glyphosate-based herbicides and their main degradation product

Glyphosate was created in 1950 by a Swiss pharmaceutical company that unsuccessfully sought its use in this field. Fourteen years later, the first patent (number 3,160,632) was granted for the use of glyphosate as a metal chelating and descaling agent to eliminate mineral deposits in pipes and boilers.[98] Following further research by different companies on some of its biological properties, the multinational Monsanto patented it as a herbicide after discovering its herbicidal properties, and introduced it in the market in 1974 with its best-known commercial formula, *Roundup*®.[99]

Today glyphosate is the substance most widely used as a herbicide worldwide, with over 750 formulations based on this compound.[100,101] Chemically, it is a molecule comprised by a glycine fraction and an aminophosphate radical attached as a substituent of one of the α-amino group hydrogens. It is the main active ingredient in several commercial herbicides used in agriculture, gardening and other domestic activities in both rural and urban environments, in addition to maintenance on highways and other thoroughfares.

Glyphosate-based herbicides (GBHs) are mixtures of several substances containing other ingredients in addition to glyphosate. These ingredients are usually excluded from the toxicity tests submitted by companies when applying for authorization to market their products, considering only the active ingredient.[102, 103] The rest of the formulation, which generally accounts for more than 50% of the herbicide, remains unknown under some form of trade secret protection. [104] As will be seen below, this is a loophole shared by pesticide regulatory systems that rely on international standards for their risk assessments. It has been found that the different available formulations of *Roundup*® can vary in toxicity by as much as 100-fold because of the different ingredients they contain.[105,106,107]

These ingredients of complete commercial formulations are classified into two groups: 1) "active" ingredients, which are the ones added intentionally to be toxic against the target species, which is the plant species that the herbicide seeks to eradicate; and 2) "inert" ingredients,[108] also called formulants, which are added to the formulation to enhance the effect of the active ingredient; in addition to adjuvants.

In the case of GBHs, the main adjuvants are surfactants, i.e. molecules that help glyphosate pass through plant membranes. The compounds most commonly used as surfactants in GBHs are ethoxylated amine molecules, also known as POEAs, chemically synthesized from amines to which ethylene oxide units are added.

Glyphosate's main degradation product is aminomethylphosphonic acid (AMPA), which is more persistent and mobile in water bodies and soils compared to glyphosate[109,110, 111] and has also been shown to have harmful effects on health and the environment, as described below.

The Inter-Ministerial Committee on Biosafety of Genetically Modified Organisms (Cibiogem) microsite currently contains the "compilation of scientific information on the harmful effects of the glyphosate herbicide", as part of the National Information System on Biosafety of GMOs (SNIB), mandated by the GMO Biosafety Act (LBOGM), which contains more than 350 records of scientific papers providing compelling evidence of the harm caused by glyphosate to human health, the environment and biodiversity.[112]

As if that were not enough, the 5th edition of the "Antología Toxicológica del glifosato (Toxicological Anthology of Glyphosate)", published in 2020, refers to 1,108 scientific investigations, free of conflicts of interest, on the effects of glyphosate, its dynamics and the impact caused by the use of glyphosate-based herbicides, as well as its main degradation product, AMPA (aminomethylphosphonic acid).[113]

Transgenic events, with emphasis on glyphosate-tolerant GM corn traits; worldwide, in the United States and in Mexico

We can confirm that the general trend in GM crop management, in particular, for GM corn involves the use of glyphosate as the main herbicide, in view of the following:

- 63% of herbicide-tolerant GM crops worldwide are glyphosate-tolerant.[114]
- NK603 corn is glyphosate-tolerant and one of the two transgenic events with the highest number of international approvals. In addition, this event is stacked in more than 20% of approvals.[115]
- 65% of GM corn events approved in the US are herbicide-tolerant, with 42% glyphosate-tolerant.[116]
- In Mexico, nearly half the authorizations granted for the importation of transgenic events are accounted for by GM corn, 90% of which are glyphosate-tolerant events.[117]

Global glyphosate use

With the spread of GM crops in countries that have approved them, GBH usage rose 113-fold in volume between 1996 and 2018,[118] an increase of 1,500%. Also, more than 55% of glyphosate used in agriculture (90% of global glyphosate use) is intended for GM crops.[119,120] Authors with experience in weeds that have developed resistance to the glyphosate herbicide have stated that the most important aspect of glyphosate's commercial success has been the introduction of GM crops tolerant to this herbicide. [121]

Glyphosate residues in products and foods with GM corn and other GM crops

Since 2012 the presence of GBH residues has been reported in edible glyphosate-tolerant GM plants, especially GM corn.[122] A study published in 2017 revealed the presence of transgenic sequences and the glyphosate herbicide in different corn-based foodstuffs that are widely consumed and readily available in Mexico. Samples included basic consumer products (tortillas, tostadas and tortilla chips) and processed products (flours, snacks and breakfast cereals). The study found that 82% of all foods analyzed contained transgenic event sequences, of which 30% were found to include glyphosate and AMPA residues. It was also found that 60% of samples with GMOs contained the glyphosate-tolerant GM corn event known as NK603.[123]

Other studies have also detected the presence of glyphosate and AMPA residues in water, as well as in foods such as grains (barley, oats, rye and wheat), processed products (bread, breakfast cereals, corn syrup, flour and baking mixes, wheat cakes and snacks, bran flour and soy-based products) and other products (pulses and pulse-based foods, peas and GM soybeans).[124] Traces of glyphosate and its derivatives have been detected in a large number of foods, especially those containing technologically-produced cereals such as oats, canola, wheat and soybeans. In these cases, glyphosate is sprayed prior to the harvest period to speed up grain desiccation, as well as on GM corn and soybean crops.[125]

The presence of glyphosate residues in small grain crops is increasing because of this growing practice of pre-harvest desiccation.[126,127,128] According to a pre-harvest preparation guide from Monsanto Company, this is considered a management strategy not only to control perennial weeds, but also to facilitate crop management and secure advantages for the following year's crop. [129]

Another study that compared the nutritional and elemental composition of GM soybeans, conventional soybeans and organic soybeans in the US found that GM soybean samples contained a considerable level of glyphosate residues (3.3 mg/K), while the other two had no residues of this agrotoxin. In addition, the findings reveal that organic soybeans had the healthiest nutritional profile with significantly more total protein, zinc and less fiber, as well as fewer total saturated fats.[130]

Analyses have also been conducted on honeys from different countries, comparing the presence of glyphosate residues in each one and classifying the samples in terms of: countries that use GMOs extensively, countries that allow the use of some GMO traits, and countries that do not allow GMOs at all. The findings indicate that glyphosate levels are generally lower in samples from countries that either do not allow GMOs at all or only allow limited use of some GMO traits, compared to countries that allow the planting of GMO traits. In addition, the presence of glyphosate was detected in products with corn ingredients (pancakes and corn syrup) and soy-based products (soy sauce, soy milk and tofu). The researchers point out that glyphosate has increased with the introduction of GM soybeans and corn and did not rule out the possibility that the products analyzed were made using these inputs.[131]

In Switzerland samples of different foods were analyzed with glyphosate residues being found in pasta (identified as a highly relevant food in terms of glyphosate residue intake in that country), wine, fruit juice and almost all honey samples. According to the researchers, the use of glyphosate on cereals or oilseeds in that country is not recorded, and nor is its use on GM crops or as a desiccant. However, several of these products come from countries where pre-harvest use as a desiccant is reported.[132] In the United States, glyphosate has also been detected in wine and beer,[133] as well as in drinking water.[134]

Additional research notes that UK Food Standards Agency (FSA) residue testing conducted in 2012 found glyphosate residues in one-third of the bread samples tested. This paper adds that tests in the US conducted by the Department of Agriculture (USDA) in 2011 revealed the presence of glyphosate and AMPA in 90.3% and 95.7%, respectively, of soybean samples analyzed. The relationship suggested by these figures cannot be ruled out if we bear in mind that most of the soybeans in that country are transgenic.[135] In Argentina the presence of glyphosate has been reported in cotton-based products such as healing materials (gauze and cotton) and personal hygiene products (tampons).[136]

In Canada a group of scientists analyzed 7,955 foodstuffs, finding glyphosate residues in 42.3% of the samples. Food samples included a wide variety of fresh and processed fruits and vegetables, cereals (e.g., wheat, corn, oats, barley, buckwheat and quinoa), beverages, pulses (beans, peas, lentils and chickpeas), soy and products for children, as well as ready-to-eat/frozen foods and meals. The study does not specify whether these products contain or are made from GMOs; however, in the case of processed or soy- and corn-based foods, this possibility cannot be ruled out.[137] In fact, other scientific research notes the introduction of thousands of tons of glyphosate into the food chain through GMO-based foods such as soybeans, which are tolerant to this herbicide.[138]

Lastly, in December 2023 the American Academy of Pediatrics (AAPP) in the United States published a clinical report by Committee on Nutrition medical specialists revealing the close relationship between glyphosate and GMOs and warning about the measurable amounts of this herbicide in a broad range of GMO-based foods available to children and adolescents. At the same time, it is emphatically clear that GM crop technology has focused on agricultural aspects related to yield, disregarding the nutritional quality of the products which are intended mainly for manufacturing ultra-processed foods. The foregoing highlights the important role of pediatricians in informing families about the potential risks of GMO and glyphosate ingestion, as well as recommending the consumption of organic foods.[139]

These results demonstrate that human exposure to glyphosate is widespread and constant and involves products containing or made from GMOs, which is an alarming insight considering the possible negative effects of this pesticide on human health.

Presence of glyphosate in fluids, tissues and excreta of animals fed with GM corn and other GM crops

A large proportion of GM crop production is used by the meat industry to feed sheep, cattle and pigs, making livestock the biggest consumer of GMO crop-based products in the world. As a result, research has been conducted in recent years to detect the presence of glyphosate in animal fluids and understand the impact of this herbicide on animal health. In the US 95% of livestock feed is made from GM crop inputs while, globally, these feeds are considered to account for 70 to 90%.[140]

Some of these investigations have reported the presence of glyphosate in the urine of dairy cows and fattening rabbits fed a GM corn- and soybean-based diet. Glyphosate has also been found in organs and tissues of GMO-fed cows, including the intestine, liver, spleen, kidneys and muscles.[141,142,143] Another investigation, in which the mycological characteristics of dairy cows were evaluated, analyzed a total of 258 dairy cows from 14 farms and found glyphosate in their urine; these findings suggest that the glyphosate herbicide seems to modulate the mycological community of these ruminants.[144]

Other studies in dairy cows, in which glyphosate was also detected, indicate that the main excretion pathway of this agrotoxin is through the feces, and that the degradation of glyphosate and AMPA by rumen microbes, as well as their possible retention in animals' bodies, needs to be taken into consideration.[145]

Presence of glyphosate in human fluids and excreta, in countries with GMO production or consumption

At this stage it is very clear that human exposure to glyphosate is widespread and constant, occurring not only in agricultural environments but also in urban and peri-urban centers. In other words, this agrotoxin is entering our bodies both occupationally and non-occupationally.[146,147]

It is known that glyphosate is being detected in the general population of industrialized countries, with a higher prevalence in children, as well as in people from agricultural areas.[148] Several of the studies that have detected glyphosate in human fluids and excreta were conducted in three countries where GM crops have spread exponentially and which rank among the world's biggest exporters of GMOs: the United States, Argentina and Brazil.

However, the presence of glyphosate in the human body is not exclusive to these nations. Studies are reporting the presence of glyphosate in countries that have lower GM crop growth rates but which import GMOs (Mexico, Spain and Portugal, as well as China) and in countries that do not plant GMOs but import them (European Union countries and Thailand). It should be noted that, in both cases, glyphosate is permitted and used on non-GM crops and in other non-agricultural activities.

United States

The majority of this country's inhabitants have glyphosate in their urine. This was reported by the US National Health and Nutrition Examination Survey (NHANES) conducted by the Centers for Disease Control and Prevention. The 2022 report revealed that 80% of urine samples taken from the child and adult population in the US in 2013-2014 contained glyphosate. The data from this National Survey are robust and reliable, and the sample size is representative of the total population, so it can be assumed that exposure to this compound in the United States is widespread.[149]

Another study by researchers at the US National Institutes of Health identified a link between the presence of glyphosate and the presence of molecular biomarkers of oxidative stress in urine samples from the agricultural health study. [150]

In 2018 another study measured urine glyphosate levels in a sample of 71 pregnant women in the state of Indiana in the US, with 93% found to have glyphosate levels above the detection limit (0.1 ng/mL), with a mean of 3.4 ng/mL. Levels were higher among women living in rural areas with a mean of 4.19 ng/mL.[151] Glyphosate was also detected in samples taken from pregnant women in California. Furthermore, studies conducted on infants found that glyphosate was associated with alterations in the development of the reproductive organs of girls, suggesting that glyphosate acted as an endocrine disruptor.[152]

Other studies were carried out to detect glyphosate in the fluids of people spraying this substance. For example, in South Carolina glyphosate concentrations in the urine of several farmers and their families were evaluated 24 hours before and 24 hours after exposure to this chemical; 60% of all the samples taken after exposure contained the herbicide.[153]

In 2017 excretion levels of glyphosate and its metabolite AMPA were measured in individuals at a Healthy Aging center. Mean glyphosate and AMPA levels and the proportion of samples with detectable levels were found to increase over time.[154] A cohort study published in 2021 noted high levels of AMPA in urine and associated this with a 4.5-fold increase in the risk of developing breast cancer among women from different ethnic groups in Hawaii.[155]

Argentina and Brazil

Glyphosate-resistant GM soybeans have been grown in Argentina for nearly thirty years, turning glyphosate spraying into a daily occurrence in this country. This has caused irreparable damage to the health of the population, where an increase in cases of cancer, congenital malformations, as well as endocrine and reproductive disorders has been reported. Alarm bells rang in Argentina when agrochemicals were found in the blood of 80% of tested children living in a suburb of the city of Córdoba, surrounded by fields growing GM soybeans and sprayed with different agrochemicals. Mothers had been complaining about the high incidence of several diseases for years.[156]

The foregoing is consistent with another study conducted in that country, which analyzed people in eight rural communities who were exposed to agricultural pesticides. The findings revealed that the incidence of cancer in these areas was significantly higher than among the general population, especially for people aged 15 to 44. Furthermore, cancer mortality rates were also higher in these rural areas compared to the national average.[157]

In the case of Brazil, in 2022 milk samples were collected from lactating women from urban and rural areas of the city of Francisco Beltrão, Paraná, during the peak period of glyphosate usage on corn and soybean crops in the region, which is noted for the presence of GM crops. The aim was to test for the presence of glyphosate in breast milk and characterize environmental exposure. Glyphosate was found in 100% of the samples analyzed, as well as in drinking water samples from the urban area and in well water in the rural area of the region where the study population lived. Breastfed babies are considered to have ingested amounts of glyphosate during the 6-month period.[158]

Mexico

For a number of years Mayan communities and civil organizations have been denouncing to the Mexican federal authorities the clandestine growing of GM soybean and corn seeds in the municipality of Hopelchén, Campeche.[159]

In 2017 the findings of an investigation into the presence of glyphosate in seven agricultural communities in the municipality of Hopelchén, Campeche – the state's biggest soybean producer – were published. Urine samples were taken from farmers and fishermen. All the tests revealed the presence of glyphosate, but the concentration of this chemical among farmers was more than double that of the fishermen. In addition, traces of this substance were found in drinking water bottles and wells in the municipality. The concentrations exceeded the maximum levels permitted internationally.[160]

The community of Muna, Yucatan, another state in which soybeans are grown, reported harm to the reproductive health of farming families, both in terms of pregnancies among wives of farmers exposed to pesticides – especially organophosphorus – and in terms of semen quality. This is due to changes in the spermatozoa and their genetic material during the sperm formation process, their motility, concentration and damage to sperm DNA, as well as neurological and genotoxic effects.[161,162,163,164,165]

Another study was conducted in the communities of Agua Caliente, near Lake Chapala, the largest lake in Mexico, and Ahuacapán, a community in the southern coastal region of the state of Jalisco, in response to alerts about kidney disease. The urine of children and adolescents was analyzed and glyphosate residues were found in 70% of the samples. Further investigation of exposure pathways found that the waters of the lagoon near the communities, where children's clothes are often washed, contained traces of glyphosate, which means that children are constantly exposed to the herbicide. [166]

Another study performed in the same area detected glyphosate in the urine of children of all ages living in the rural community of Lake Chapala in Jalisco, regardless of whether they had direct contact with this substance or not. Glyphosate levels in urine were found to be highest in May, which is the season of soil preparation using pesticides, with glyphosate being widely used to kill weeds. Children and women in this area perform a specific role in these activities.[167]

Furthermore, another study conducted in the area in connection with the high prevalence of chronic kidney disease appearing from early stages found that this condition was associated with social and environmental factors, including exposure to pesticides.[168] The same research group conducted another study in the area – identified as a region where chronic kidney disease and malnutrition are endemic – with the aim of measuring glyphosate levels in the urine of children in a rural community, including children between 6 and 16 years of age. Alarmingly, all the samples tested positive for glyphosate, which was even present in individuals who had no direct contact with it.[169]

China

A recent study – the first of its kind in China – concluded that there is a high prevalence of glyphosate in the urine of children (92.05% of samples) living near the country’s main vegetable-producing regions. In addition, a positive correlation was found between continuous exposure to glyphosate and the presence of kidney damage biomarkers.[170]

European Union countries

Spain and Portugal are the only EU countries where GMO planting is permitted. Spain in particular is one of the EU's biggest users of agrochemicals, not only in agricultural areas but also in cities and towns. It has been reported that its legislation on the use of glyphosate is very flexible, which is why a study conducted in 2013 reported the presence of glyphosate in 40% of urine samples in Spain.[171]

In the case of Portugal, a study was conducted to detect glyphosate in the urine of children (aged 2 to 13) and to identify possible determinants of exposure. Glyphosate was found in 95.1% of the samples. Concentrations were higher in the urine of children aged 7 to 9 living near agricultural areas, with a higher percentage of home-produced food consumption and whose parents used herbicides in the backyard.[172]

France is Europe's biggest user of pesticides, so a team of scientists set about the task of determining glyphosate levels in the country's general population and look for an association with seasons, biological traits, lifestyle, dietary habits and occupational exposure. Their findings reveal that there is widespread contamination among the French population, with glyphosate quantifiable in 99.8% of urine samples; the highest levels were found in men and children. It was found that contact takes place through food and water intake, since the lowest glyphosate concentrations are associated with the consumption of predominantly organic food and filtered water. Higher occupational exposure among farmers working in wine-growing environments was also confirmed.[173]

A study conducted with children and adolescents living in rural areas with intensive agriculture in northeastern Slovenia analyzed the presence of glyphosate and AMPA. Sampling was performed in two separate periods, in accordance with the presumed seasonal use of pesticides. The first period was winter (January-March), when pesticide use is not common, and the second was late spring-early summer (May-June), when pesticide use is more intensive. Glyphosate and AMPA were detected in 27% and 50% of urine samples from the first period, respectively, and in 22% and 56% of the second period. Urine samples from children indicated a greater degree of exposure that did not differ significantly between the two sampling periods. However, the frequency of extensive food consumption revealed higher exposure to glyphosate and AMPA only among individuals with higher consumption of nuts and brown rice.[174]

United Kingdom

In the United Kingdom urine analysis was performed in 2022 to investigate exposure to 186 common insecticide, herbicide and fungicide residues. Glyphosate was found in 53% of urine samples, with only 10 cases (8%) in which glyphosate levels were below the quantification limit. Residue levels of glyphosate, pyrethroids and other organophosphates were comparable to those of previous studies conducted with other European populations. In the study, the researchers highlight that the consumption of fruits and vegetables has health benefits, but if they are grown by conventional means they can lead to a higher intake of pesticides. They also note that people who regularly consume organic products have higher healthy eating index values, although other lifestyle choices are also contributing factors.[175]

Thailand

A 2017 longitudinal study measured glyphosate and paraquat concentrations in maternal and umbilical cord serum in pregnant women who gave birth in three provinces of Thailand. Glyphosate concentrations in the serum of pregnant women at the time of delivery were significantly higher than in the umbilical cord serum. Women with serum glyphosate levels above the detection limit were 11.9 times more likely to be working in farming, 3.7 times more likely to live near agricultural areas, and 5.9 times more likely to have a family member who worked in agriculture. These findings confirm that pregnant women who work in agriculture or live in families that work in agriculture have greater exposure to the herbicides studied.[176]

These studies acquire great relevance thanks to the sizeable accumulation of scientific research published on the health risks and harm associated with glyphosate, glyphosate-based herbicides and their degradation metabolite. Details of these risks and harm will be presented in detail in a later section. Knowledge of the health risks posed by exposure to the glyphosate herbicide, its formulations or its degradation metabolite (AMPA) requires an assessment of the general population exposed to the herbicide, along with data on glyphosate and AMPA levels in urine and/or blood, as these are the main biomarkers of exposure. Such data are, however, currently scarce.[177]

For example, an evaluation of the presence of glyphosate in urine can provide reliable estimates of actual internal human exposure that can be compared to appropriate benchmark values, such as Acceptable Daily Intake (ADI) or Acceptable Operator Exposure Level (AOEL).[178]

The existence of reports revealing higher glyphosate levels in children means it is crucial to conduct analyses aimed at these populations that are more vulnerable to the effects of environmental contamination because of their greater nutritional requirements, physiological development and intense outdoor activities.[179]

A review of the scope of international scientific literature with international evidence of the presence of pesticides in children's urine samples, and their effects on health, identified a number of studies of pesticides in the urine of populations from various parts of the world. These studies included several investigations showing that infants are exposed to pesticide residues through food intake and the use of these toxic substances. Reported effects included neuronal damage, diabetes, obesity and lung impairments. The same research reports international evidence that organic diets for children are a successful way to reduce urine pesticide levels.[180]

Occupational and non-occupational glyphosate exposure by environmental settings, in GM crop fields

In different countries glyphosate and/or AMPA have been detected in the soil, the atmosphere and surface and ground water bodies, including drinking water, in urban, peri-urban and agricultural environments, as well as in marine environments. Several investigations of this type have been reported in countries with a high prevalence of GM crops, as well as affectations among people occupationally exposed to the herbicide and non-occupationally exposed because of their presence in contaminated environments. A few examples are outlined below.

United States

Glyphosate and AMPA were detected in soil, surface and ground water, as well as in the atmosphere, by the US Geological Survey, which analyzed the presence of pesticides in the Mississippi River. It was found that 77% of rainwater samples contained glyphosate.[181, 182] Another study also revealed extensive volatilization of amines, which are often included in glyphosate, 2,4-D and dicamba formulations to boost herbicide solubility and reduce its volatilization. Amines can impact atmospheric chemistry, human health and climate. The release of herbicides containing amine salts is responsible for the release of 4,000 metric tons of amines a year in the United States.[183]

Argentina and Brazil

Glyphosate-tolerant GM soybeans have been grown in Argentina for almost thirty years, turning glyphosate spraying into a daily task in that country. This has resulted in irreparable damage to the population's health with reported cases of cancer, congenital malformations, and endocrine and reproductive disorders on the rise.[184]

One study investigated eight small rural communities that were exposed to agricultural pesticides. The findings revealed that the incidence of cancer in these areas was significantly higher than among the general population, especially among people aged 15 to 44. In addition, cancer mortality rates were also higher in these rural areas compared to the national average. These results highlight the need to implement pesticide reduction strategies, especially in areas with dispersed rural populations.[185]

In Argentina glyphosate accounts for 76% of the total package of chemicals used in agriculture, and there are several examples of research addressing the problem of GBH accumulation in soils and water bodies, as well as their harmful impact and relationship with GM soybean cultivation.[186,187] Glyphosate has been found in soil and in peri-urban horticultural areas. Furthermore, Argentine specialists from La Plata National University analyzed rainwater in urban and peri-urban areas in the Las Pampas region between 2012 and 2014 and found glyphosate in 90% of samples.[188,189]

There are also studies in Brazil addressing the issue of excessive herbicide use in urban systems, especially for controlling so-called "weeds" in roads, parks and gardens.[190]

Mexico

The presence of glyphosate has been reported in coastal waters of the Yucatan Peninsula, especially in areas close to zones with more intense agricultural activity.[191] In 2017 significant levels of glyphosate were found in groundwater and bottled water in Campeche, Mexico, confirming the herbicide's excessive use in agricultural areas where, as mentioned, there have been complaints of illegal planting of GM soybeans and corn.[192] A pilot study provides important data on glyphosate exposure in the region and raises concerns about the potential impact on human health and the environment.[193]

In the state of Sinaloa in northern Mexico, where agriculture is the main activity and GM cotton crops (mostly HT) are grown, glyphosate concentrations were detected in the main rivers of the region, posing a health risk for the human population and wildlife.[194]

As has already been mentioned, groups of scientists have demonstrated the presence of glyphosate in human fluids of farmers, children and adolescents from agricultural communities in the Bajío region. These findings were made in rural, urban and peri-urban settings in which there is occupational and non-occupational exposure.[195]

Colombia

Sierra Nevada de Santa Marta is home to the Kogi, Sanha, Kakuama and Ika peoples, who are beset by a number of issues including violence caused by the planting of illegal crops and the spraying of glyphosate on these crops. This has led to the poisoning of people as a result of the ingestion of glyphosate upon eating local endemic vegetables, commonly harvested by these communities as part of their food system and contaminated with the herbicide.[196]

The eradication of illicit coca cultivation with glyphosate was suspended by the Constitutional Court in Ruling T-236-2017 to guarantee the rights of the ethnic communities of the department of Chocó – in particular to a healthy environment and good health – against impacts on the physical, cultural, social and economic integrity of these communities by the spraying of glyphosate. In this case the substantial impact on the vital relationship between ethnic communities and the land, water sources and the environment in their territories was analyzed. The ruling stated that the "precautionary principle requires that the State control risks to human health by means of constitutionally reasonable regulation, when there is objective evidence of such risks, even if the evidence is not conclusive".[197]

2. Scientific evidence of effects on human health

2.1 Effects on human health of consumption of the main GM corn events

Chronological overview of scientific studies on the negative effects of GM corn consumption and its potential impact on human health

In 2000, when GM crops of Bt corn and HT soybeans had already made an exponential leap in terms of adoption with planting for commercial use in the US (percentage of cropland acres used for each crop)[198], the scientific literature was largely devoid of studies by biotech companies demonstrating the safety or nontoxicity of GMOs accessible to the scrutiny of the international scientific community.[199]

Nor was there any information on long-term effects but, according to a group of experts convened by the WHO and FAO, "the pre-market safety assessment already ensures that the food (GM) is as safe as its conventional counterpart".[200] Since then, there has been a reported lack of transparency on the part of the regulatory authorities in the US regarding risk assessments and scientific evidence of the safety and security of GMOs.[201]

Since the beginning of the 21st Century, some studies were conducted on the risks for allergic patients and the possible allergenicity of new GM foods.[202] Apart from this, only two other studies could be found: [203,204,205]

- One, carried out in rats on the toxicity of GM potatoes, with the Cry1 BtK gene strain HD1 insert, found possible structural alterations in the intestinal cells of individual rats. Several villi appeared with an abnormally large number of enterocytes; fifty percent of these cells were hypertrophied and multinucleated; the mean enterocyte area increased significantly; several forms of secondary lysosomes or autophagous vacuoles were detected in these cells; the basal lamina along the base of enterocytes was damaged in several foci; several microvilli associated with variable shape cytoplasmic fragments were found ruptured and some of these fragments contained endoplasmic reticulum as well as ring-shaped annulate lamellae; in addition, Paneth cells were highly activated and contained a large number of secretory granules. The researchers suggested thorough testing of all GMOs to avoid risks prior to commercialization.
- Another study evaluated whether standard broiler chicken diets prepared with GM Bt corn (Event Bt176, which expresses tolerance to the glufosinate herbicide, insect resistance and antibiotic resistance) had any adverse effects on broilers. In this case, no significant differences were found between individuals fed with and without GMOs. However, this study compared only body measurements and survival, without evaluating physiological or toxicological effects.

In 2000 the group of experts convened by the FAO and WHO agreed that if a GM food contained a gene from a source with known allergenic effects, the genetic product should be assumed to be allergenic unless proven otherwise.[206] Since 1999, it had been shown that exposure to *Bacillus thuringiensis* triggers allergy-associated immune system responses among agricultural workers exposed to Bt pesticides, including both spores and vegetative extracts of the bacterium, concluding that exposure to Bt aerosols can cause allergic skin rashes and induce antibodies (immunoglobulins), or both.[207]

Cry proteins act like microscopic needles piercing the cell membranes of the insect gut. This causes the intestinal cells to rupture and release their contents, including bacteria and toxins that cause sepsis. The piercing of cell membranes and subsequent sepsis lead to the death of the pest insect. This usually happens a few days after ingestion of the Cry protein.[208] Bt transgenes, which express insecticide-acting protoxins, can be toxic or allergenic for humans.[209]

In addition to allergenicity and immunogenicity, other effects of *B. thuringiensis* have been described in subsequent years, including the induction of oxidative stress in mice livers,[210] selective hematotoxicity and a significant reduction in bone marrow cell proliferation that demonstrated cytotoxic effects.[211] Furthermore, Cry1Ac can cause anaphylaxis.[212]

In 2001 the famous case of GM corn known commercially as StarLink came to the attention of the public. This was an event expressing HT and Bt traits that had been approved in the US for animal consumption, but which was also marketed in that country, as well as in Mexico and other Latin American countries, for human consumption.[213] The event expresses the insecticidal protein Cry9c. Despite being recognized as an allergenic protein, StarLink was permitted for use in animal feed and was subsequently detected as an ingredient in food for human consumption.[214]

It was never demonstrated that the consumption of this GM corn was safe because the Cry proteins it expressed remained in the gut. Its license was definitively revoked only following social pressure in the form of a campaign by organizations. Several people reported adverse effects from the consumption of foods containing this corn, and cases were reviewed by the US Centers for Disease Control which concluded that that the affectations were possibly linked to StarLink.[215] The StarLink story sets a paradigm because it illustrates a number of flaws in the regulatory systems that have been permissive of the use of this recombinant DNA biotechnology. Experiences with this GM corn suggest that regulatory approaches should be strengthened.[216]

The marketing of Bt corn has continued despite scientific evidence continuing to identify adverse effects associated with Cry proteins. For example, in 1999 the *Bacillus thuringiensis* recombinant Cry1Ac protoxin was shown to be highly immunogenic, as has been confirmed repeatedly by a number of studies in subsequent years.[217,218,219,220]

In 2007 GM crop adoption in the US was still on the rise. By that time, HT and Bt corn GMOs had already taken up 50% of cropland area earmarked for these crops in the United States, while HT soybeans were already close to 90% and HT and Bt cottons were around 60%.[221] At that time, international regulatory bodies were claiming that all GM products on the market had undergone risk assessments by national authorities without any risk to human health being detected.[222]

However, a comprehensive 2007 review of scientific literature on the potential toxicity of GM plants found a "surprisingly limited" number of studies, and almost none of them had been published by the biotech industry, which would have been helpful for the scientific community to read and review. The aim of the investigation was to critically review the scientific literature published on the possible toxic effects of GM plants and the risks for human health. What was found was a paucity of experimental data: studies of short duration, mainly nutritional studies with very limited toxicological information, nothing that guaranteed the long-term safety of GM food consumption.[223]

Of all the publications studied, three review articles were found (the only ones available: one from 2001 and two from 2003) that concluded that: 1) the assessment of concordance between the chemical composition of GM and that of conventional crops ("substantial equivalence") was insufficient; 2) subchronic *in vivo* studies are necessary, given the incipient nature of crop genetic engineering; 3) there was no full understanding of the physiology, genetics and nutritional value of GM crops. Since then, much was said about the lack of knowledge of toxic substances in GM crops, which might not be "substantially equivalent" to non-GM crops in terms of genome, proteome and metabolome. Given the scant number of studies (only 10 in 2003) on the health effects of GM food and feed, much more scientific research and effort would be necessary before any guarantees could be made as to the long-term safety of consumption. The lack of transparency in the testing of each GM product prior to its introduction on the market was also called out.[224]

That same review – particularly in the case of GM corn – failed to find any studies that included specific toxicology analyses. However, a reported industry study conducted on rats fed NK603 GM corn concluded that diets based on GM HT corn were as safe and nutritious as diets based on available hybrid corn. This was despite the fact that the research was conducted over a relatively short period of GM corn management[225], the total level of corn protein in the diet provided was only 3.3%, and the presence of GM protein (several times lower) may not be sufficient to cause any adverse reactions.[226]

At this stage it is crucial to point out that it is not possible to conduct experimental studies on humans to demonstrate the effect of GMO-based diets. The potentially negative effects on human health are based on studies carried out with experimental animal models, which are an indispensable scientific source for the design of GMO biosafety measures and regulations. These are the studies explored in this section. Another important source is comprised of studies based on statistical models, which are presented in another section of this document.

In 2008 the intestinal and peripheral immune response from diets containing GM Bt corn (MON810) was evaluated in mice under vulnerable conditions (recently weaned or old). MON810 corn triggered intestinal and peripheral immune responses (alterations in the percentage of T and B cells and CD4+, CD8+ subpopulations, γδT and R T, in the gut and peripheral zone sites, in addition to increased serum IL-6, IL-13, IL-12p70 and MIP-1).[227]

By 2009 the growing adoption of GM crops in countries approving this technology continued, while scientific research showed that: GM crop risk assessments for human health and nutrition were not systematic and lacked detailed methodologies and comprehensive guidelines for testing their safety; the analyses featured different feeding periods, animal models and parameters; regulatory agencies were advised to adopt developments and recommendations made by advisory committees and scientific organizations, presented in scientific publications.[228]

At the same time, there were reports of adverse microscopic and molecular effects of some GM foods on different organs or tissues, especially in transgenic events of insect-resistant corn and herbicide-tolerant soybeans. It cannot be ruled out that the feed given to experimental animals contained glyphosate residues. The most relevant findings on the effects of GM corn are summarized below:[229]

- Diets with GM Bt corn events:[230,231,232] Changes within the variability of the reference population in male rats, there was a slightly higher white blood cell count, lymphocyte count and absolute basophils count and a decrease in chloride, while in female rats there was a slight increase in glucose. Statistically significant incidence of renal tubule mineralization (female rats) and a high incidence of focal inflammation and tubular regeneration changes in the kidneys (male rats) among rats fed with GM Bt corn. Under more specific statistical analyses to make correlations, slight but significant dose-related variations in growth were observed; chemical measurements revealed signs of hepatorenal toxicity, increased triglycerides in females, and decreased urinary excretions of phosphorus and sodium in males. This means that the two main organs of detoxification (liver and kidneys) were altered, all with diets based on very low levels of GM protein. In salmon, there were small changes in stress protein levels and activities, significant changes in the number of white blood cells, associated with an immune response.

Another study performed with rats fed with GM Bt corn for three generations showed different levels of minimal granular degeneration in the liver, increases in the parietal layer of Bowman's capsule and minimal tubular degeneration in the kidneys, alterations in the amounts of creatinine, globulin and total protein. These histopathological and biochemical effects were reported by the researchers as minor changes.[233]

Another investigation into the reproductive assessment by continuous breeding (RACB) discovered that GM corn with the stacked event NK603 x MON810 affected reproduction in mice, showing differences in the number of offspring, size and weight, with greater interindividual variability in the group fed with GM corn. In terms of organ weight, targeted dietary effects were found in kidneys while an electronic histological study of cell nuclei revealed differences in terms of fibrillar centers, dense fibrillar components and pore density in hepatocytes, possibly indicating effects on metabolic parameters. Metabolic pathway analyses revealed differences between the groups regarding certain important pathways, including the interleukin signaling pathway, cholesterol biosynthesis and protein metabolism.[234]

A comparative analysis of blood and organ system data from trials with rats fed the three main commercialized GM corn events (NK603, MON810, MON863), found in food and feed worldwide, where approximately 60 different biochemical parameters per organ were classified and measured in serum and urine, clearly revealed sex-dependent and often dose-dependent effects for the three GM corn events. The effects were associated primarily with the kidneys and liver, although they were different across the three GMOs. In addition, other harmful effects were observed for the heart, adrenal glands, spleen and hematopoietic system.[235]

A fresh review of scientific literature in 2011 reported a considerable increase in the number of available publications on the potential toxic effects and health risks of GMO consumption. The review reported research that raised serious concerns about the lack of safety of GMO-based foods. It also pointed out that some research groups were suggesting that GM products (primarily corn and soybeans) were as "safe and nutritious" as the respective conventional non-GM plants, specifying that most of these studies had been carried out by biotech or associated companies that commercialize GMO plants.[236] This paves the way for further controversy in the science of GMO risk: conflict of interest and the involvement of biotech industry companies in scientific malpractice and manipulation of information. We will explore these topics later on.

A subsequent short-term comparative study of weaned male pigs investigated the effects of feeding with GM Bt corn (MON810). The findings of potential harm to the animals' health included reports that pigs fed with GM corn consumed more feed than control pigs and were less efficient in converting feed into profit; their kidneys tended to be heavier than those of control pigs; even though the morphology of their small intestine was the same, the duodenal villi tended to have fewer goblet cells,[237] which secrete mucus to protect and lubricate the internal surface of that organ. Other research by the same group of scientists found alterations in the immune responses of weaned pigs also fed with GM Bt corn (MON810), although the researchers have no conclusive opinions on the biological relevance of these findings.[238]

Another investigation conducted with pigs, but in this case in a long-term study to comparatively evaluate the toxicological effects of a diet based on a combination of GM soybeans and corn (with stacked events), showed that the GMO-based diet was associated with gastric and uterine differences (the uteruses of females fed with GMOs were 25% heavier), a higher incidence of severe stomach inflammation (a rate of 32%) among males and females. The GM corns used in this investigation were: a triple stack of Bt and HT (NK603, MON863 and MON810), a double stack of Bt and HT (NK603 and MON810), plus single HT events. In addition, soybeans tolerant to the glyphosate herbicide *(Roundup Ready*) were used.[239]

In 2012 an analysis was performed of toxicological response variables among rats on diets containing insect-resistant GM corn (Ajeeb YG, with the MON810 trait), compared to its isogenic counterpart. Rats fed GM corn underwent histopathological changes: cytoplasmic vacuolation of centrilobular hepatocytes occurred in the liver with fatty degeneration of hepatocytes; blood vessel congestion occurred in the kidneys along with cystic dilatation of renal tubules; necrosis was observed in the testes along with desquamation of spermatogonial germ cells lining the seminiferous tubules; the spleen showed mild lymphocytic depletion and splenic congestion; and the small intestine revealed hyperplasia, with hyperactivation of mucous glands and necrosis of the intestinal villi also detected.[240] The research group also measured some visceral organs (heart, liver, kidneys, testes and spleen) and serum biochemistry: the sample of rats fed with GM corn underwent a number of changes with increased or decreased organ and body weight or serum biochemical values.[241]

A study was conducted of the cytotoxic effects of stacked Bt and HT events, in which glyphosate residues were present, to analyze synergistic effects in human cells. It was demonstrated that Cry1Ab (present in different transgenic events in corn and other GM plants) caused cell death beyond 100 parts per million; that GBHs of the *Roundup®* brand trigger necrosis and apoptosis at doses well below agricultural dilutions; that Cry1Ab and Cry1Ac reduced GBH-induced caspase activations, which could delay the activation of apoptosis. These findings show that modified Bt toxins are not inert in human cells and may have side effects in combination with glyphosate-based pesticide residues.[242]

During the 2010s there were further findings on the relationship between GM corn consumption and negative impacts on experimental animal models. One such case was the onset of oxidative stress, as demonstrated by a comparison published in 2013, in which GM Bt corn (MON810) and its near-isogenic counterpart were used in salmon feed. Fish fed with Bt corn utilized feed less efficiently (lower protein and mineral digestibility and lower lipid and energy retention efficiency); increased intestinal weight; increased interferon-g and decreased sodium-glucose cotransporter mRNA expression; a transient increase in the presence of T-helper cells. In addition, Bt corn appeared to enhance oxidative cellular stress in the distal intestine of immuno-sensitized fish.[243] Omic science analysis led to the subsequent discovery that GM HT corn (NK603) produces compounds (putrescine and cadaverine) that can trigger allergic reactions and the formation of free radicals, also causing oxidative stress, which is linked to a number of chronic diseases such as diabetes and cancer. This is an unforeseen effect of transgenesis.[244]

A 2013 scientific study investigated the effects of GM corn on rat pups between the start of dry food consumption and puberty. The rats were fed with GM Bt corn and conventional corn. After the experimental period, the length, height and weight of the liver, spleen, lung and kidneys in the group of rats fed with Bt corn were found to be different. Some obvious differences were observed in the mean values of serum chemistry and hematology parameters, namely glucose, urea, total protein, cholesterol, triglycerides, very low-density lipoproteins, low-density lipoproteins, calcium, phosphorus, sodium, potassium and chlorine. These findings demonstrated that GM corn can impact organ length, height and weight, as well as causing alterations in serum chemistry and hematology values.[245]

At the same time, immune reactions following the inhalation of pollen and plant debris from GM Bt corn (MON810) have also been studied, revealing an influx of lymphocytes and eosinophils in bronchoalveolar lavage and an increased release of cytokines in mediastinal lymph node cells. Furthermore, exposure to purified Cry1Ab proteins was tested and confirmed to cause inherent immunogenicity and allergenicity.[246]

It has also been shown that GM Bt corn that produces Cry1Ab has toxic effects on crayfish *(Orconectes rusticus*) commonly found in headwater streams near GMO cultivars, among which survival was 31% lower.[247]

In 2014 a compelling study was republished, following strict peer review and thorough scientific and biotech industry scrutiny, on the long-term health effects in mice of consuming glyphosate-tolerant GM corn (NK603) in the form of kernels grown with and without *Roundup*®, as well as the effects of ingesting *Roundup*® diluted in water. [248]

This is the first study on the effects of this type of corn in which all observations are reported chronologically. It includes follow-up on findings in 34 organs observed and 56 parameters analyzed, at 11 time points for most organs. The results of biochemical analyses confirmed severe chronic renal deficiencies for all treatments and both sexes, in which 76% of the altered parameters were kidney-related; in treated males, liver congestion and necrosis increased 2.5 to 5.5 times; the incidence of marked and severe nephropathies was also generally 1.3 to 2.3 times higher; in females, all treatment groups showed a 2- to 3-fold increase in mortality with earlier deaths. These findings were also evident in three groups of males fed GM corn. All the results were sex-dependent and pathological profiles were comparable.[249]

It should be noted that this same study was not designed, per se, as a carcinogenicity study; however, it clearly demonstrated that females developed large mammary tumors more frequently and earlier than the control group. Males, on the other hand, developed up to four times as many large palpable tumors, starting 600 days earlier than the control group, in which only one tumor was observed. The pituitary gland was the second most impaired organ, with sexual hormone balance undergoing alterations due to the consumption of GM corn and *Roundup®* treatments. These results can be explained not only by the nonlinear endocrine disrupting effects of *Roundup®*, but also by the overexpression of the EPSPS transgene or other mutational effects of GM corn and their metabolic consequences.[250]

The paper's first version in 2012 had a major media impact and, at the same time, aroused controversy, prompting the authors to publish specific responses to detractors in 2013[251] in the same scientific journal as the 2012 original. In 2017, following a court order forcing Monsanto, the patent's owner and marketer of NK603 and the GBH *Roundup®* formula, to disclose its internal communications, it became known that the company had orchestrated a campaign to discredit the 2012 study and its authors, in particular Gilles-Eric Seralini.[252,253,254] This matter is explored further in later sections which present additional aspects of information manipulation and scientific malpractice by the company in question, based on these same documents that were dubbed the "*Monsanto papers*".

In 2015 there were more studies on the biological effects of GMO-based diets in mice, analyzing the impact of a GMO-based diet (GM corn and soybeans) compared to a non-GMO diet. A range of combined parameters, including biochemical, histopathological and cytogenetic, were used to assess the impact on animal health. The results of all the parameters evaluated were consistent and confirmed that the GMO-based diet has harmful histopathological and histochemical impacts. Biochemical alterations were observed in alanine aminotransferase, aspartate aminotransferase, creatinine, uric acid and malondialdehyde concentrations, while genotoxicity was detected in germ cells, along with a higher number of cells with chromosomal aberrations, and in liver cells, and higher proportions of DNA fragmentation.[255]

In 2016 a long-term pathology report was published on the effects on German cows fed with GM Bt176 corn (with expression of glufosinate herbicide tolerance, insect resistance and antibiotic resistance), the first GM corn to be commercially released in Europe. The data come from an independent, modern farm conducting the first and longest ever on-farm observation of mammals by an experienced farmer and certified veterinarians over a specific period of time. Farm-grown GM Bt176 corn was introduced progressively into the controlled diets over the years, coinciding with regular increases in the diet's GMO content (0-40%). The proportion of healthy cows with high milk production fell from 70% (normal rate) to only 40%. Mortality peaked in 2002. The GM corn, later withdrawn from the market, was at that time the only administrative change planned in the cattle's diet. At the peak, 10% of the cows died, preceded by long-lasting paresis syndrome, without hypocalcemia or fever but with biochemical renal failure and mucosal or epithelial complications. No microbial origin was identified, even though it was intensively investigated.[256]

By 2016 there were also studies confirming that GM Bt corn can affect the characteristics of the gastrointestinal tract, profoundly altering its functioning and structure. A study evaluated the impact of this type of GM corn on the histological structure of the jejunal mucosa of adult male albino rats using different histological, immunohistochemical and morphometric methods. Specimens from the GM corn-fed group showed different types of structural changes; focal destruction and loss of villi, leaving a denuded mucosal surface alternating with stratified areas, while some crypts appeared completely altered; congested blood capillaries and focal infiltration with mononuclear cells; significant positive up-regulation of PCNA expression, an increase in the number of goblet cells and a significant increase in both villus height and crypt depth; marked ultrastructural changes in some enterocytes with focal loss of the microvilli border; some enterocytes had vacuolated cytoplasm, swollen mitochondria with ruptured cristae and dilated rough endoplasmic reticulum (rER); some cells had irregular dark nuclei with abnormally clumped chromatin.[257]

Another study investigated the impact on rat stomachs of a triple-stacked GM corn variety containing modifications for insect resistance (through cry1Ab and cry3Bb1 genes) and glyphosate tolerance (through an EPSPS gene). The study examined the stomach mucosa of rats fed GM corn, finding alterations in the apposition of tight junctions, gland dilatation with epithelial elongation and dysplasia among GMO-fed rats.[258]

Other prominent effects of different Bt and HT GMOs, other than GM corn, reported in the scientific literature are as follows:

- GM soybean.[259,260,261,262,263,264,265,266,267,268,269,270] Minor pathological findings in female mice, such as corneal opacity, renal and pituitary lesions, and uterine

hydrometer randomly distributed among all groups; irregularly shaped cell nuclei and an increased number of nuclear pores; decreased nucleoplasmic and nucleolar splicing factors, accumulation of perichromatin granules; reduced Sm antigen, hnRNP, SC35 and RNA polymerase II; cell proliferation in distal intestine compared to the control diet; decreased somatic index in spleen and distal intestine; increased LDH1 in kidneys and heart, moderate swelling in distal intestine and increased lysozyme activity in kidneys; depletion of zymogen granules, disorganization of acinar cells. Significant decrease in growth of kids from mothers fed GM soybeans; stunted growth starting immediately after birth, presumably due to the lower percentage of protein in colostrum and milk at 15 days of lactation; in addition, there was lower immunoglobulin concentration, closely linked to a number of growth and maturation factors.
- GM Bt eggplant.[271] Affections were found in the chemistry and blood cells of goats (alterations to prothrombin time and biochemical parameters such as total bilirubin and alkaline phosphatase) and rabbits (alterations to prothrombin time, higher levels of bilirubin in some cases, albumin, lactate dehydrogenase and the hepatic markers alanine and aspartate aminotransferase; sodium, glucose, platelet count, mean corpuscular hemoglobin concentration and hematocrit levels were also modified); in the case of cows, milk production and composition changed by between 10 and 14%; in rats there was a decrease in liver weight and a relative decrease in the liver to body weight ratio; glucose alterations in broilers; changes in average feed conversion rates and efficiency in fish.

Lastly, a recent systematic review (2022) of studies on the consumption of GM foods by animals and humans, in terms of effects and adverse events, searched for in vivo, animal and human scientific studies published between January 1, 1983, and July 11, 2020. The review independently identified eligible studies by assessing study quality (as uncertain or with a high risk of bias) with parameters such as author and affiliation, type of literature, study topic, funding, sample sizes, characteristics of the target population, type of intervention/exposure, results and result measurements, as well as details of adverse effects and events. Minor illnesses were reported in one human crossover trial, and out of 204 animal studies, 59.46% reported 22 adverse effects (out of 37), of which 16 were reported as serious adverse effects (mortality, tumors or cancer, significantly low fertility, decreased learning and reaction ability and certain organ abnormalities). The adverse effects were linked to GM foodstuffs involving 5 transgenic events with corn (NK603×MON810; NK603; MON863; MON810; and MON863×MON810×NK603), one with soybeans (GTS40-3-2) and one with rice

(Shanyou 63), all of which had obtained regulatory approval in certain countries/regions.[272]

Lack of precision in transgenesis, at the genomic level, translates into unexpected and undesired consequences, at the epigenetic level: the myth of substantial equivalence under the microscope of omic science

Biotechnological breakthroughs in the obtaining of genetically modified seeds, together with the keenness of producing companies to market them with the fewest possible restrictions, prompted the Organization for Economic Cooperation and Development (OECD)[273] to encourage the adoption of the principle of substantial equivalence as the primary basis for assessing the risks of these new biotechnologies, leaving as a secondary concern the need to carry out the pertinent analyses to detect metabolic changes which, as we saw in previous sections, given the imprecise nature of transgenesis techniques, are inherent to the process of genetic transformation.[274]

The principle of substantial equivalence is intended to be as flexible, malleable and open to interpretation as required. The FAO/WHO note that the determination of substantial equivalence "does not constitute a safety assessment per se, but is a dynamic, analytical exercise in the safety assessment process of a novel food, taking an existing food as a reference… it is not a safety assessment per se; it does not characterize the hazard, but is used to structure the safety assessment of a genetically modified food in relation to its conventional counterpart" and that "the characteristics taken as a reference for making equivalence comparisons must necessarily be flexible and will change over time as the needs of the food processing industry and consumers, as well as experience, change".[275]

Another highly relevant criticism of substantial equivalence is that it is mainly based on the comparison of data obtained through very limited chemical analyses, such as compositional analysis and the detection of certain substances or certain phenotypic and agronomic traits, lacking clarity on the biological relevance of these data to prevent potential harm.[276] These analyses almost always turn out to be limited and deficient when targeted analyses are performed, focusing only on the things whose detection is sought.

As we will see later on, modification of biosynthetic pathways can cause alterations at unexpected sites, thereby giving rise to unintended or unpredictable effects, or altered levels of metabolites not detected by the targeted analysis on which the concept of substantial equivalence is based. This means that such an analysis will be incapable of detecting changes in the expression patterns of the organism's own (endogenous) genes, such as positive or negative regulation or the silencing of these genes, nor will it determine whether the inserted constructs or parts of them move within the recipient genome.

The findings of various investigations conducted in the more than 20 years during which GMOs or their products have been used for human consumption, have documented several cases of unexpected modifications that are not detected using the principle of substantial equivalence. One example is the use of RNA interference techniques on GM beans to create resistance to the golden mosaic virus, even though the compositional analysis of the GM variety was "similar" to that of the conventional variety. Nuclear magnetic resonance (NMR) analysis found significant differences in the content of bioactive compounds such as flavonoids, caused by an unexpected modification in the plant's metabolic pathways.[277]

Another study conducted in 2003, in which GM wheat lines containing additional copies of genes that synthesize high molecular weight gluten proteins were analyzed by nuclear magnetic resonance, found significant differences in the levels of certain sugars such as maltose and sucrose, as well as differences in the content of free amino acids.[278]

At the same time, it has also been discussed that GM crops may express unwanted novel proteins that are very difficult to quantify, isolate or purify with the methods used in substantial equivalence assays. These so-called "intractable or undetectable" proteins are usually transcriptional factors, membrane proteins responsible for nutrient transport, signaling proteins or glycosylated proteins that may be involved in allergic reactions whose effects are not estimated in comparative analyses.[279]

As for the detection of unknown allergens in GM varieties, there is the very well-known case of StarLink corn, which expresses an insecticidal protein (Cry9c) that was recognized as potentially allergenic and was therefore restricted in its use in animal feed. It was subsequently detected as an ingredient in food for human consumption.[280]

Breakthroughs in omics techniques have made it possible to study together a large number of molecules involved in the functioning of an organism, e.g. genomics, proteomics, metabolomics, among others. This has, in turn, facilitated more comprehensive comparative studies between conventional plant varieties and their transgenic counterparts. For example, mass spectroscopy-based metabolomics assays demonstrated that conventional corn has a higher bioactive compound and antioxidant content than GM corn.[281]

Multiomics analyses have revealed significant differences – neither expected nor desired – in the metabolites produced by each type of variety. For example, significant disparities have been found in the content and chirality of amino acids such as arginine, serine and aspartic acid between conventional and Bt corn.[282] In addition, differences have been identified in the production of metabolites such as L-carnitine and L-proline-betaine, which are involved in metabolic pathways unaffected by genetic modification.[283]

A comparative proteomic analysis of GM corn (MON810, a Bt-type GMO) and the closest non-GM hybrid under different agronomic conditions revealed a total of 32 differentially expressed proteins between GM and non-GM samples, whose molecular functions are mainly associated with energy and carbohydrate metabolism, genetic information processing and stress response. This study is relevant because it was carried out under field conditions.[284]

Another study investigated undesirable changes in GM corn (MON810) by comparing the proteome of the field-grown transgene and its closest isogenic counterpart. The data showed that energy metabolism and redox homeostasis were unequally modulated in samples of GM versus non-GM corn varieties. Additionally, an allergenic protein was identified in the GM corn.[285]

According to another study, the glyphosate-tolerant GM corn event NK603 produces significantly higher amounts of cadaverine and putrescine. These are free radical activity molecules that can cause oxidative stress, a factor linked to various chronic and degenerative diseases such as cancer and diabetes. The nutrient composition equivalence of NK603 corn to its non-GM counterpart was analyzed. Significant differences in protein and metabolite profiles were revealed, including imbalances in energy metabolism and increased oxidative stress. An increase was observed in polyamines, which can have protective or toxic effects depending on the context and are related to cell death. Taken together, these findings show that NK603 and its counterpart are not substantially equivalent.[286]

A systematic review and meta-analysis of 60 scientific studies found that all of them (except three in which no comparative analysis was performed as such) discovered statistical differences in GM versus non-GM omics profiles. The metabolic pathways most frequently affected were the ones related to carbohydrate, energy, lipid and amino acid metabolism, as well as the processing of genetic and environmental information.[287]

This was also the case with other GM crops, such as GM soybeans (MON87701 x MON89788, a glyphosate-tolerant crop with cry1Ac and cp4epsps transgenic inserts) which have been authorized under the principle of substantial equivalence, even by more stringent legislations such as the European. A 2023 study conducted field trial experiments with this GM soybean, comparing its proteomic profile against reference varieties and its non-GM counterpart. Comparisons revealed six GMO proteins outside the 99% tolerance ranges of the reference varieties in the equivalence test. An evaluation of proteomic and metabolomic data based on a systems biology approach found 70 proteins and the metabolite xylobiose differentially expressed between the GMO and its non-GMO comparator, along with alterations in several metabolic pathways related to protein synthesis and processing. In addition, an allergenicity analysis was performed identifying 43 proteins with allergenic potential that are differentially expressed in the GM soybean variety. The study conclusively found that GM soybeans are not substantially equivalent to their non-GM counterparts.[288]

The authors of this study point out that, according to the respective guidelines of the European Food Safety Authority (EFSA), one of the pillars of GMO risk assessment in the European Union is the comparative analysis of agronomic and compositional characteristics, under the principle of substantial equivalence. The approach proposed in this innovative research provides a better, broader and more accurate way to understand the specific undesired effects of genetic modification on plant metabolism, the biological networks involved and their interactions, thereby paving the way for the formulation of specific risk hypotheses.[289]

In view of the huge potential of omics techniques, several authors have proposed that these techniques be included in the evaluation of GM crops, both current[290] and novel.[291] Disturbingly, however, certain researchers working directly as employees of the biotech industry have expressed a contrary view.[292,293]

Studies on horizontal transfer of antibiotic-resistant transgenes, a public health concern

Horizontal transfer events, which are very common among prokaryotic species, have irreversible consequences in evolutionary dynamics, not only in the species receiving the genetic material, but also in the other species with which the recipient species interacts. The insertion of a single new function inside a gene, in a new environment, can cause a diversification of the same sequence, since, given the absence of a historical functional commitment, there is no negative selection pressure so most of the mutations occurring there are kept instead of eliminated. The ecological-evolutionary consequences for species capable of acquiring new genetic material involve changes in ecological interactions such as increased diversification speeds, more opportunities for exploiting certain resources, or an increase in biotic or abiotic conditions due to the exploration of new ecological niches.

Two cases can reinforce the effects and, especially, the consequences of acquiring a new function:

- *Vibrio cholerae* is a free-living Gram-negative bacterium that lives in aquatic environments; however, the acquisition of two pathogenicity islands causes this new information to transform the bacterium into a highly virulent pathogenic organism, specifically, for the human species.[294]
- *Yersinia pestis* is a Gram-negative bacterium that has caused two major pandemics throughout human history: the Justinian Epidemic in the 6th to 8th centuries and the Black Death in the 14th to 19th centuries. Most pathogenesis systems have been acquired from other bacteria and viruses, including adhesins, secretion systems and insect toxins. *Y. pestis* is a rodent pathogen that is usually transmitted to humans subcutaneously through the bites of fleas parasitizing both species. It has been proposed that *Y. tuberculosis* underwent several horizontal transfer events about 15,000 to 20,000 years ago, which caused speciation by obtaining toxin genes from a plasmid from Salmonella enterica, a plasminogen activator and insect toxins.[295]

In the specific case of transgenesis, one of the hazards associated with the production of genetic modifications – and one the scientific community has been warning about for decades – is the likely spread of DNA fragments from a GMO to the receptor cells of another organism of an unrelated species through horizontal gene transfer.[296,297]

The genetic constructs introduced into plant cells are, in addition to genes that code for insect resistance or herbicide tolerance, antibiotic resistance marker (ARM) genes. ARM genes in GM plants intended for food production have been questioned for safety reasons, as they could be transferred and spread to bacteria in the gastrointestinal tract (GIT).[298,299]

ARM genes such as kanamycin, neomycin, ampicillin, streptomycin or spectinomycin have been used to construct some recombinant DNA plants, thereby raising justified concerns over possible horizontal gene transfer to clinically important intestinal bacteria, given that acquired antibiotic resistance may compromise the therapeutic value of relevant antibiotics used for the treatment of pathogenic microorganisms.[300]

An analysis of 83 studies to detect "foreign" DNA in animals found GM crop-related DNA in 35 of the studies carried out in livestock species (cattle, chickens, pigs, fish, sheep, rabbits and goats).[301]

In 2014 a study was published on the incorporation of transgenes into tissues of different organs and the blood of rats fed for three months on diets containing GM components with DNA segments of the cauliflower mosaic virus promoter -35S, present in a large number of GM crops. The analysis revealed that: 1) fragments of the CaMV-35S promoter had been incorporated into the blood, liver and brain tissues of experimental rats; 2) the mean total transfer of genetically modified target sequences increased significantly as the feeding period increased; and 3) the affinity of different transgene fragments from the ingested GM diet to be incorporated into different rat tissues varied from one target sequence to another.[302]

More recently, this team of researchers studied the transfer of the antibiotic resistance marker (ARM) genes *nptII* and *aadA* from a GMO-based diet into the blood cells and enteric microbiome of Wistar rats. The results unequivocally demonstrated the transfer of *nptII* and *aadA* gene DNA from the GM diet to rat blood and enteric microbiome bacteria cells. The authors believe that these results highlight the importance of exploring the possible effects of horizontal transfer of antibiotic resistance genes from GM products to consumers, as well as drawing attention to the importance of having a broader and better understanding of the factors involved in this phenomenon.[303]

Internationally, 161 approved transgenic events have been recorded with the antibiotic resistance trait, several of which are edible plants such as corn (34 events), potato (27 events) and canola (19 events). To a lesser degree, we also find tomato, alfalfa, sugarcane, apple, chicory, papaya, rice, soybean, melon, safflower, pumpkin, plum and eggplant, as well as cotton (37 events) which can be used to produce edible oils, although this is not its main use.[304]

Moreover, contrary to the standard paradigm claiming that proteins and DNA degrade into small constituents during digestion, robust scientific evidence since 2013 (over 1,000 human samples in four independent studies) has shown that DNA fragments large enough to carry genes from food can avoid degradation and enter the human circulatory system. Studies in animals (trout, goats, pigs and mice) fed GMO-based diets support the hypothesis that recombinant DNA fragments can pass into the bloodstream and even reach various tissues: these fragments have been found in the digestive tract and leukocytes.[305]

Robust statistical evidence of the unsafety of GMO-based foods

A global meta-analysis of the relationship between the glyphosate herbicide and the development of non-Hodgkin's lymphoma cancer has shown a worrying increase in the incidence of this disease in recent decades.[306] A pooled analysis of case and control studies revealed a statistically significant increase in the risk of non-Hodgkin's lymphoma (NHL) associated with glyphosate exposure.[307] Furthermore, another large, consolidated study also identified a link between glyphosate and follicular lymphoma.[308]

Robust statistical data from official US sources, such as the aforementioned National Health and Nutrition Examination Survey (NHANES) of the National Center for Health Statistics, have been used to make epidemiological analytical models that correlate the increase in different diseases with increases in the area of land planted with GMOs and the use of glyphosate in that country. This has shown that, if causality exists, glyphosate and GMOs are linked to the increased incidence of more than 20 chronic diseases (oncological, endocrine, metabolic and neurodegenerative, as well as systemic disorders) in the US.[309,310]

The development of these diseases is complex and multifactorial, but the vast scientific evidence provides elements that systematically point to all the harmful effects of glyphosate on health and how these, in turn, are very closely linked to the development of a large number of diseases and illnesses.

These correlations raise questions about the safety of glyphosate and GM crops, suggesting the need for further research to better understand their impact on human health.[311]

2.2 Other human health considerations regarding the consumption of GM corn

Studies on the advantages of Mexican corn over GM corn for human consumption in the context of the country

Self-sufficiency in high quality corn for human consumption in Mexico

Mexico is the center of origin, domestication and diversification of corn. The domestication process began at least nine thousand years ago.[312,313] Corn holds a key position in Mexican culture and history; it is an essential component of the traditional diet and plays a crucial role in food security [314]

The cultivation of Mexican corn, including native corn, involves agricultural practices that prioritize nutritional quality and culinary diversity. Its value lies in its potential to meet the preferences of consumers seeking products with suitable organoleptic properties. Today farmers engage in integrated or agroecological pest and fertilization management practices to ensure healthy plant growth and optimal yields, in order to provide not only healthy food and feed, but also raw materials for industry.[315]

The corn crop has been studied extensively in genetic research due to its monoecious nature.[316] The importance of corn in Mexican culture and its monoecious flowering have led to the creation of high-quality hybrids by public institutions and universities in the country for more than 70 years.[317]

The production of hybrid corn varieties in Mexico follows an agricultural process, from the selection of seeds with characteristics of interest to their cultivation in fields using conventional agricultural methods.[318] These genetic improvement methods have produced Mexican corn hybrids with increased production yields and improved crop traits, including resistance to diseases, pests and climatic conditions such as drought, and efficiency in the use of resources such as water and fertilizers, with high-protein kernels.[319, 320, 321, 322,323, 324, 325, 326, 327]

According to the Agrifoods and Fisheries Information Service (Servicio de Información Agroalimentaria y Pesquera or SIAP), of the Ministry of Agriculture and Rural Development (Sader), estimates for the 2022/2023 period indicate an output of around 26.7 million tons (Mt) of grain, of which 23.54 Mt were white corn (including white, blue and colored corn), with an average yield of 3.7 tons per hectare (t/Ha), and 3.183 Mt of yellow corn, with an average yield of 6.3 t/Ha. In this same period, estimated human consumption of white corn came to 18.626 Mt. It was used for nixtamal (10 Mt) and flour production (3.554 Mt), with the rest (5.03 Mt) used for self-consumption (food uses by producers and seeds for planting in the following period). [1, 328] These data may vary depending on the source and the time of consultation.

Human consumption of yellow corn came to 2.988 Mt, with 1.448 Mt for various industries (this includes SIAP records of yellow corn for human consumption, used in snacks and cereals and for the fructose industry), 1 Mt for the starch industry and 0.54 Mt for self-consumption (the so-called industrialized yellow corn includes that used in various industries and the starch industry). Meanwhile, animal consumption estimates for the same period come to 15.49 Mt of yellow corn and 4.334 Mt of white corn. Calculations based on these estimates reveal that Mexico imported 16.526 Mt of yellow corn and 0.777 Mt of white corn during this period. In addition, 17.2 million tons produced in 2022 are reported for green fodder.[329,330]

These data confirm the existence of:

- A 4.913 Mt surplus in the production of white corn;
- A 15.49 Mt deficit of yellow corn for animal consumption.

In Mexico alternatives are available for growing high quality corn (non-GM, without agrochemicals such as glyphosate). For example, in June 2021 a corn producer in the state of Sinaloa certified before a notary public and demonstrated to a verification commission, comprised by producers from different Mexican states, the production of white corn without the use of agrochemicals or glyphosate during the 2020-2021 fall/winter period.[331]

This production was carried out using the Farmer Agriculture with Integrated Knowledge and Integrated Management of Induced Crops (Agricultura Campesina de Conocimientos Integrados y Manejo Integral de Cultivos Inducidos or ACCI-MICI) model, which combines scientific knowledge with the traditional knowhow of

[1] Calculations based on SIAP, data from the Regulatory Council for the Corn Supply Chain (Consejo Regulador de la Cadena de Maíz) (2007-2012) and the National Chamber of Industrialized Corn (Cámara Nacional del Maíz Industrializado).

farmers and producers. The model includes the constant measurement of soil conditions, biological pest control and the implementation of agroecological practices such as the use of stubble and organic matter, microorganisms, vermicompost and other bio-inputs. The results showed a 14.2 t/Ha yield, with a 17.2% lower cost, compared to a test plot grown using agrochemicals with a yield of 14.7 t/Ha.[332]

Adaptive advantages of native corn under different environmental and climatic conditions

The great genetic richness of corn in the world today exists thanks to the millenary legacy comprised by the wisdom, knowledge, technologies and culture of native peoples, as well as the fact that farmers continue to plant and select hundreds of local native populations year after year[333] for cultural, social, technical and economic reasons.

Local varieties are grouped into 64 breeds (this number varies depending on the author)[334] comprising a crucial genetic reservoir, independent of any form of private ownership, to cope with adverse environmental and ecological conditions (e.g. particular soil characteristics, climate change scenarios, pests) and to continue with the native genetic improvement of corn. The entire Mexican territory can be considered a center of genetic diversity.[335] Mexican teocinte *(Zea spp.*) is the closest wild relative of corn and offers an important source of genetic variability for the genus, as it maintains the flow of genes.[336,337]

Corn stands as a pillar of the biological and cultural legacy of the Mexican people; it is the backbone of our food system[338] and a large proportion of social, economic, cultural and religious practices in our country are linked to this crop.[339] Furthermore, the process of corn diversification is still alive and its survival requires the conservation of the germplasm, as well as the ecosystems surrounding the croplands, with their biotic and abiotic interactions, the knowhow behind them and the people who sustain them.[340]

The process of sharing genetic material from native corn, evaluating the results of crosses and selecting desired traits has played a fundamental role in the domestication of corn in Mexico, giving rise to an impressive genetic diversity.[341] This diversity is of immense value as a source of genetic variation for the creation of new varieties adapted to diverse conditions and with different utility characteristics.[342] The subsistence farming of native corn is still an important activity to this day.[343]

The specialization of native seeds in farmer and indigenous agriculture is centered on small differences in climate, soil and plot location, while industrial agriculture uses commercial seeds selected and crossed from native seeds under generic controlled conditions, along with a considerable use of external inputs such as agrotoxins and fertilizers.[344] The foregoing factors provide native corn varieties with important adaptive advantages for diverse agroclimatic regions, compared to industrial seeds.[345]

Some of the advantages of native corns that we can mention are their superior adaptation to local climatic conditions, stability to climatic variability, lower costs of the inputs needed for their production and a very significant suitability for preparing traditional dishes as the staple of a people who feed mainly on native corn.[346, 347]

At the same time, the hardiness of native corns under farmer management eliminates the need for complex hybridization processes. They are tolerant to diverse environmental and climatic conditions and have been demonstrated to provide good yields in adverse contexts, certain tolerance to pests and diseases, as well as tolerance to plant bending.[348, 349]

Native corn production also involves seed conservation practices and agricultural biodiversity, which preserve native and traditional corn varieties.[350, 351] In addition, the quality of this corn is associated with sustainable and environment-friendly farming practices; farmers use cultivation methods that minimize reliance on synthetic chemicals, thereby fostering soil health and biodiversity. [352]

For example, according to the theory of trophobiosis put forward by Francis Chaboussou, a healthy, well-fed plant is less prone to attack by pests and diseases because it does not provide the sustenance necessary for the latter to develop, mainly free amino acids and other soluble substances.[353, 354] This principle may partially explain how interaction between crops in the traditional *milpa* system provides a more balanced environment for plants and can reduce the need for synthetic pesticides, as soil nutrition is improved, weed competition is reduced, and the variety of nutrients and bioactive compounds in the growing area makes it more difficult for pests to thrive. [355, 356, 357]

Native corn needs to be protected against the planting and importing of genetically modified corn, which have caused transgenic contamination and ushered in unacceptable risks from a scientific, social and ethical point of view.[358]

Differences in nutritional quality between native and GM corns

The quality of corn, in terms of yield and the nutritional and nutraceutical properties of crops and foods, is a concern both globally and nationally, as demand for it increases in step with population growth and evolving food preferences.[359]

Corn kernels are comprised of three main structures: the pericarp, the germ (embryo) and the endosperm. The proportions of these structures vary in accordance with the intended use of the kernel.[360] They also contain components that make them nutritionally valuable, such as proteins, oils, fiber and minerals, among others. This composition is influenced by factors such as the kernel's physical structure, its genetics, the environment, production processes and other elements of the food chain.[361]

Its protein content (7% to 12%) can be used as an energy source, with prolamins being the most abundant.[362] It also contains approximately 3% to 5% oils, with a higher concentration in the germ of 25-30%. The main fatty acids contained in corn kernels are oleic acid, which accounts for 24% and is monounsaturated, and linoleic acid of the Omega 3 family with 62%.[363] These fatty acids help keep saturated fat levels in the arteries low and play an important role in children's growth and neurological development.

Native varieties are valued for their natural and authentic properties.[364,365] Their genetic and nutritional composition is prized by consumers and markets seeking more natural products. [366,367] The quality of this type of corn is measured in terms of its nutritional content, flavor, texture and suitability for different uses, such as the production of food for human consumption, animal fodder and the manufacture of derived products, such as flours and starches. [368,369]

Native corn contains an array of bioactive compounds, including carotenoids, anthocyanins, phenolic compounds and flavonoids.[370,371,372] Blue, purple and black corns have high concentrations of anthocyanins and flavonoids, [373,374] which provide a higher antioxidant capacity compared to other white corn varieties, [375,376] although the latter also has pigments, albeit in lower concentrations.

In addition, corn quality studies have found that the quality of native corn is superior with its softer and larger kernels, and higher beneficial oil, protein and pigment content.[377] Documented evidence reveals that native varieties also contain levels of protein that can be used as energy, ranging from 8% to 12%.[378]

Native corns with pigmented genotypes (white, yellow, red, pink, orange, black, blue, purple, etc.) are the best option for making tortillas, a staple food for Mexicans.[379] Among other characteristics, they are more resistant to aflatoxins,[380] which are highly toxic, carcinogenic and teratogenic mycotoxins produced by *Aspergillus flavus* and closely related fungi that infect corn cobs and kernels.[381]

These corns, as previously mentioned, contain bioactive compounds that confer antioxidant properties and major health benefits, including antimutagenic, anticarcinogenic and chemoprotective effects.[382,383,384,385] Handmade blue corn tortillas, for example, are high in dietary fiber, phenolic acids and anthocyanins, and even have 4.5 times more ferulic acid, an important antioxidant, anti-inflammatory, intestinal barrier protector and a stimulant of beneficial gut microbiota growth and activity, compared to tortillas made from commercial white corn. [386, 387]

Mexico is the world’s biggest tortilla consumer. This food plays a crucial role in the population's diet thanks to its versatility, flavor and recognized health benefits. Tortillas are made in different ways in this country, the most noteworthy being the traditional manner which is based on nixtamalization, a traditional preparation process that involves soaking them in water with lime (calcium hydroxide) and cooking them.

The main purpose of the nixtamalization process is to soften the corn, facilitate the removal of the husk and improve its nutritional value by increasing the bioavailability of certain nutrients such as niacin (vitamin B3), reducing the presence of mycotoxins in the raw kernel, increasing the resistant starch content, and reducing the amount of phytate, a P-rich compound that can form complexes with elements such as Ca, Fe, Zn, Mg and reduce their bioavailability in the finished product.[388,389,390,391]

On the other hand, there is GM corn which, apart from being inextricably linked to agrotoxins such as glyphosate and glufosinate-ammonium as part of the technological package used for their planting, is of inferior nutritional quality. It contains lower levels of protein, fiber and antioxidants compared to criollo corn varieties.[392, 393,394] Most of it is derived from commercial hybrid lines of white or yellow corn, so it has lower phenolic compound and anthocyanin levels and, therefore, a lower antioxidant capacity [395,396]

Relationship between ultra-processed food and GM corn

So far, the focus has been on the different effects on human health, the environment and social and cultural aspects, which have been widespread in different parts of the world. These conditions began to arise with the so-called "green revolution" which, in Mexico, entailed the imposition of the industrial agrifood system that incorporated certain technologies and agrochemicals, and favored hybrid seeds and large-scale monocultures.[397] Subsequently, since 2000, people's eating habits in Mexico have changed, with so-called junk food becoming a major feature in cities and towns. The result of these trends has been an increase in the number of diet-related diseases, as well as the diminished viability of rural lifestyles and dwindling access to traditional foods,[398] accompanied by the demise of the rituals involved in the growing, harvesting, preparations[399] and forms or types of consumption, as enshrined in a number of festivals.

As mentioned earlier, intensive GM crop farming systems are geared more towards producing large quantities of ultra-processed, high-calorie, but nutritionally deficient food, than combatting hunger.[400,401,402]

The myth that GMOs provide the population with a healthy food supply falls apart when we understand that their main purpose is the manufacture of ethanol and animal feed, as well as to produce inputs for the food industry to make fructose syrups and edible oils for use as ingredients in the production of food of very low nutritional quality.[403, 404]

Latin America ranks fifth in the world in the sale of ultra-processed products,[405] with a high consumption of instant soups, sweet rolls, snacks, processed meats, among other things. In the case of ultra-processed liquids, Mexico ranks first in sales, consuming mostly carbonated beverages, juices, sugary drinks and nectars, with per capita sales of almost 450mL/day.[406] This reveals how our diet shifted towards the consumption of high levels of fat and sugar with negative health consequences.

Considerations about corn consumption patterns in Mexico

Since 2004 the conclusions and recommendations of the Commission for Environmental Cooperation of the Free Trade Agreement – set out in the Ministry's report together with the conclusions and recommendations of the report it prepared with the help of an Advisory Group on "Corn and Biodiversity" – warned that Mexico stands out for the manner and amount of corn consumed as a fundamental foodstuff for diet and culture. The report also stated that GMOs required special attention, since the toxicity of GM corn would be especially high given the Mexican population's consumption patterns, thereby warranting a public policy response.[407]

It also specified that the production of certain drugs and industrial compounds, unfit for human and animal consumption, entailed unique risks to human health. This is a matter of particular concern in the case of corn, which is the staple food in Mexico, produced through open pollination.[408]

In Mexico average per capita consumption of corn as food comes to around 128 kg/year, the highest in the Americas. In the case of tortillas, consumption stands at 328 g/day/per person, totaling almost 12 million tons of tortillas a year.[409,410] These data may vary depending on the source and the time of consultation. FAO data for 2021 comparing Mexico to the US reveal that 10 times more corn and corn products are consumed in the former than in the latter country, the energy supplied by corn is also 10 times greater, while the protein provided by corn is almost 15 times greater (Table 1).[411] Any effects associated with GM corn consumption must take this special circumstance into consideration, including in the case of non-occupational exposure due to the ingestion of agrotoxins residually present in GMOs such as glyphosate.

Table 1. 2021 corn and corn product consumption figures for Mexico and the United States.[412]

US	Quantity of food supply	12.46 kg/person/year
	Energy supplied by food	92.21 kcal/person/day
	Amount of protein supplied	1.59 g/person/day
Mexico	Quantity of food supply	123.47 kg/person/year
	Energy supplied by food	1,024.83 kcal/person/day
	Amount of protein supplied	21.04 g/person/day

2.3 Exposure to glyphosate, a pesticide immanent in GM corn and other GMOs; its effects on human health, even at low doses

As noted above, glyphosate is the most widely used herbicide in the world.[413,414] This implies that the risks associated with exposure to it are extremely high, since the standard sets forth that risk is equal to hazard multiplied by exposure. In other words, as exposure increases (due to the exacerbated use of the pesticide), regardless of whether the hazard (in this case represented by toxicity level) is low, then so does the risk.

It has already been shown in previous sections that human exposure to glyphosate is high and constant. It will now be demonstrated that glyphosate is, in principle (according to FAO and WHO guidelines),[415] a highly hazardous pesticide, given that, in the light of scientific evidence, it meets several of the criteria defining such substances.

As mentioned above, the toxicity of glyphosate and GBHs is significantly higher (as much as 100 times) than other compounds containing commercial formulations.[416] For example, GBH formulations induce apoptosis and necrosis in umbilical, embryonic and placental cells, as well as altering placental integrity in humans.[417, 418, 419] Scientific publications have shown that commercial brand GBHs such as *Roundup*® contain toxic agents including petroleum derivatives[420] and heavy metals.[421]

The toxicity of a substance can be acute or chronic, depending on the dose and exposure time. In the case of glyphosate, both toxicity types are widely documented, along with proven and potential harm to human health, as outlined below.

Acute toxicity

The acute toxicity of glyphosate and GBHs is evident in cases of poisoning that can be classified as voluntary, by self-poisoning – there are several reported cases of deaths caused by this practice, mainly in Asia[422,423] – or involuntary, for example, in several Latin American countries there have been documented cases of poisoning from the spraying of glyphosate on GM crops by light aircraft. Symptoms of exposure include vomiting, diarrhea, respiratory complications and skin rashes. [424,425]

Chronic toxicity

A broad range of scientific studies reveal the potential harmful effects of glyphosate and GBHs on human health following prolonged exposure, causing what is known as chronic toxicity.[426, 427, 428]

About the harmful effects of glyphosate and GBHs

Regarding the adverse effects of exposure to glyphosate, GBHs or its main degradation product, AMPA, from a vast review of scientific studies,[429] we found that:

1. They have high carcinogenic potential (myeloma, leukemia, melanoma, multiple myeloma, non-Hodgkin's lymphoma, as well as cancer of the oral cavity, colon, lungs, rectum, pancreas, kidneys, bladder and prostate) through different pathways such as genotoxicity and oxidative stress. The latter process is, in turn, associated with the development of a multiplicity of chronic degenerative diseases.
2. They act as endocrine disruptors and agents that cause serious disorders in the reproductive system.
3. They can cause damage to organs and systems, metabolic alterations and neurological diseases.

These effects can occur even from exposure to "low doses", i.e. doses much lower than the ones used in most toxicity tests, which are the limits established as safe for animals and humans and can easily be found in the environment. The concept of "low dose effects" refers to effects that, according to scientific evidence, occur at dose levels lower than the ones tested in standardized toxicology studies.[430]

Evidence of carcinogenicity

In 2015, following an extensive review of all the scientific literature available at the time on glyphosate, the International Agency for Research on Cancer (IARC), a research body of the World Health Organization (WHO), evaluated glyphosate and glyphosate-based commercial herbicide formulations and classified them as a probable human carcinogens (Group 2A).[431] This review of nearly 1,000 studies was conducted by an interdisciplinary group of experts in the field who had no conflict of interest that could bias their research,[432] and demonstrated – with compelling scientific evidence – that glyphosate can operate through two key characteristics of known carcinogens affecting humans: genotoxicity (damage to DNA) and oxidative stress.[433]

Confirming the IARC's position, in 2019 the Agency for Toxic Substances and Disease Registry (ATSDR), an entity of the US Department of Health and Human Services, published a toxicological profile of glyphosate with more than 300 references, that supported the report published by the IARC and highlighted a strong correlation between exposure to glyphosate (in its pure state or as a commercial formulation) and the development of different types of cancer, as well as other pathologies, such as stunted development, intestinal diseases, and liver and kidney toxicity.[434]

A systematic scientific review in 2023 mapped key carcinogenic traits in all *in vivo*, *ex vivo* and *in vitro* human and experimental animal (mammalian) studies comparing exposure to glyphosate and GBHs with low or no exposure counterparts. This review identified with compelling evidence that, in addition to genotoxicity and oxidative stress (glyphosate carcinogenicity pathways identified by the IARC in 2015), glyphosate and GBHs are carcinogenic through the modulation of receptor-mediated effects and the induction of epigenetic alterations and chronic inflammation.[435]

Genotoxic effect

An extensive range of studies, both prior and subsequent to the IARC report, demonstrates the genotoxic potential and the relationship between exposure to glyphosate, GBHs or AMPA and the development of different types of cancer. These include very recent cohort studies based on observational and analytical designs, making them the most valuable or closest studies in terms of the search for causal relationships.[436]

Glyphosate's genotoxicity (the ability of a substance to cause damage to genetic material) leads to chronic and irreversible harmful effects on the health and development of exposed organisms.[437,438,439] GBHs cause death in human mononuclear white blood cells, as well as damage to the DNA of these cells. Once metabolized, glyphosate causes DNA damage to increase, although the mechanism by which this occurs has not been explained. [440]

At the same time, effects on epigenetic mechanisms (which regulate genes without altering DNA; they are heritable) that regulate the expression of peripheral blood mononuclear cells have been observed following exposure to environmental concentrations of glyphosate or occupational exposure. These effects include a significant reduction in global levels of DNA methylation (a mechanism involved in gene regulation)[441,442]. There were also reports of methylation of promoter regions of certain tumor suppressors, which is associated with the silencing or overexpression of key genes in the regulation of cancer initiation and progression[443], and changes were identified in the expression of genes regulating the cell cycle (cell growth and reproduction stages) and apoptosis (programmed cell death). The alteration of these processes is linked to the development of cancer.[444]

Epidemiological, medical and toxicological studies have also been published confirming the toxicological profile of glyphosate drawn up by the ATSDR and linking the herbicide to an increased incidence of different types of cancer such as leukemia, melanoma, multiple myeloma and non-Hodgkin's lymphoma, as well as oral cavity, colon, lung, rectal, pancreas, kidney, bladder and prostate cancer.[445,446] This link depends on the dose and time of exposure; for example, a case study in North Dakota, United States, reported that the incidence of prostate cancer in men under 50 years of age is higher if they have been exposed to herbicides throughout their lives. [447] Subsequently, it was found that the herbicide affected non-tumorous prostate cell lines by increasing the expression of urokinase, a protease (protein-degrading molecule) that facilitates invasion and metastasis (the spread of cancer cells) in prostate cells.[448]

Another investigation in 2021 reviewed animal studies that demonstrated glyphosate's association with genotoxic effects in several animal and cellular models, such as human lymphocytes, even at low doses, in addition to its relationship with the development of Non-Hodgkin's lymphoma (NHL). The studies determined that up-regulation of cytidine deaminase induced by activation of the B-cell genome mutating enzyme was the underlying mechanism for the development of this type of cancer. The authors of this review recommend that pesticide regulatory agencies reevaluate the classification of this herbicide.[449]

Recently, researchers from the United States evaluated the damage caused by the agricultural use of glyphosate in pregnant women, five-year-old children, and 14- and 18-year-olds from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS). The findings show that exposure to glyphosate and AMPA causes effects in early childhood among children living near glyphosate usage sites, such as an increased risk of liver disorders and metabolic syndrome in adulthood. Such disorders can lead to cancer of the liver, diabetes and cardiovascular disease in the future.[450]

In early 2023, two paradigmatic studies were published reaffirming the IARC's conclusions regarding the two pathways along which the carcinogenic effects of glyphosate and GBHs operate, namely genotoxicity and oxidative stress.

In the first one, researchers from US and British universities demonstrated, with 80 studies published since 2016, that 87% of research corroborates the genotoxic effects of glyphosate and glyphosate-based herbicides. They also suggest that the US Office of Pesticide Programs should make legal changes to assess the oncogenicity of glyphosate-based herbicides.[451]

The second study features research by scientists from the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC), the US government agencies in charge of health and public health research, respectively, who found an association between human exposure to glyphosate by farmers and the presence of oxidative stress indicator molecules.[452] This study, which was published in the journal of the US National Cancer Institute, further swells the vast body of scientific evidence highlighting the carcinogenic potential of the glyphosate herbicide.

Evidence of oxidative stress

Several studies have found that glyphosate causes inhibition of numerous enzymes, metabolic alterations and oxidative stress leading to excessive membrane lipid peroxidation, as well as cellular and tissue damage.[453] Studies with the freshwater crustacean *Macrobrachium nipponensis* showed that commercial formulations of glyphosate cause DNA damage by inducing oxidative stress and inhibiting the antioxidant response in the cells of this model.[454]

Studies with model animals have revealed that glyphosate increases oxidative damage indicator levels in the intestine. It also increases the expression of enzymes involved in the response to oxidative stress conditions, such as catalase and superoxide dismutase, and heightens intestinal permeability, which reduces the expression of proteins forming the barrier through which ions and molecules pass into the intestinal cells.[455]

Additionally, glyphosate and its commercial formulation *Roundup*® have been reported to cause genetic damage in human lymphocytes and liver cells. Studies in liver tumor cells found alterations in mitochondrial membranes affecting the cell's respiratory capacity and which may be related to premature aging, as well as in cytoplasm damage-induced lysosomal activity.[456]

A study with rats in 2021 compared standard histopathology and serum biochemistry measurements and conducted a multi-omics analysis in a subchronic toxicity test of mixtures of various pesticides detected in food, such as glyphosate, and including low-dose exposure. There was little impact on the feed and water intake ratio, body weight, histopathology and serum biochemistry. However, metabolomics, in serum and blind, revealed nicotinamide and tryptophan metabolism affectations, which have implications for oxidative stress. Liver transcriptomics showed 257 genes with expression changes, with affectations including steroid hormone responsiveness regulation and the activation of stress response pathways. Genome-wide DNA methylation analysis of the same liver samples showed that 4,255 CpG sites were differentially methylated. The researchers stress the importance of in-depth molecular profiling in laboratory animals exposed to low concentrations of pesticides to detect metabolic alterations.[457]

In 2022 another paper was published evaluating the carcinogenicity of GBHs and confirming that glyphosate causes DNA damage and leads to activation of DNA repair mechanisms in a mammalian system *in vivo*. As for the carcinogenicity assessment, the paper showed that *Roundup®* can activate oxidative stress and an unfolded protein response, even at concentrations at which glyphosate is considered safe under international regulations. The study highlights the usefulness of omics methods and recommends their use by agencies to more accurately assess the toxicity of chemicals for the benefit of public health.[458]

Cohort studies, evidence of carcinogenicity in humans after the IARC report

Although cancer is a multifactorial disease that can be aggravated by many factors, epidemiological or cohort studies conducted in different countries in recent years have demonstrated a strong link between glyphosate exposure and the incidence of cancer. [459,460]

Cohort studies identify individuals on the basis of the presence or absence of exposure to a risk factor of interest. At the beginning of the study, all individuals are free of the disease to be studied and are monitored over a period of time long enough to observe the frequency of occurrence of the expected event (disease). At the end of the monitoring period, some individuals will have developed the disease under study, and, in such cases, there will be certainty that this happened after being exposed to the risk factor.

In the case of glyphosate and AMPA, and their relationship with the incidence of cancer, there are three highly relevant cohort studies. The first one was published in 2018 by the US National Cancer Institute (NCI); the second was published in 2019 by the international consortium AGRICOH (Consortium for Agricultural Cohort Studies), and the third was a 2021 multi-ethnic pilot cohort study also conducted in the US. It should be noted that these studies are subsequent to the IARC's determination to classify glyphosate as a "probable human carcinogen".

The AGRICOH study pooled data from 316,270 farmers or farm workers in France, Norway and the US. The average follow-up period was 16 years. During this time, a total of 2,430 cases of non-Hodgkin's lymphoma (NHL) associated with the use of different agrochemicals were diagnosed. The study calculated specific risk values (HR) indicating an increased risk of developing some type of NHL following exposure to agrotoxins, compared to a control group that was not exposed, with 95% reliability. For glyphosate, average HR levels of 1.36 (with lows of 1.00 and highs of 1.85) were found to develop diffuse large B-cell lymphoma, which is the most common type of NHL and is characterized by rapidly growing tumors in the lymph nodes, spleen, liver, bone marrow and other organs. This finding reveals that exposure to glyphosate increases the risk of developing this type of cancer by 36%, with reported cases of increases of as much as 85%.[461]

The NCI study considered 54,251 agrochemical sprayers in the states of North Carolina and Iowa in the US, who were monitored between 1999 and 2005. A total of 82.8% (44,932) of the sprayers reported using glyphosate, and 5,779 cases of cancer were found. This study points out that the relative risk (RR) of developing Acute Myeloid Leukemia (AML) was higher among sprayers reporting the highest exposure levels than among sprayers who did not use glyphosate.[462]

The findings of these cohort studies are consistent with other case studies that have reported a 50% increase in RR for NHL due to glyphosate exposure,[463] as well as with a meta-analysis reporting a 30% increase in the likelihood of developing this lymphoma in people exposed to glyphosate-based herbicides.[464]

A 2021 pilot cohort study investigated the link between urinary AMPA excretion before diagnosis and the risk of breast cancer in 250 predominantly postmenopausal women: 124 cases and 126 healthy controls (individually matched by age, race/ethnicity, urine type, date of urine collection, and fasting status). The researchers found that the presence of high levels of AMPA in urine is associated with a 4.5-fold increase in the risk of developing breast cancer in women of different ethnic groups in Hawaii. Ninety percent of the women volunteers did not work in the fields, yet they still had traces of AMPA in their urine, demonstrating the constant level of exposure we face on a daily basis.[465] These findings are similar to the ones reported in another 2018 case study with pregnant women from the state of Indiana in which glyphosate was detected in the urine of more than 90%.[466]

Also, in 2021 another cohort study, called *Puerto Rico Testsite for Exploring Contamination Threats (PROTECT)*, revealed that glyphosate and AMPA levels in urine samples collected from pregnant women near the 26th week of gestation were associated with a higher likelihood of preterm delivery.[467]

Evidence of endocrine disruption and reproductive disorders

There is abundant evidence confirming glyphosate's behavior as a disruptor of endocrine (a molecule capable of altering the hormonal balance of an organism) and as an agent that causes toxicity in reproductive systems.[468] In addition, there are many other studies that conclude that GBHs affect the reproductive systems of various species. The mechanisms by which this damage occurs are numerous and are not limited only to interaction with hormone receptors.

Effects on reproductive and hormonal systems

A test with mice demonstrated that exposure to glyphosate destroys the growth and developmental capacity of the oocyte (the germ cell that helps produce eggs), interfering with maturation by generating oxidative stress, reducing membrane potential, and causing DNA damage and early apoptosis, which in turn leads to cytotoxicity in these cells.[469]

In the case of females, exposure to glyphosate or herbicides containing this molecule as the main ingredient during a critical period of development, such as the first days after birth, has been found to affect important processes that predispose the organism to develop chronic diseases during the rest of its life, especially those related to ovarian and uterine development and functionality, along with reproductive cycle complications and a high miscarriage rate.[470]

In the case of male rats, glyphosate has been reported to cause oxidative stress in cells involved in sperm production, which negatively impacts male fertility.[471] Exposure to glyphosate also reduces testosterone synthase protein levels in testicular cells and inhibits testosterone secretion.[472] In addition, exposure to this herbicide in rats produced changes in the functional structure of the testes and reduced serum testosterone concentrations.[473] It has also been demonstrated that long-term exposure to glyphosate in male rats negatively affects the integrity of the blood-testis barrier which hinders spermatogenesis by activating the ER-α/NOX1 axis.[474] Furthermore, glyphosate is also reported to be a metal chelator and, since manganese affects sperm motility, it is believed that glyphosate could partially explain higher levels of infertility and birth defects in humans.[475]

As far as other animals are concerned, herbicide exposure in sheep was found to alter ovarian histomorphology (the shape of ovarian tissues) and molecular parameters in sex organs, similar to the findings reported for other xenoestrogens (endocrine-disrupting chemicals or EDCs).[476] This same study detected glyphosate in the blood serum of neonates 14 days after exposure. Also, exposure to glyphosate in male lizards decreased sperm production, caused changes in testicular morphology and affected estrogen receptors and their expression.[477]

Glyphosate has been identified as causing dysregulation of a large number of genes (an expression either increased or decreased in 680 out of 1,550 genes studied) in human breast cancer cells grown *in vitro* under environmentally acceptable exposure levels. The herbicide can substitute and work symbiotically with estrogen (necessary for the growth of breast cancer cells), highlighting glyphosate's high endocrine-disrupting capacity in this type of hormonal environment.[478] Estrogen-dependent breast cancer cell proliferation is increased by exposure to pure glyphosate through estrogenic mechanisms *in vitro*.[479]

Another study supporting this line of research was an evaluation of the safety of glyphosate in pregnant sows, which investigated the effects on placental angiogenesis and the mechanism of exposure to low (20 mg/kg) and high (100 mg/kg) GBH concentrations, based on the limit established as safe under the international standards used by regulatory agencies. The findings revealed that gestational exposure to GBHs reduced placental vessel density and cell multiplication by interfering with the expression of the VEGFA, PLGF, VEGFr2 and Hand2 angiogenesis indicators. The foregoing may, in turn, be related to mitochondrial fission and fusion disruption triggered by oxidative stress, as well as impaired functioning of the mitochondrial respiratory chain. In addition, GBHs affected the transfer of nutrients across the placenta and its function as a protective barrier, and oxidative stress was detected in the jejunum of newborn piglets.[480]

Some research links glyphosate exposure to altered expression of important enzymes in humans and other mammals, such as glutathione transferase, CYP3A4 and CYP1A2, as well as the disruption of sex hormones in animals and human cells in vitro.[481, 482] The implications of such endocrine-disrupting effects can be profound and far-reaching, and include a range of developmental impacts such as alterations in sexual differentiation, bone metabolism, liver metabolism, reproduction, pregnancy, growth, brain and organ development, cognition, as well as endocrine-related diseases such as breast, testicular and prostate cancer, along with neurodegenerative and metabolic disorders such as diabetes or obesity. [483]

Additionally, rainwater runoffs from GM corn crop fields sprayed with *Roundup*® and 2,4-D have been found to contain substances that affect androgen production, which means that chronic exposure to this water may lead to endocrine disruption in humans.[484]

Transgenerationally inherited effects

The main effects observed as a result of exposure to low doses of glyphosate include affectations to hormone levels and the reproductive system. A number of these harmful effects have been reported following exposure to glyphosate and GBHs by an initial generation, with consequences occurring up to two generations later without exposure to the herbicide.

Recent studies – both *in vitro* and *in vivo* – have shown that glyphosate and GBHs can act as endocrine disruptors at low doses, commonly present in the environment. For example, alterations in the development and differentiation of ovarian follicles and the uterus have been observed in female rats and mice exposed to glyphosate before puberty, affecting their fertility.[485] In pregnant animals, F1 and F2 generation offspring have also been affected; and in fish, various reproductive and epigenetic effects have been reported involving egg maturation, causing reproductive toxicity and compromising the dynamics of the exposed populations.[486]

A subsequent study was designed to identify the epigenetic biomarkers of glyphosate-induced transgenerational diseases using an epigenome-wide association study (EWAS) in connection with transient glyphosate exposure of pregnant female rats (generation F0) during the developmental period of gonadal sex determination. The results showed that the next generation, without direct exposure, aged more rapidly and animals were found to have developed specific pathologies such as prostate disease, renal disease and obesity. Alarmingly, the dose that pregnant females with the described affectations were exposed to is equivalent to half the "no observable adverse effect level" (NOAEL), which is a toxicity index determined in toxicological evaluation processes from which the other toxicity parameters are derived. [487]

Other environmentally relevant harmful effects of glyphosate at low doses have also been documented in recent years. For example, a study of mouse neural stem cells found that exposure to low concentrations of glyphosate, such as the ones permitted in drinking water by the environmental protection authorities, caused environmental neurotoxicity in the nervous system.[488] Furthermore, it has been found that glyphosate can damage the epigenomic structure (DNA methylation patterns) of organisms and that the harmful effects of exposure to glyphosate can appear after three generations. A recent study found that the F2 and F3 generations (great- and great-great-grandchildren) of rats exposed to glyphosate concentrations below the NOAEL dose, a parameter referring to the highest dose with no observable adverse effects, suffered a higher incidence of abnormalities such as prostate disease, obesity, kidney disease, ovarian disease and abnormalities during parturition.[489]

Exposure to glyphosate-based herbicides, even at very low doses, can cause reproductive complications including miscarriages, premature births, low birth weight and birth defects. Laboratory studies have demonstrated that very low levels of glyphosate, *Roundup*®, POEA and the metabolite AMPA kill human umbilical, embryonic and placental cells. For example, it has been shown that *Roundup*® formulations can kill testicular cells, reduce sperm count, increase abnormal sperm, stunt skeletal development and cause deformities in amphibian embryos.[490] Changes in organ histology and decreased copulation frequencies and reproduction rates have also been observed in fish.[491]

Endocrine affectation caused by exposure to low doses of glyphosate in humans was demonstrated in assays on MDA-kb2 cell lines to detect hormone receptor antagonists[492] and on JEG3 placental cell lines, which revealed that GBHs disrupt the activity of aromatase, the enzyme responsible for estrogen synthesis, at concentrations lower than those recommended for agricultural use.[493]

A pilot study of 94 mother-infant pairs (45 females and 49 males) was published in 2021 as part of The Infant Development and Environment Study (TIDES) carried out by the National Institute of Environmental Health Sciences (NIEHS) in the United States. This study seeks to examine how maternal exposure to everyday chemicals during pregnancy can affect the developing fetus. The pilot study measured glyphosate and AMPA levels in the urine of mothers with no occupational exposure, in the second trimester of pregnancy. Glyphosate was detected in 95% of the samples and AMPA in 93%, which correlated with alterations in the embryonic development of the fetuses, particularly in the anogenital distance of the babies, which is an indicator of androgen concentration during prenatal development in mammals. The finding was that anogenital distances in female infants of mothers with high urinary glyphosate or AMPA concentrations were significantly greater than normal, suggesting that glyphosate is a female-specific endocrine disruptor with androgenic effects in humans.[494]

In 2022 researchers conducted a human reproductive health study, collecting urine samples from women with high-risk pregnancies and from diverse cultural backgrounds, with glyphosate levels found to be above the detection limit in 99% of the pregnant women. Postpartum, higher maternal glyphosate levels were associated with an increased risk of neonatal intensive care unit admission and low newborn weights, which are linked to glyphosate levels in the first trimester of pregnancy.[495] In the same year, links between prenatal glyphosate exposure and gestational duration (The Infant Development and the Environment Study, TIDES) were investigated based on a multicenter cohort of pregnancies in the United States. The findings showed that widespread exposure to glyphosate in the population can affect reproductive health by shortening the gestation period.[496]

The scientific evidence available at present confirms that the toxic effects of glyphosate and herbicides containing it are manifested even at low doses, mainly affecting the functionality of sex hormones and, therefore, causing reproductive complications in organisms exposed to these substances. In addition, an alarming study conducted in the municipality of Muna, in the state of Yucatan, found reproductive alterations in men and women exposed to pesticides, including glyphosate.[497]

Evidence of organ and system damage, metabolic alterations and neurological diseases

Effects on the digestive system and alteration of the intestinal microbiota

All orders of bacteria comprising human and animal microbiota require aromatic amino acids from the shikimate pathway for their growth and development. As indicated previously, this is the metabolic pathway affected by glyphosate and serves to explain its herbicidal effect according to information provided by companies promoting and marketing glyphosate. In fact, most beneficial bacteria for humans, animals and soil are sensitive to the glyphosate herbicide and can be affected by its presence.[498] The results of experimental animal studies presented below can also apply to humans since we have bacteria in the intestinal tract that use the shikimate pathway.

Intestinal microbiota is the community of living microorganisms residing in the human intestine, composed of trillions of bacteria that, due to their abundance, organizational complexity and specific functions, are fundamental to maintaining good health and have been called by experts in the field a new organ requiring great care. The bacterial community forming the intestinal microbiota of humans includes more than 1,000 species of bacteria that together perform vital functions such as regulating the supply of energy to cells, promoting proper body growth, and developing immunity and nutrition, among others.[499]

Existing evidence indicates that the glyphosate molecule is a toxic substance that has multiple effects on the digestive system. Experimental studies have reported that glyphosate induces an inflammatory response in the small intestine of laboratory rats leading to a decreased expression of antioxidant enzymes and an alteration in the balance of ions in the intestine, including decreased iron absorption, which may be associated with neurological problems or anemia.[500] Furthermore, exposure of intestinal microbiota components to glyphosate results in the suppression of cytochrome P450 enzymes (involved in the detoxification of environmental toxins, the activation of vitamin D3, the catabolization of vitamin A, the maintenance of bile acid production and the supply of sulfate to the intestine) and the biosynthesis of certain amino acids leading to a reduction of beneficial bacteria in the gastrointestinal tract favoring the proliferation of pathogens.[501]

Another study, on glyphosate exposure during peripartum in rats, demonstrated changes in the behavior of the mothers associated with alterations in neuroplasticity. The study also reported an imbalance in the intestinal microbiota of the mothers linked to alterations in the Central Nervous System.[502]

The herbicide also alters the composition of intestinal microbiota, reducing the abundance of the *Lactobacillus* genus while enhancing the proportion of potentially pathogenic bacteria.[503] In addition, when harmful bacteria such as *Escherichia coli* or *Salmonella spp.* were exposed to *Roundup®* their resistance to antibiotics such as kanamycin and ciprofloxacin increased,[504] which could exacerbate the problem of antibiotic resistance that is currently a global public health issue.

Considering the above, numerous studies have been performed using animal models to understand the effects of the herbicide on the gut microbiome and the effect this has on the health and behavior of animals. Some studies have highlighted the intricate relationship between intestinal microbiota and behavioral alterations.[505,506] Consequently, studies conducted in mice, in which the microbiome is very similar to that of humans, demonstrate that chronic and subchronic exposure to GBH increases behaviors related to depression and anxiety in response to the altered composition of gut microbiota since there is a reduction in the abundance of key bacteria genera and this may increase the prevalence of behavioral alterations. [507]

Furthermore, the effect of glyphosate and the GBHs *Roundup Pro®* (MON52276) and *Roundup GT® plus* on the gut microbiome of rats has been investigated using multi-omics techniques. In one study these were found to inhibit the shikimate pathway and it was shown that treatment with glyphosate and MON52276 resulted in higher levels of harmful bacteria such as: *Eggerthella* spp., *Shinella zoogleoides*, *Acinetobacter johnsonii* and *Akkermansia muciniphila*. *Shinella zoogleoides* was higher only with exposure to MON52276. It was also detected that, for *in vitro* culture assays using *Lacticaseibacillus rhamnosus* strains, *Roundup GT® plus* inhibited growth at concentrations at which MON52276 and glyphosate had no effect.[508]

Another scientific group analyzed effects in mice exposed to doses of glyphosate below those allowed by US regulatory authorities. It was shown that intestinal microbial alterations were apparent after 90 days since the abundance of beneficial commensal bacteria was reduced and anti-inflammatory functional pathways diminished. This can lead to colitis, multiple sclerosis or obesity.[509] As for other organisms, studies have shown that water and animal feed contaminated with glyphosate affect intestinal microbial communities, depending on the form and concentration of glyphosate.[510] Furthermore, *in vivo* assays in pigs showed that glyphosate causes dose-dependent intestinal toxicity as well as increased permeability of the intestinal membrane and a deregulation in the expression of genes involved in the antioxidant response during digestion.[511]

In recent years, it has been suggested that the use of glyphosate is the most important causal factor linked to the development of celiac disease, known as gluten intolerance, a growing problem worldwide but especially in North America and Europe.[512] Symptoms include nausea, diarrhea, skin rashes, macrocytic anemia and depression. This is a multifactorial disease associated with numerous nutritional deficiencies and reproductive problems. The characteristics of celiac disease point to a deficiency of essential amino acids such as tryptophan, tyrosine, methionine and selenomethionine as well as impairment of many cytochrome P450 enzymes.

Celiac disease is associated with imbalances in gut bacteria that can be fully explained by the known effects of glyphosate on gut bacteria. Glyphosate is known to inhibit cytochrome P450 enzymes, while deficiencies of iron, cobalt, molybdenum, copper and other rare metals associated with celiac disease may be attributed to the powerful capacity of glyphosate to chelate these elements (a chelating agent is a heavy metal sequestering agent, a substance that forms complexes with heavy metal ions). Deficiencies in essential amino acids coincide with the known depletion of essential amino acids by glyphosate.[513]

Today this approach is supported by even more robust evidence. Based on a critical review of scientific literature on the effects of glyphosate on the gut microbiome, a research group concluded that glyphosate residues in food may indeed cause alterations in the gut microbiome (dysbiosis) associated with such conditions as celiac disease, inflammatory bowel disease and irritable bowel syndrome, since opportunistic pathogens are more resistant to glyphosate than to commensal bacteria.[514] The authors also point out that the effect of glyphosate on dysbiosis is not considered when regulatory authorities make safety recommendations.

Effects on numerous organs and systems

Some reports indicate damage to cells of other species such as: bovine lymphocytes and bone marrow cells, mouse liver and kidney; fish gill cells and erythrocytes; alligator erythrocytes and fruit fly embryos among others.[515, 516]

All of these alterations negatively affect the body, although the impact is subtle and manifests itself slowly over time since the inflammatory processes damage cellular systems throughout the body and may ultimately be correlated with the onset of gastrointestinal diseases as well as obesity, diabetes, heart disease, depression, autism, infertility, cancer and Alzheimer's.[517]

Polyoxyethylene tallow amine (POEA), the main adjuvant agent of GBH, was the first surfactant incorporated in glyphosate formulations. These formulations have been associated with acute eye toxicity since the 1970s and 1980s and were identified as a serious worker safety concern by the California Department of Pesticide Regulation.[518] According to data collected between 1981 and 1985, the two main causes of diseases associated with occupational exposure to pesticides were eye

(50%) and skin (35%) lesions. In fact, glyphosate ranked third among all pesticides as a cause of occupational disease in the US state of California.[519]

Despite the fact that, according to the manufacturers' own information,[520] certain commercial brands of GBH have been reformulated to replace animal fat-derived POEA with other less irritating POEAs, these surfactants continue to have alarming toxic effects on multiple non-target organisms, including mammals and aquatic organisms. Several recent studies have shown that POEAs can increase the toxicity or uptake of glyphosate into human cells and generate more severe toxicological symptoms[521,522] such as cytotoxicity or toxicity in cells,[523] effects on different sex hormones,[524] as well as genotoxicity or DNA damage.[525,526]

An Italian study on the adverse effects of glyphosate on thyroid cells *in vitro (Fisher-rat-thyroid-cell line-5*, FRTL-5) showed reduced cell viability, mitochondrial respiration and cell proliferation. This can lead to various conditions, including thyroid cancer, hypothyroidism and alterations in the oxidative phosphorylation process affecting the development of autoimmunity. This research is further supported by other studies that expose pesticides as thyroid-affecting compounds, mostly in people directly exposed to glyphosate.[527]

Urine and feces are two of the main routes of elimination of substances such as glyphosate, which makes the kidneys vulnerable to its toxicity. Brazilian researchers orally administered low doses of GBH (0, 0.5 or 5 mg/kg) to rats from weaning to adulthood. They measured serum levels of urea, creatinine, and examined the histological morphology of the kidneys, the mRNA expression of related genes and the biomarker Kim 1 as well as lead levels. The results showed mild kidney damage in the presence of glyphosate-based herbicides. However, long-term evaluation is required as it may also contribute to the development of chronic kidney disease.[528]

In another Brazilian study, toxicological research into four pesticides, including glyphosate, conducted using animal models was reviewed from 2014 to 2019. The predominant model were fish and the evaluation focused on mortality, blood cell and developmental abnormalities, as well as behavioral alterations.[529]

Research on *Rhinella arenarum* tadpoles to evaluate the toxicity of glyphosate and glufosinate-ammonium indicates that the most frequently reported anomalies were abdominal edema, teratogenicity, DNA damage, hormone disruption and oxidative stress. This study also demonstrates considerable chemical interaction between the active components of the two herbicides.[530] Furthermore, a model evaluated using zebrafish showed that environmental exposure to glyphosate causes damage to DNA and to cardiomyocyte mitochondria while also increasing endoplasmic reticulum stress.[531]

As for humans, research conducted by scientists from East China and the United States using human aortic vascular smooth muscle cells and a zebrafish model showed that glyphosate has toxic effects on blood vessels which can lead to atherosclerosis. Since glyphosate is a potential factor in this disease, protecting populations chronically exposed to this pesticide for cardiovascular risk is suggested. In addition, this research shows that glyphosate induces cell aging through DNA damage and mitochondrial deterioration.[532] Another study related to cardiovascular health indicates that glyphosate causes senescence and inhibits the proliferative capacity of cardiomyocytes, which manifests itself in a reduction of cardiomyocytes and can cause arrhythmias, cardiomyopathies and arteriosclerosis in humans.[533]

In view of the growing number of reports of diseases such as recurrent miscarriages and increased malformations, autism, behavioral disorders and cancer in Argentine agricultural areas, a group of researchers conducted a study which found that the glyphosate-based herbicide, the most widely used in Argentina, produces severe cephalic malformations, alterations of the cardiac area and the embryonic trunk in amphibian and chicken embryos.[534]

Recently, the skin sensitization generated by exposure to glyphosate and its commercial formulations has been studied in greater detail using multi-omics techniques. The findings are that in the presence of these herbicides an immune system response is triggered that causes cellular autophagy, i.e. the destruction of the cells themselves to prevent the accumulation of toxins or toxic substances.[535]

Acute effects from exposure to this herbicide observed in laboratory studies include respiratory difficulties, ataxia and convulsions.[536] The herbicide *Roundup*® has also been associated with cardiac depression.[537,538] In aqueous environments, glyphosate causes eye irritation and penetrates cell membranes causing alterations.[539,540] There are also reports on the effects of occupational exposure involving mucosal or skin affectations such as allergies, irritations and chemical burns.[541] Other reports suggest that glyphosate promotes skin cancer.[542]

As indicated above, the fact that many commercial formulations of glyphosate contain other ingredients that companies are not required to report, or that are handled as trade secrets, makes it more difficult to estimate their toxic effects and the likely risks associated with their use. These other ingredients, as seen in the case of POEA, can increase the toxicity or absorption of glyphosate in human cells and lead to more severe symptoms, such as respiratory failure, which complicates severe cases of poisoning caused by glyphosate-based herbicides.[543, 544]

Neurological damage and diseases of the nervous system

There is evidence that glyphosate may affect areas of the brain associated with Parkinson's disease, particularly dopaminergic neurons. Epidemiological and clinical case studies link glyphosate exposure to premature mortality due to Parkinson's disease, although further research is required on the subject. It was also found that the cases of premature death were geographically close to regions with intensive agriculture (less than one kilometer away from application points of glyphosate, atrazine, diazinon and paraquat).[545] Furthermore, very recent evidence suggests that glyphosate may affect areas of the brain associated with Parkinson's disease.[546] In 2022 it was detected that glyphosate crosses the blood-brain barrier and causes neurodegenerative disorders such as cognitive impairment, Alzheimer's disease, anxiety and depression.[547]

In another study, scientists demonstrated certain negative impacts of glyphosate on the nervous system of vertebrates. The study was based on a comparison of seizures caused by the glyphosate component and a commercial version *Roundup®*. This research concluded that the nervous system of *C. elegans* was affected to a greater extent by *Roundup®*, prolonging the duration of seizures. It is therefore concluded that it acts on GABA-A brain receptors which may generate toxicity and neurodegenerative diseases such as Parkinson's disease in humans.[548]

A 2022 review of the toxic effects of glyphosate in several animal species and humans demonstrates the capacity of this herbicide to induce oxidative stress, neuroinflammation and mitochondrial dysfunction. These processes lead to neuronal death by autophagy, necrosis or apoptosis, as well as to the onset of behavioral and movement disorders. The findings suggest that glyphosate and GBHs can produce significant alterations in the structure and function of the nervous system of humans, rodents, fish and invertebrate animals.[549]

Studies performed on mice have found that chronic and subchronic exposure to formulations using glyphosate as an active ingredient increases behaviors related to depression and anxiety due to hyperactivation of brain areas related to anxious behaviors. [550]

In addition, a pioneering 2023 research project conducted by scientists at the US National Institutes of Health (NIH) revealed effects in male farmers exposed to glyphosate. The results show that prolonged use of the herbicide may be associated with relevant genetic or selective effects in addition to mechanisms related to hematological, cardiovascular and neurodegenerative effects such as Alzheimer's disease.[551]

Also in 2023, another study was published linking glyphosate to neurological damage. This study was based on official statistical data obtained from the National Health and Nutrition Examination Survey (NHANES) conducted by the US National Center for Health Statistics (NCHS). Based on these reliable and comprehensive data, the authors demonstrated a correlation between the presence of glyphosate and a neurofilament light polypeptide (NfL) which serves as a molecular biomarker indicating neurological disorders.[552] The authors stated that the potential causality indicated by the observed correlation raises serious concerns about the possible effects of glyphosate exposure on neurological health among US adults. This is even more significant since, as indicated above, the NHANES took a representative sample of the US population and showed that 80% of people have glyphosate in their urine.[553]

2.4 Evidence of corporate malpractice by biotech companies involving GM seeds, information concealment and scientific manipulation

As indicated above, Bayer (which bought *Monsanto Company*) and Syngenta Group (ChemChina bought Syngenta AG) are among the four companies globally that account for 65.8% of the world market share in agrochemicals and 53.2% in seeds. This means they have a major influence in the food chain.

This oligopoly is built on false narratives about food systems, where on the one hand it claims its food production system is the only one capable of performing the necessary transformation to meet the challenges of climate change, while on the other they undermine the world's three billion indigenous and campesino producers, rural and urban, fishermen and pastoralists, who not only feed the majority of the world's population and the majority of the world's malnourished, but also create and conserve most of the planet's biodiversity.[554]

In addition, corporations have imposed a certain degree of control over the agricultural research and development agenda to satisfy their own interests while still influencing trade and agricultural policies to drive growth and profits.[555] The following is evidence of how these companies have implemented strategies that have included one or more examples of corporate malpractice, including: manipulation of science, concealment of information, attacks on critical scientists and journalists, and exerting influence on regulatory authorities.

The *Monsanto papers* and the lawsuits against Monsanto Company for damage to human health

The Monsanto papers

The *Monsanto papers* are documents that formed part of the evidence used in the biggest lawsuits brought by individuals against the Monsanto Company in the United States for punitive damages related to cases of cancer. The documents were obtained by means of a pre-trial civil procedure that allows the parties to obtain evidence from each other (Discovery).[556, 557]

In essence, these are internal company communications (internal emails, text messages, company reports, studies and other memos), which show how, in various ways, the transnational devised a scenario to pass off its glyphosate-based herbicide formula, Roundup, as harmless for four decades. It is important to point out again that more than half of global glyphosate use is for GM crops and that its usage volumes have increased 1,500% since the scaling up of marketing for GM soybeans and corn, to which glyphosate is inextricably linked.[558, 559]

The law firm BH Baum Hedlund, one of the leading groups representing individuals from the US in lawsuits against Monsanto, has a website granting access to the declassified documents up to December 2, 2019. The firm specializes in taking on high-risk litigation against corporations for toxic torts, among other issues and, for the first time in history, managed to win a trial in which it was proven that the glyphosate herbicide had caused Non-Hodkin's Lymphoma, a type of skin cancer, in the case of a gardener exposed to this product for a period of 5 years. These documents demonstrate the following:[560,561]

- Since the 1990s, Monsanto has concealed the findings obtained by its own consultants concerning the herbicide *Roundup*®. These findings show that it causes DNA damage and that dermal absorption of the chemical is higher than the rates reported to regulatory agencies.

- Monsanto deliberately used the scientific malpractice of *ghostwriting* by means of which company employees wrote manuscripts claiming glyphosate was harmless to health. Regulatory agencies have based their conclusions on this type of document for years, subordinating science to the lucrative interests of industry.[562,563]

- Monsanto is behind attacks seeking to discredit the World Health Organization's International Agency for Research on Cancer (IARC) and its members. This stems from a study by this international body which, in 2015, concluded that glyphosate was a probable human carcinogen.[564,565]

- Monsanto orchestrated a campaign to discredit French scientist Gilles-Eric Seralini, who in 2012 published a study showing how the most highly marketed GM corn HT (NK603) and glyphosate caused tumors in mice.[566,567]

- Monsanto influenced EPA (US Environmental Protection Agency) officials to issue findings in favor of the safety of *Roundup*®. The EPA issued a draft risk assessment in which it concluded that glyphosate did not pose a serious risk to human health and recently a US Court of Appeals, in response to a lawsuit, found the EPA's conclusion on glyphosate to be inconsistent and concluded that the opinion of the EPA was not supported by substantial evidence. It therefore vacated the human health portion of the EPA's decision and ordered that further analysis and explanation be conducted and that the ecological section of the assessment also be redone.[568,569,570,571,572]

Concise evidence of Monsanto's conflict of interest has emerged during lawsuits in the United States, with the company deploying a series of strategies to exert corporate influence and discredit studies that proved its herbicides were harmful.[573,574]

This "corporate war"[575] has been designed to neutralize economic losses, create a negative public opinion of the IARC, strengthen Monsanto's position and rally industry associations against the IARC,[576] the intention of these measures being to protect the reputation of *Roundup*®, prevent the popularizing of claims and studies regarding its carcinogenicity, and provide cover for regulatory agencies to continue allowing the use of glyphosate. The foregoing can be confirmed by the following:

- The formation of a network of business partners[577,578,579,580,,581,582,583,584] that closed ranks with Monsanto to discredit IARC scientists. This network included the largest organizations in the food and pesticide industry: CropLife International, the Grocery Manufacturers Association[585] and BIO; industry-funded spin-off groups: GMO Answers and the International Food Information Council; and groups lacking scientific rigor: Sense About Science, the Genetic Literacy Project and Academics Review,[586] who waged a rigorous pseudoscientific campaign to discredit IACR and defend the use of glyphosate.

- A series of documents revealed how Monsanto public relations agents Bruce Chassy[587] and David Tribe, Monsanto executive Eric Sachs, former Monsanto communications director Jay Byrne and former biotech industry trade group vice president Val Giddings spoke openly in emails[588,589,590] about setting up Academics Review as a shock group to promote industry interests. The heads of Academics Review, Chassy, Tribe, Byrne, Sachs and Giddings, are also members of *AgBioChatter*,[591] a private list server mentioned in Monsanto's PR plan as a Tier 2 industry partner. The Genetic Literacy Project, led by chemical industry public relations agent Jon Entine[592] also partnered with Academics Review to promote and improve GMOs and herbicides.

- Another significant case illustrating the influence Monsanto has for generating ghost articles is that of Henry I. Miller,[593] a renowned academic and advocate of genetically modified crops, who was asked by Monsanto to write an article for them which appeared under his name on the Forbes website in 2015. Forbes removed the story from its website and said it had terminated its relationship with Mr. Miller amid revelations that emerged regarding the conflict of interest.[594]

- There are other emblematic cases featuring the involvement of scientists with conflicts of interest who use their positions of authority to favor companies either by lobbying, serving as their spokespersons or publishing articles[595,596,597,598,599]

The findings of the US District Court for the Northern District of California point to Jess Rowland, the former deputy division director of the health effects division of the EPA's Office of Pesticide Programs,[600] as being instrumental in Monsanto's efforts to refute IACR findings. Rowland managed the work of scientists evaluating the human health effects of exposure to pesticides such as glyphosate, in addition to chairing the EPA Cancer Assessment Review Committee (CARC), which issued the internal report[601] in October 2015 discrediting the IARC's findings.

This report determined that glyphosate "is not likely to be carcinogenic to humans." However, the handling of the report raised questions when it was posted on a public EPA website on April 29, 2016, and remained on the site for only three days before being taken down. Shortly after the report was removed from the EPA website, Rowland terminated his 26-year career at the EPA. The plaintiffs' attorneys requested that Rowland clarify this situation and other dealings with Monsanto but the EPA rejected this request, along with Monsanto's objection to releasing documents related to conversations with Rowland.[602,603]

In view of this background, the EPA has been under scrutiny for its ties to Monsanto.[604] An article in Environmental Sciences Europe in 2019[605] documented how the EPA had ignored a large number of independent peer-reviewed studies linking glyphosate to cancer in humans while using Monsanto-funded research to support the agency's position that glyphosate is not carcinogenic.

The evidence presented on the political influences exerted by Monsanto to favor its position is as follows:

86. Email Confirms Monsanto's Efforts to Overcome Regulatory Hurdles Using Political Influence[606]
No: MONGLY01061857
Date: 2/18/2009 - 2/22/2009
Documents Released: 8/1/2017
This document contains e-mail correspondence between various Monsanto personnel. It demonstrates the strategy adopted by Monsanto to overcome regulatory hurdles by effectively deploying political leverage to ensure that regulatory authorities have "no doubts" regarding the safety of glyphosate.

87. Email Correspondence Further Confirming Monsanto's Close Ties with Former EPA Official, Jess Rowland[607]
No: MONGLY02162507
Date: 1/15/2010 - 1/16/2010
Documents Released: 8/1/2017
It confirms Monsanto's intimate relationship with Mr. Rowland of the EPA, who helped Monsanto circumvent the regulatory process

88. Text Messages Detailing Monsanto's Collusion with EPA[608]
No: MONGLY03293245
Date: 2/11/2013 - 3/10/2016

Documents Released: 8/1/2017
This document contains text message correspondence between Mr. Daniel Jenkins, various Monsanto employees and various EPA officials regarding the regulatory aspects of glyphosate

91. Document Details Monsanto's Goals After IARC Report - 'Orchestrate Outcry with IARC Decision...'[609]
No: MONGLY02913526
Date: 2/23/2015
Documents Released: 8/1/2017.
This document details a series of objectives for Monsanto to pursue before and after the anticipated IARC decision, demonstrating Monsanto's intention to discredit the IARC prior to the second classification of glyphosate.

94. PowerPoint Presentation Showing Monsanto's Efforts to Influence State of California on Glyphosate 'No Significant Risk Level.'
No: MONGLY03320237[610]
Date: 3/24/2015
Documents Released: 8/1/2017.
PowerPoint submitted by Monsanto to the California Office of Environmental Health Hazard Assessment on October 7, 2015, reflecting a No Significant Risk Level (NSRL) for glyphosate. It demonstrates Monsanto's efforts to limit the OEHHA's consideration of data for determining the appropriate NSRL to high-dose exposure animal bioassays.

101. Email Showing Communications Between Monsanto and EPA in Furtherance of Avoiding Roundup and Glyphosate Testing[611]
No: MONGLY02060344
Date: 6/24/2015
Documents Released: 3/14/2017.
This email demonstrates communications between Monsanto and regulatory agencies (EPA) in support of efforts to prevent the evaluation of Roundup® and glyphosate.

106. Monsanto Executive Confirms in Email to CropLife America That Company Pressured EPA Not to Convene Scientific Advisory Panel on Glyphosate[612]
No: MONGLY03379079
Date: 2/2/2016
Documents Released: 8/1/2017.
This document contains email correspondence between Monsanto's regulatory affairs employee, Mr. Daniel Jenkins, and members of Croplife America, in which Mr. Jenkins informs Ms. Janet Collins (Croplife) that Monsanto has been urging EPA not to convene the Science Advisory Panel to review the 2016 Glyphosate Issue Papers.

Furthermore, a 2022 investigation into how Monsanto conducts its product defense strategies analyzes the tactics used to manipulate science, attack scientists and journalists, and influence regulatory agencies to protect profits.[613]

Lawsuits against Monsanto Company, based on damage to health caused by glyphosate

One of the health harms most evident from exposure to glyphosate is the development of the cancer known as non-Hodgkin's lymphoma.[614,615,616] In the United States, Monsanto Co. faces more than 125,000 lawsuits in state and federal courts from users of glyphosate (farmers, day laborers, gardeners, landscapers, and government workers) in its best- known formulation, Roundup. [617] In all cases, use of the herbicide is linked to the development of Non-Hodgkin's Lymphoma without the company warning of this pernicious effect on its labeling.[618] Three California juries, in particular, found Monsanto, now owned by Bayer, guilty of causing this type of cancer in the plaintiffs due to their exposure to the glyphosate herbicide.[619] These cases are described below:

1. Dewayne Johnson[620,621,622]

The plaintiff, Dewayne Johnson, was a gardener who used the herbicide *Roundup®* as part of his work activities in a school district. In 2014, when he was 42 years old, he was diagnosed with non-Hodgkin's lymphoma type cancer. In 2015 he sued Monsanto Company and in 2018 the court ruled in his favor ordering Monsanto to pay him $289 million in damages and compensation. During the trial it was determined that the herbicide *Roundup®* was the cause of Johnson's non-Hodgkin's lymphoma and that Monsanto had failed to issue warnings about the health damage caused by exposure to its herbicide.[623] On August 10, 2018, a San Francisco jury ordered Monsanto pay $39.25 million in compensatory damages and $250 million in punitive damages. After a series of appeals, on July 20, 2020, the Court upheld the verdict against Monsanto but reduced Johnson's award to $20.5 million. In August 2020, Monsanto filed a petition against this decision.[624] Finally, on March 18, 2021, Bayer announced that it would not file a new appeal.[625]

2. Edwin Hardeman[626]

Beginning in 1980, Edwin Hardeman used *Roundup®* to control weeds on his property. Despite following safety precautions, Haderman developed non-Hodgkin's lymphoma. In 2019, a US jury ruled that glyphosate was a significant factor in Hardeman, aged 70, developing cancer. The jury in the case concluded that Monsanto was negligent in failing to take reasonable care to warn about the risk of developing non-Hodgkin's lymphoma when using *Roundup®*.[627] Hardeman was initially awarded $5.1 million in compensation and $75 million for damages in this case. Following a motion by the attorneys for Bayer/Monsanto, the judge modified the damages awarded to $20 million dollars with total compensation of $25.3 million dollars. The judge hearing the case acknowledged Monsanto's aggressive conduct to influence scientific discourse and publications on glyphosate.[628]

3. Alva and Alberta Pilliod[629]

Alva and Alberta Pilliod, an elderly couple, used *Roundup®* herbicide several days a year for about two decades on their rural properties. Both were diagnosed with non-Hodgkin's lymphoma. In 2019 Alva and Alberta Pilliod initially received compensation of $2 billion. In response to motions filed by the company's attorneys, the judge reduced the damages awarded to $69 million.[630]

In all three cases, juries found that although Monsanto knew that the herbicide *Roundup®* was dangerous and could harm health, the company still proceeded to market a product that was harmful to human health. The juries also found that Monsanto was negligent in marketing the product without providing adequate warnings about the dangers of using *Roundup®*, which is why it was found to have acted with malice and forced to pay large amounts of money for damages.[631] This is pointed out in the documents of these trials, as follows:

- The Ruling of Judge R. Nelson, dated May 14, 2021, number 19-16636, filed in the United States Court of Appeals for the Ninth Circuit, in the case of Hardeman v. Monsanto Company, stated that the company had acted with malice by ignoring the carcinogenic risks of its most popular brand of herbicide, which contains the active ingredient glyphosate.

- Whereas, in the Decision of the Court of Appeal of the State of California, First Appellate District, Second Division, dated August 9, 2021, number A158228, in the case of Pilliod et al. v. Monsanto Company, it was revealed that the company knew, or was likely to have known in light of the scientific evidence, that its herbicide had carcinogenic potential and that it had also worked for decades to suppress knowledge of the risk.

The IARC report classifying glyphosate as a "probable human carcinogen" was decisive for the lawyers and plaintiffs in the aforementioned lawsuits. The EPA assessment of glyphosate was not considered as it does not take into account high levels of exposure, nor exposure to the commercial mixture, but to pure glyphosate. Nor was it necessary to prove that a plaintiff's lymphoma was caused exclusively by exposure to *Roundup®* since it was sufficient to show that the preponderance of scientific evidence supports the conclusion that exposure to *Roundup®* sped up the development of the plaintiff's cancer or made it more difficult to control and treat.[632]

These are not the only legal cases Monsanto has faced in relation to health damage caused by glyphosate. In June 2020, Bayer announced it would pay US$10.1 million to settle nearly 125,000 liability cases related to the use of *Roundup*®.[633] In addition, the Monsanto Company had already been the subject of legal considerations in connection with the marketing of toxic products.[634]

Other GMO and pesticide companies have also been accused of scientific malpractice, in particular concealing information on the health damage caused by some of their products; evidence of these cases was also obtained in court proceedings during litigation for punitive damages.[635,636,,637,638,639,640,641,642]

2.5 Examples of restrictive or permissive GMO regulations, transparency and opacity under scientific scrutiny

Restrictive measures for GMOs

As explained above, the release of GMOs into the environment and their importation are not widespread practices around the world (80% of countries do not plant GMOs, 78% do not import them).[643]

Furthermore, several countries have imposed explicit bans on environmental release, either on a temporary basis, such as Peru[644] and Switzerland;[645] partially, such as the EU;[646,647] or totally, such as Austria, [648] Luxembourg[649,650] and Russia. [651,652]

A total of 64 countries have implemented some form of mandatory labeling and traceability for products containing GMOs. These countries have established different tolerance levels for the percentage of GMO present in a product, ranging from 0% to 5%. Only six countries have opted for a voluntary labeling system for GMOs.[653,654] As explained above, the level of GM corn consumption in Mexico is extremely high, so these types of measures are insufficient.

The Cartagena Protocol on Biosafety is the main international legal agreement regulating GMOs,[655] but has only been ratified by 157 countries.[656] A key feature of the Cartagena Protocol is the precautionary principle that has served as a guide for dealing with the uncertainty inherent in scientific progress concerning protection of environmental and human health.

Restrictive measures for glyphosate

In the case of glyphosate, there is an extensive list of countries where glyphosate has been banned or restricted, either totally (Vietnam, Malaysia, Sri Lanka, the United Arab Emirates, Oman, Saudi Arabia, Slovakia, Austria, Slovenia, Germany, Luxembourg, Kuwait, Qatar, Bahrain); or partially in some provinces/states or in relation to certain uses (Thailand, India, Denmark, the Netherlands, Belgium, Spain, the United Kingdom, Costa Rica, Argentina, Canada, the United States of America, Australia, the Czech Republic, Malawi, Colombia). [657,,658,659,660,661,662,663,664]

In recent years, legislation has played a notable role in promoting the development and use of chemicals that are compatible with the environment and the protection of human health has been notable, instead of encouraging the use of and reliance on harmful substances such as glyphosate.[665] This is favorable for the population in general, but especially for social groups that have historically lived in conditions of heightened social vulnerability, since susceptibility to the toxicity of a chemical substance is related to the degree of vulnerability of an individual.

As indicated earlier, in 2019 the US government's Department of Health published a toxicological profile of glyphosate that confirmed and underpinned the report published by the IARC. Similarly, for environmental matters, the EPA determined that 93% of plant and animal species in the risk category, as well as 96% of their habitats, are at risk because of the use of the glyphosate herbicide in that country, even when it is used in accordance with recommendations on the label and appropriate regulations.[666]

The case of California:

While California has not imposed a statewide ban on glyphosate, on July 7, 2017, it did become the first state in the US to issue a warning about glyphosate.[667,668] The decision to warn consumers about glyphosate was in accordance with the Safe Drinking Water and Toxic Enforcement Act, better known as California Proposition 65, [669] a ballot initiative approved by voters in 1986 to address issues of exposure to toxic chemicals.

The case of Florida:

The Florida Fish and Wildlife Conservation Commission has stopped using aquatic herbicides, including glyphosate, and is consulting public opinion.[670]

Although some sponsors and promoters of biotechnology never tire of claiming there is scientific consensus on the safety of GMOs, such a claim, in the light of scientific evidence and free of conflicts of interest, is completely illusory. [671]

Some scientists and decision-makers have used their reputation and position alone to claim that marketed and even non-marketed GM crops are inherently safe for human consumption and do not require testing. However, at this point in the document, it is clear that the alleged safety of GMOs has been called into question by years of a lack of independent evidence to reliably demonstrate that they cause no harm. This is in addition to poor industry practices and a body of scientific evidence that, over time, has shown various detrimental effects of GM foods on animal health and their potential to affect human health, including pesticide-induced harm associated with GM crops.

While some animal feeding studies have shown adverse health effects, respected scientists who conducted this peer-reviewed research have suffered mistreatment. This raises serious concerns about possible political and ideological influences on science. In risk assessments, the number of studies that reveal a risk may be more significant than a larger number of studies that do not, and when there is controversy about the risk of a consumer product, negative results should be replicated to see if they hold up to rigorous testing.[672]

In the case of GM crops, this pattern has not been followed and they are still on the market without having been proven safe for human consumption despite the scientific community expressing serious concerns from the moment their marketing commenced. Given the lack of certainty concerning the safety of GMOs, it was indicated that the most reasonable course of action would be to require that all GMO products intended for human consumption be subjected to long-term toxicity and carcinogenicity tests before being marketed and that industry should examine these products more thoroughly before continuing to introduce them into the food supply.[673]

Nearly 30 years later, any claim of consensus on the safety of GMOs is not supported by an objective analysis of the scientific literature. A broad community of independent researchers and scientists consequently issued a joint statement claiming that any alleged consensus on the safety of GMOs for human health has been falsely perpetuated as an artificial construct.[674] Furthermore, the statement acknowledges that rigorous safety assessments of GMOs have been hampered by a lack of independent funding and the involvement of private interests. Scientific research focused on the public good is significantly limited by proprietary rights issues and the denial of access to research material for researchers unwilling to enter into contractual agreements with developers, giving private interests unacceptable control over scientific publications. [675]

At the same time, the lack of transparency and strictness of regulatory authorities, whose duty is to monitor the safety of this type of food while ensuring human health and environmental protection at all times, has been criticized.

GM crops were approved for commercial use despite the fact that, at the time, the regulators themselves openly acknowledged that certain effects of GMOs and GM-based foods could not be predicted or observed. For example, in 2001, when GM crops of Bt corn and HT soybeans were already being planted in the US for commercial use and the trend was exponential,[676] the FDA acknowledged that recombinant DNA insertions could alter or inactivate an important gene or regulatory sequence, thereby affecting the expression of one or more genes when the gene is inserted into a genetically active chromosomal location. Furthermore, it was recognized that users of these technologies could not control the precise location in the genome of the target plant into which the GM material was inserted.[677]

During this period the FAO and the WHO simultaneously recognized that observational epidemiological studies were unlikely to identify the human health effects of GM foods in a complex context where the undesirable effects of conventional foods are also present. Adding that "experimental studies, such as randomized controlled trials (RCTs), if properly designed and conducted, could be used to investigate the medium- and long-term effects of any food, including GM foods. These studies could provide additional evidence of human safety but would be difficult to conduct. In this regard, it is also important to recognize the wide variation in diets and dietary components from day to day and year to year."[678]

In 1998, the FDA had been involved in a landmark case amid a spate of legal disputes related to GM crops when it was sued for lack of transparency in the approval process for GM foods. When the Administration was forced to make its files on GM foods public, it came to light that it had concealed warnings from its own scientists concerning the risks and potential harm of GMOs in order to speed up the entry of these products into the market in violation of federal legislation.[679]

The following facts were disclosed in the documents declassified as a result of this litigation[680,681, 682, 683]:

- The FDA issued a policy statement on GM foods in 1992, long before any were ready for the market and also before regulators in other nations had established official positions. This statement paved the way for the marketing of these products by stating there was an overwhelming consensus among scientists that they are so safe they can be marketed without any testing.[684]
- The FDA covered up the crucial fact that its own scientists had concluded that: (a) GM foods come with abnormal risks, especially their potential for unintended harmful side effects that are difficult to detect; and (b) none can be considered safe unless they have passed tests capable of detecting such effects.[685]
- These concerns of FDA scientific staff are supported by a memo from the head of Regulatory Affairs who protested that the Agency was "...trying to fit a square peg into a round hole ... [by] trying to force an ultimate conclusion that there is no difference between foods modified by genetic engineering and foods modified by traditional breeding practices." She then declared: "The processes of genetic engineering and traditional breeding are different, and according to the technical experts in the agency, they lead to different risks."[686]
- FDA officials also knew there was no consensus on the safety of GM foods among scientists outside the agency, and this was acknowledged by its Biotechnology Coordinator in a letter to a Canadian health official in which he further admitted that "the question of the potential for some substances to cause allergic reactions is particularly difficult to predict."[687]

Nevertheless, the FDA continues to operate with a lack of transparency and use an approach that favors the GM crop and food industry to the detriment of human rights. This can be corroborated from an in-depth 2016 study on FDA regulation of GM foods which concludes that:[688] 1) the scientific scrutiny of these foods is not fully transparent and appears to be plagued by conflicts of interest, which prevents manufacturers from effectively demonstrating that they are safe; 2) the FDA omits the risks inherent in the production processes of GM crops from its food safety analyses, such as the use of glyphosate in 90% of GM soybeans and corn in the US, despite having the authority to do so and growing concern in the scientific community and among consumers that at least some GM foods pose such risks; 3). The FDA's limited interpretation of "material" information for food labeling purposes exacerbates the lack of transparency in the regulatory process and interferes with consumers' ability to decide what they consume in order to avoid allergens, respect religious requirements, implement personal ethical choices and avoid risks.

Furthermore, the authors add that although the FDA has broad authority to "regulate by disclosure" and label products, its process allows manufacturers to evade disclosure of the genetic identity of food products and the process continues to lack transparency and is potentially fraught with conflicts of interest.[689]

In addition, the coexistence of GM and non-GM foods on the market poses a problem of asymmetric information. Producers know the composition of their products, but consumers depend on the information provided by producers. Governments must establish standards to ensure safety against threats to life and health as well as prevent adulteration and mislabeling of food.[690]

The regulatory framework addressing biotechnology, in which the FDA plays an important role, faces significant challenges. These challenges are due to existing laws being outdated and not adequately adapted to effectively address the specific risks associated with genetic engineering.[691] This fact has raised concerns about the possible long-term effects of GMO use and its potentially adverse impact on the environment and consumer health.[692]

In addition, international standards and regulations governing biotechnology are fragmented and do not comprehensively cover all aspects necessary for efficient regulation.[693] This fragmentation often means that multiple agencies are involved in the oversight and regulation of biotechnology which complicates the consistency and effectiveness of regulation as a whole. The need for a comprehensive review and update of the international regulatory framework for biotechnology has become critically important to address emerging challenges and ensure the safety and protection of consumers and the environment.

In the case of GM corn, there are several technical, economic, regulatory and socio-political constraints that need to be addressed.[694] In addition, the safety of GM corn as a food and in relation to environmental aspects is a major concern.[695] Commercial cultivation of GM corn has raised safety concerns, and regulations that take into account the impact of GM crops on human health and the environment are crucial.[696]

With respect to the environment, these regulations should consider such factors as genetic suppression, gene expression or the possibility of resistance to the Bt protein.[697] A shared understanding of resistance risks among government regulators, producers and other stakeholders is also critical for effective governance and monitoring of producer compliance with resistance management requirements, resistance surveillance and mechanisms to support rapid implementation of corrective actions.[698]

3. Scientific evidence of the impact on the environment and biodiversity

3.1 About the environment and biodiversity, which includes the biocultural richness of native Mexican corn

Mexico as a center of origin, domestication and diversification of corn: genetic reservoir and biocultural legacy to be protected for the sake of humanity

Not only is Mexico a country of significant biological richness and multiculturalism, it is also a major point of origin for the domestication and genetic diversification of approximately 15% of all important species in the world food system.[699] As indicated above, Mexico is the center of origin and diversification of corn.[700, 701]

Molecular analyses have shown that the domestication of corn started in Mesoamerica approximately 9,000 years ago using an annual species of teosinte (*Zea mays* ssp. *parviglumis*) native to the Balsas River valley on the Pacific slope[702, 703,704] and spread and diversified throughout the Americas.[705] The oldest archaeological evidence of corn was found in the cave of Guilá Naquitz, Oaxaca, and is about 6,250 years old,[706] approximately 700 years older than the oldest corn specimens previously reported in the Tehuacán Valley.[707] The evidence found to date, as well as archaeological and anthropological studies, indicates that Mesoamerican civilizations based their food and culture around the cultivation of corn.[708,709]

The greatest diversity of corn in the world is currently found throughout Mexico along with populations of its wild relatives, teosintes, and another set of related poaceae, species of the genus *Tripsacum*,[710] which are the wild relatives and ancestors of corn.[711]

According to the definition given in Article 1 of the Federal Law for the Promotion and Protection of Native Corn, section VII, native corn is understood as landraces of the taxonomic category *Zea mays* subspecies *mays* that indigenous peoples, campesinos and farmers have cultivated and cultivate from seeds they select or obtain through exchange, and which are constantly evolving and diversifying, as identified by the National Commission for the Knowledge and Use of Biodiversity (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad).[712]

Native corn has been conserved in farming communities thanks to the traditional management practices of seed selection by farmers cycle after agricultural cycle. A total of 64 landraces of corn have been reported in Mexico, of which 59 are native according to the most recent classification based on morphological and isoenzymatic (genetic) characteristics.[713,714] An impressive level of genetic diversity of these native corn landraces is maintained even within individual strains. [715]

This number represents a high percentage of the 220 to 300 corn landraces reported in the Americas.[716,717,718] In the state of Oaxaca alone it has been reported that 35 native corn landraces are cultivated, accounting for more than half the landraces reported for the entire country. This is of particular significance for the *in situ* conservation of the most important genetic reservoir of corn in Mexico.[719,720] The term "landrace (*raza*)" in corn has been used to group individuals or populations that share common morphological, ecological, genetic and cultivation history characteristics that allow them to be differentiated as a group. [721,722,723]

Based on morphological, genetic, adaptive and geographic distribution traits as well as a common evolutionary history, native landraces of corn in Mexico have been grouped into 7 racial groups or complexes (Table 2).[724, 725, 726,727, 728]

Table 2. Strain groups and strains of native corn in Mexico.

Strain groups	**Native corn strains**
Cónicos	Arrocillo, Cacahuacintle, Chalqueño, Cónico, Cónico Norteño, Dulce, Elotes Conicos, Mixteco, Mushito, Mushito de Michoacán, Negrito, Palomero de Jalisco, Palomero Toluqueño and Uruapeño
Sierra de Chihuahua	Apachito, Azul, Complejo Serrano de Jalisco, Cristalino de Chihuahua, and Gordo
Ocho hileras	Blando y Onaveño, Harinoso de Ocho, Tabloncillo, Tabloncillo Perla, Bofo, Elotes Occidentales, Tablilla de Ocho, Jala and Zamorano Amarillo, Ancho and Bolita
Chapalote	Chapalote, Dulcillo del Noroeste, Elotero de Sinaloa and Reventador
Dentados tropicales	Celaya, Tepecintle, Tuxpeño, Tuxpeño Norteño, Vandeño, Zapalote Grande, Nal-Tel de Altura, Pepitilla, Chiquito, Choapaneco and Cubano Amarillo
Tropicales precoces	Conejo, Nal-Tel, Ratón and Zapalote Chico

Maduración tardía	Dzit-Bacal, Comiteco, Coscomatepec, Motozinteco, Olotillo, Olotón, Tehua, Negro de Chimaltenango, Quicheño, Serrano, Mixeño and Serrano Mixe

The great diversity of native corn in Mexico can be explained by the presence of traditional agricultural systems for which native seeds are cultivated, mainly in campesino and indigenous territories and populations,[729] as well as by the deep knowledge and culture of these communities.[730,731,732,733]

Indigenous peoples and campesino communities are the main heirs, custodians and breeders of native corn germplasm engaged in a constant process of managing the genetic diversity of native corn for over 9,000 years.[734] Given the processes of continuous farmer selection of seeds, corn is one of the species with the greatest genetic plasticity in terms of environmental adaptation, capable of growing at high and low altitudes, and in tropical, subtropical and temperate climates.[735]

The cultivation of native corn has expanded geographically with the crop being found at sea level on the coasts of almost the entire American continent as well as in the highlands and mountains of the Andean region. This means it grows in a wide range of agroclimatic conditions within an altitude range of 0-4,000 meters above sea level.[736] Corn cultivation has adapted to different ecological contexts, including areas with low rainfall, temperate regions, the foothills of high mountains, in very hot and humid environments, in areas with poor soils, on steep slopes or in wide fertile valleys, at different times of the year and using various traditional and conventional management systems.[737,738]

In this great diversity of environments, indigenous farmers have accumulated experience and generated knowledge and plant breeding techniques for centuries. This has helped them adapt to extreme climatic situations where most rainfed agriculture is found, while conserving *in situ* the great diversity of native corn to this day.[739]

The diversification of native corn in Mexico is mainly due to the fact that primitive strains of this group of grasses exist as living varieties, interacting with all types of corn. Mesoamerican corn varieties have been enriched with South American varieties and vice versa. In particular, the teosintes have naturally crossed with corn, introducing new varieties and characteristics in both regions. [740741.] This has given rise to an important genetic reservoir of native corn cultivated by indigenous peoples, Afro-descendants and campesino communities using traditional farming methods, ensuring continuous reproduction within traditional agroecosystems.[742,743]

Relevance of the genetic variability of native Mexican corn in the face of climate change scenarios

Based on the adaptive advantages of native corn described above, we can affirm that its genetic variability makes it an important reservoir of collective biological and genetic wealth generated and safeguarded by indigenous peoples, Afro-descendants and other agricultural communities. The *in situ* conservation of this heritage foments development not only of the country's food system but also the global system as it responds to the need to face future adversity brought by global climate change.[744, 745]

To ensure successful harvests two variants of corn are frequently planted: early varieties, which almost all types have, to make the most of available water and the alternation of dry and rainy seasons, as well as offering protection against freezing; and long-cycle varieties with greater productivity. These processes occur year after year for all live native types and varieties thereby ensuring that the process of domestication and diversification continues.

Cultural and biocultural importance of native corn

The cultivation of corn has played a central role in the origin and spread of agriculture and in all indigenous cultures in Mexico.[746,747] The various cultural groups of Mesoamerica have relied largely on the cultivation of corn. The abundance of anthropological and archaeological evidence, the variety of *metates*, *comales*, agricultural tools and ceramics, attest to the profound value of this crop in the past[748,749] while also demonstrating that today it continues to be the central and guiding crop in Mexico's food supply, society, culture and economy.[750,751] Similarly, the traditions and knowledge of Mexico's indigenous peoples are rooted primarily in the culture of corn, reflected in traditional, culinary and religious preferences. [752]

Small-scale producers tend to prefer their local varieties given the advantages identified in native breeds, which are mostly planted in agroclimatic terrain with greater agricultural constraints[753]. It is fair to say that campesino and indigenous communities have more generations of corn adapted to different ecological zones than any scientific or technological group or institution.[754]

For Mexican society and the multicultural diversity of Mexico, corn represents a basic and sacred food as well as cultural identity.[755,756] The great genetic diversity of corn in Mexico is possible because hundreds of native varieties continue to be planted for cultural, social, technical and economic reasons.[757]

The *milpa* system is adapted to a great diversity of ecosystems in which farmers, indigenous or mestizo, through their knowledge and skill and based on their preferences, have managed to adapt and maintain an extensive diversity of native corn in a biocultural context[758] characteristic of indigenous agriculture. In this context, an interconnection between cultivated plants and their wild counterparts is maintained in such a way that it is possible to find cultivated, fostered and tolerated plants growing together on their plots.[759]

Milpas are a biocultural expression of knowledge, technologies and agricultural practices that meet the basic needs of the farming family.[760] Most of the corn varieties we know today were diversified in Mexico, Central America, and the Andean region.[761] The great diversity of corn is not only due to the different climates and ecosystems where it is cultivated, but also to the presence of a significant number of native peoples who have managed its genetic diversity for generations.[762]

It is essential to preserve and safeguard traditional knowhow and expertise in the use and management of native corn varieties, as well as the practices of saving and exchanging seeds within communities from one agricultural cycle to the next.[763,764] This allows alleles to pass from one generation to the next, thereby continuing the evolutionary processes that sustain and generate the genetic diversity of crops.[765,766,767,768,769]

In addition, indigenous and campesino agroecosystems in Mexico and the rest of the world serve as genetic reservoirs for the most important domesticated plant agrobiodiversity.[770] In this regard, it is important to reflect on the great importance of the indigenous genetic improvement process for native breeds of corn, the intellectual and cultural generators of which are the collectives of indigenous peoples and campesino communities.[771]

Foods made from native corn in Mexico

Corn is fundamental to the diet of the Mexican people, accounting for nearly half of the total volume of food consumed in the country. As indicated above, average per capita human consumption of corn comes to approximately 128 kg/year, the highest in the Americas.[772] The numerous varieties of native corn are used to make an enormous number of traditional culinary preparations in addition to tortillas, making corn the sine qua non of Mexican cuisine. All parts of the corn plant are used in some way and there are 605 different ways of cooking and preparing corn-based foods (Table 3).[773,774,775]

Table 3. Corn-based foods

Baby corn	Corn on the cob, tamales, corn bread, toqueras de elote, pictes de elote, uchepos, cuitlacoche, esquites, pozoles, menudos, chacales, chicales, huachales and soups.[776]
Corn kernels	Tlayoyos, memelas,[777] tlaxcales, tortillas,[778] tlacoyos, sopes, itacates, picaditas, quesadillas, tacos,[779] tostadas,[780] tlayudas,[781] remekes,[782] totopos oaxaqueños,[783] gorditas, popcorn, totopos, chilaquiles, enchiladas, enfrijoladas, entomatadas, garapaches, panuchos, papatzules, enjococadas, chopas de perico, chalupas, molotes, peneques, tlatloyos, salbutes, nachos, frituras. Tamales: de nixtamal, de cazuela, joroch, nacatamales, *kehil hua*, *buulil hua*, zacahuil, pibipollo, *tobi holoch*, colados, chanchamitos, corundas, agrios, colados, with beans, with chickpeas, cacahuete, tortilla, tismiche, ceniza, chaya, juacane, chipilín, with fruit (pineapple, coconut, orange, almond, hazelnut, prune guayaba). Pinoles: tascalate, "alfajores", batarete yaqui, with burritos, blue corn manjar, "maria gorda", melcocha, memenshas, tepopoztes, pemoles, totopos de huetamo, boronitas, buñuelos, traditional gorditas, curd, piloncillo, cacahuacintle corn, gondoches de pabellón, Zacazonapan cookies, corn bread, corn cakes, corn turuletes,[784] ponteduro,[785] coricos,[786] *tzualli,*[787] popcorn.[788]
Beverages and fermented drinks	Atoles, puchas or mazamorras,[789] atole agrio or morado,[790] usua, champurrado, chileatole, cuatole, nicuatole, malarrabia, tanchucuá, nixteme, de pinole, bean, peanut, hazelnut, fruit, chiles, pepita, aguamiel, coyol, grano, common of various flavors (chocolate, vanilla, among others),[791] pozol or chorote tabasqueño,[792] tejate, chicha, tesgüino (teshuino or tejuino), yorique, chilote, elisquiate, menjengue, piznate,[793] sende[794] and pox.[795]

3.2 Evidence of damage associated with GM corn, other GM crops and glyphosate to the biocultural richness of Mexico's native corn, biodiversity and the environment

Damage and risks to the biocultural richness of native corn and its wild relatives with the release into the environment of GM corn and the use of glyphosate in Mexico

Evidence of the presence of GMO sequences in native corn and its wild relatives, from risk warning to proof of damage

Between 1996 and 1998 there was an increase in applications for the authorization of GM corn planting trials which posed a risk to the genetic wealth of corn in its center of origin and genetic diversity (centro de origen y de diversidad genética or CODG).[796] At that time forums were held to discuss the risks of releasing GM corn into the Mexican environment, focused mainly on the following: the possible gene flow between GM corn, native corn and its wild relatives, and what this would imply for the CODG; and the required characteristics of national biosafety regulations and risk assessments of GM corn.[797,798]

Although GM corn events had been authorized in Canada, the United States and Mexico, these conferences recognized that information on the potential impacts of GM plants was not available in Mexico. A "full evaluation" was therefore needed, contextualized in accordance with Mexico’s agroecological, socioeconomic and cultural characteristics given the wide diversity of breeds and varieties as well as the two wild relatives of corn: teosinte *(Zea mexicana*, *Z.*mays ssp. parviglumis, *Z.* perennis, *Z.* diploperennis) and Tripsacum spp.[799]

The most significant conclusions and recommendations of these forums were as follows: [800,801]

- With the introduction of GM corn, the biggest question involved the impact these plants would have on the agricultural systems of a country with a high diversity of native germplasm and wild relatives. This was "a serious problem in Mexico due to the deregulation of GM corn in the United States, its geographic proximity, its commercial relations and the importance of corn in Mexican agriculture".
- Research in multidisciplinary groups was required to study the quantitative aspects of gene flow, hybridization and introgression between GM plants, native

crops and their wild relatives, and to assess the risks associated with this technology, as well as consolidate a national biosafety system.

These academic discussions were brought before the National Agricultural Biosafety Committee and led to recommendations to impose a *de facto* moratorium. The Ministry of Agriculture, Livestock and Rural Development (SAGAR, today Sader) responded by implementing this measure from 1999 to 2009, although the issuing of licenses for the planting of GM corn in the experimental phase was resumed in 2003.[802,803,804,805]

The year after the moratorium was imposed, reactions from stakeholders in these techno-scientific developments were not long in coming, with two academics arguing that there was no need to worry about the possible introgression of GMOs into native corn and its wild relatives.[806] Their claims were refuted that same year by a multidisciplinary group of researchers who stressed the importance of precautionary measures in view of the incipient scientific information on the potential impact of GM corn in the plant's country of origin.[807]

In 2001 a scientific study published in one of the world's most prestigious scientific journals demonstrated, for the first time, the presence of GM sequences in samples of native corn varieties from two communities in the Sierra Norte of Oaxaca, a state where there had been no authorizations for GM corn planting trials, and in a sample of loose corn kernels from local stores belonging to the government-run Diconsa, all of which were collected during the last quarter of 2000.[808] These developments reaffirmed the warnings of the scientific community and social sectors, nationally and internationally, concerning the possible consequences of releasing GM corn into the Mexican environment (whether intentionally, accidentally or illegally).

As this news travelled the world, reactions were conflicting.[809] Attempts were made to discredit the findings of the 2001 study the following year using a series of assumptions – referred to in a couple of brief comments published in the same scientific journal – based on information about the corn genome and empirical inferences that questioned the results, stating that they were not GMOs but artifacts produced by defects in DNA amplification techniques for sample analysis (inverse polymerase chain reaction, i-PCR).[810,811]

Opposition to the study was countered by its authors, who pointed out that the criticisms raised only addressed one of its conclusions, which they explained in greater detail. They also elaborated on the characteristics of their research and even provided additional data from the original study.[812] Furthermore, the pair of brief comments was challenged by others in the scientific community, who revealed the existence of "networks of political and financial influence" behind the controversy, given that the authors of the published criticisms had received full or partial funding for their research from a subsidiary of the agricultural biotech company Novartis (now Syngenta).[813]

The controversy was the subject of analysis under the scrutiny of the international scientific community and the journal's editors were even questioned about their own standards regarding conflicts in issues related to political and economic interests.[814] Another comment made emphasized that the GM biotech industry was concerned about the search for the benefits, not risks, of GMOs; in other words, it was pointed out that industry scientists could not be expected to publish experiments showing whether GMOs pose a risk to the environment or human health as this was not their priority. The search for risks was left to other researchers who had a reason to hypothesize about them.[815]

There is documentation showing that researchers participating in the 2001 study in the Sierra Norte of Oaxaca had previously been in contact with the Mexican authorities (the National Institute of Ecology, INE; the National Commission for the Knowledge and Use of Biodiversity, Conabio; and Cibiogem) and that two studies were consequently conducted: [816,817,818,819]

1. INE and Conabio conducted an investigation to corroborate the findings and to evaluate and quantify levels of gene flow between GM corn and native varieties, which confirmed the presence of GMOs in 95% of the locations sampled with a total of 7.6% positive samples.
2. The Ministry of Agriculture formed an *ad hoc* committee and tasked it with carrying out research using samples of native corn from areas of Oaxaca and Puebla. This analysis also confirmed the presence of GMOs in 40% of the sampled plots. The long version of this research has been withheld.

Furthermore, some researchers trivialized the implications of the findings in biological, environmental, social and cultural terms, while applauding the introgression of GMOs detected in native corn, stating that this was not contamination. Their argument was that the spread was not unexpected, undesirable or uncontrollable, claiming that the corn now possessed traits that would probably be preferred by Mexican campesino farmers,[820,821] claiming some kind of representation of these communities.

However, the reactions of campesino and indigenous communities came promptly on two closely related fronts: to defend native corn and to express their rejection of GM corn. These communities were joined by social organizations and groups of committed scientists.

One social organization reported in its digital publication that groups of indigenous and campesino producers, with the support of NGOs, had analyzed more than 1,500 corn plants from 104 locations in eight Mexican states. The analyses were performed using immunological tests with positive results for GMOs in 18 locations in the states of Oaxaca, Puebla, San Luis Potosí, Chihuahua, Veracruz, Tlaxcala, Morelos and Estado de México. It was even reported that several samples contained the Cry9c protein, present in the StarLink transgenic event which, as outlined in previous sections, triggers allergenic reactions to the extent that it has been banned internationally.[822, 823, 824]

These findings were not well received by the public sector, who dismissed them under the "gold standard" because they had not been published in an international scientific journal; nor did they make any effort to even verify them; and, at the same time, they systematically ignored the concerns, opinions and knowledge of the campesino communities. Nevertheless, the findings did spur a national and international mobilization that soon linked up with grassroots campesino, social and scientific organizations around the world.[825, 826, 827]

In 2002, 21 indigenous and campesino communities in Oaxaca asked the Commission for Environmental Cooperation (CEC), created after the signing of the North American Free Trade Agreement (NAFTA), to conduct an analysis of the effects of the introgression of GM sequences in native corn varieties. This request was backed by more than 90 letters from organizations and institutions from the Agreement's three member countries.[828]

The CEC considered that the "matter could be of great environmental importance, since Mexico is a center of origin and diversity of corn and the grain is intrinsically linked to Mexican culture, especially that of the indigenous communities". Consequently, an advisory group was formed with the mandate to prepare a report with the highest levels of scientific accuracy and objectivity, transparency, communication and participation, to provide recommendations to the three NAFTA countries for defining policies.[829]

It was in this context that a first document was published[830] addressing, with scientific rigor, the question of the impact of GM corn on ecological, biological and agrobiodiversity aspects. It highlighted the importance of corroborating the presence of transgenic sequences in the native corn of Oaxaca and extending research to other states, noting that:

- The unintentional movement of GMOs into corn populations, for which such genetic transformations were not designed, entails risks on two levels: the possibility that GMOs could enter and persist (introgression) in native corn strains and other cultivated varieties, as well as their wild relatives; and the biological consequences of this introgression.
- The scope of impact is ecosystemic;
- Through gene flow, GM varieties can alter biodiversity because of their impact on the environment and on other unrelated species (such as teosinte, a wild relative of corn);
- There is a negative effect on non-target organisms, with a particular impact on beneficial insects, pollinators (the best known case is the impact on monarch butterfly populations) and other organisms that act as a natural control of "pests" targeted by GMOs.
- Further research is needed on the long-term effects in this and similar cases.
- There are other effects such as: the potential for accumulation of recombinant DNA in the environment, with ecological implications; the horizontal transfer of any of the transgenic sequences to bacteria, viruses or other organisms; the possible impact of new developments in corn for the production of drugs and substances for industrial use; as well as unknowns regarding the genomic instability of GMOs. The need for further research on this matter was noted.

Also, the priority was set to step up measures to prevent gene flow from reaching more native corn varieties, along with teosintes, as well as to determine the spatiotemporal dynamics of the presence of GMOs and their origin. In this regard, it was noted that a "possible source of entry is the sale in rural stores of imported fertile corn kernels, some of which come from GM varieties deregulated in the United States".

Based on these data, in 2004 the CEC recommended, among other things: that the moratorium be kept until "adequate research and assessments of the risks and benefits of the effects of gene flow from GM corn to local strains and teosinte" was conducted and more information was shared with farmers and rural communities; that the Mexican government should reinforce the moratorium on the commercial cultivation of GM corn and prevent the entry of viable GM corn grain, as well as its planting; and that research should be conducted into the impact of GM corn consumption on human health, taking into account the characteristics of the Mexican population with its very high corn consumption.

Research on the subject continued, mainly in the hands of the academic community. In 2004, a model based on corn production conditions in Mexico was used to show possible scenarios of GMO dispersal, considering the characteristics of traditional open systems with free exchange of seeds, concluding that the most likely outcome of releasing GM corn would be the incorporation of GMOs in the genome of Mexican germplasm and possibly in that of teosinte, with multiple biological, agronomic, legal and cultural implications if GM corn was not regulated.[831] Another study concluded that analyses of the potential impact of GM corn and the spread of GMOs should take into account the characteristics of Mexican farmers' management practices, in addition to noting the influence of socioeconomic conditions on the conservation of corn landrace diversity in traditional farming systems.[832]

In 2005 a group of INE officials published a study based on new samples of corn from Oaxaca, collected between 2003 and 2004, and reporting that no evidence of GMO introgression had been found in local corn varieties in the Sierra de Juárez of Oaxaca. The authors argued that concerns about unintended or unknown effects of GMO introgression in native corn varieties could be dismissed, at least in the region sampled, and that GMOs do not remain in the environment due to processes they speculate about in their findings.[833]

Once again, the scientific community's reactions were set out in academic publications. On the one hand, there were a couple of reports that did not go beyond the collection of a few impressions and opinions of their subscribers and others.[834, 835] On the other hand, some authors took the time to analyze the methods and results presented in the 2005 publication, arguing that the claims about the supposed non-existence of evidence of GMOs, at detectable levels or for introgression, in the corn of the Sierra de Juárez of Oaxaca, were not scientifically justified given that: their samples were not representative and their statistical analyses were inconclusive; there was a viable possibility of false negatives inherent to the characteristics of the analyzed material and its standardization processes; they did not take into account the reduced likelihood of detection because of the expected skewed frequency distribution of GMOs in the study area. [836,837,838]

In 2005 the Biosafety of Genetically Modified Organisms Act (LBOGM), nicknamed "The Monsanto Law", was published. Under this law, from 2005 to 2012, 196 permits were issued for the release into the environment of GM corn, most of them in the experimental phase; 80% were transgenic events tolerant to the glyphosate herbicide.[839] In addition, from 2005 to 2018, 90 authorizations were granted for the importation and use of GM corn in grain, 79% of which were glyphosate-tolerant transgenic events.[840] This GM corn grain, which is imported into Mexico from the United States, has germination potential.

Three 2006 reports demonstrated, respectively, the presence of GMOs: in the state of Sinaloa, with five out of 157 samples of native corn testing positive; again in Oaxaca, this time in three regions different from the previous studies, with positive samples in native corn from five plots in the Sierra Norte region; and, also in Oaxaca, samples from municipal and local markets, of corn and non-industrialized food products made from corn, with transgenic sequences detected in 76% of them.[841,842,843] In 2007 a new scientific article was published illustrating how the spread of GMOs may be occurring in Mexico. In this case, GM proteins were detected in samples of native corn from the conservation zone of Mexico City, an area with a strong presence of farmers, which takes up most of the surface area of this territory. [844]

Two years later, a new study came to light confirming the presence of GMOs in corn in the same localities of the state of Oaxaca where the first detection had been made in 2001. The study used robust sampling from corn obtained in 2001 and 2002 comprising 23 localities in the state of Oaxaca (with 3 samples from a local market, a Diconsa store and a Conasupo store, respectively) and two from Puebla; in addition to targeted sampling carried out in 2004 in 60 plots in the two study localities that demonstrated GMO contamination in 2001. Analyses of the samples from 2001 reveal consistently positive results for GMOs in corn from three locations, including the two from the previous 2001 study; also, targeted sampling in 2004 revealed the presence of GM DNA in corn samples from 18.3% of the plots, providing evidence of the persistence or reintroduction of GMOs in the localities. On this point the authors note that, although further studies are needed, reintroductions are unlikely because, according to information provided by the farmers, the seeds from each sampled lot excluded local varieties; also, the experimental results underlined the importance of the effects of sampling.[845]

As before, there were clashes in the scientific community over these findings, especially involving personnel from the private laboratory where the analyses of the 2005 study were performed, pointing to the non-existence of GMOs in Oaxaca through a series of short statements. However, the research was well received by one of the authors of the 2005 study, who described the work as a masterpiece and praised the robustness and sharpness of the analytical method, both in the detection and the statistical model, and added that these new findings demonstrated the presence of GMOs in the 2001 and 2004 samples thereby resolving apparent contradictions in the scientific literature, while at the same time raising the bar for subsequent studies on the subject.[846]

The authors closed the debate with a forceful rejoinder in which they expanded on the explanation of their findings and conducted an in-depth examination of their opponents' statements, clearly discerning the qualities and nature of the sampling methods and laboratory tests used in the studies on this topic. They concluded that "the monitoring of GM DNA in the environment should be carried out by independent, non-commercial and transparent institutions with a clear mandate of public good rather than profit".[847]

A report sets out the findings of participatory biomonitoring of traditional *milpas* conducted during the period 2003-2007 which found GM proteins present in corn from farming plots in localities in the states of Chihuahua, Puebla, Veracruz, Oaxaca, Hidalgo, Tabasco and Chiapas. This is the first extensive sampling effort performed with the active participation of campesino and indigenous people.[848] In 2010 a study was performed with hybrid and native corn samples from 2006 and 2007 in 18 municipalities in the north, center and south of the state of Veracruz. The analyses revealed the presence of GMOs in the corn samples.[849]

In 2013 a class action civil lawsuit was filed with the federal courts demanding a declaration on the human right to the biodiversity of Mexico's native corn. This lawsuit decried the violation of human rights such as: the right to a healthy environment, to the conservation and fair and equitable sharing of natural resources, as well as their sustainable use guaranteeing the availability of the biodiversity of native corn for future generations; to adequate, nutritious, sufficient and quality food; to cultural rights and to health.[850] Given that this was a matter of public interest involving human rights, a judge decided to grant an injunction, effective as of September 17, 2013, preventing the release of GM corn in the Mexican countryside, pending the resolution of the class action lawsuit.

In recent years, eight more studies have again confirmed the presence of GM DNA in samples of mostly native corn, in addition to their detection in corn-based foods, grains and flours. In the first, from 2017, samples were taken from two communities in Oaxaca, obtaining seed lots from 40 farmers and 13 samples from local stores and markets. Using a statistical approach, positive results for GMOs were obtained in 6 samples from one of the communities: four of them were from farmers, one of whom sold seeds at the largest producers' fair in the municipality of Ocotlán de Morelos, the other three were locals participating in a seed exchange network; the other two samples came from local stores (Diconsa and a market). In this same study, the authors argued that sociobiological factors were determinants of the spread of GMOs within a community, so that social practices and arrangements can also be used as a way of minimizing the potential or scale of GMO flow.[851]

A second report from 2017, analyzed samples of predominantly native corn from 18 states in Mexico, with greater sampling performed in Nayarit, Michoacán, Oaxaca and Puebla. Positive results were obtained for the presence of GMO markers in 10.7% of the samples from 12 states: Chiapas, Colima, Mexico City, Estado de México, Michoacan, Morelos, Nayarit, Oaxaca, Puebla, Sinaloa, Tlaxcala and Veracruz.[852] Furthermore, in 2017 the presence of transgenic sequences was demonstrated in 82% of corn-based foods, which are in high demand and readily available (tortillas, flour, tortilla chips, breakfast cereals and snacks). In the case of tortillas, GMOs were detected in 90% of all samples analyzed, and 30% of these contained glyphosate residues.[853, 854]

In another study, published in 2018, the samples came from communities in Chiapas, Mexico City, Michoacán, Oaxaca and Veracruz, which are Mexican states with high diversity of native corn varieties. GMOs were found in 13%, 2%, 5%, 7% and 15%, respectively, of the samples. The size and distribution of the samples were representative of each state.[855] The last in this series of studies, published in 2019, identified the presence of transgenic sequences in 11 out of 192 samples of native corn in the state of Coahuila, revealing the presence of GMOs at the intra and inter-population levels and warning of a critical situation as there is a notoriously extensive dispersion of transgenic sequences in zones supposedly protected as "centers of origin and diversity" established under federal decree.[856, 857]

In 2023 transgenic sequences were found in samples from four Mexican states (53 in Jalisco, 96 in Michoacán, 46 in Oaxaca and 20 in Puebla), with the detection of the cry1Ab gene reported in all states, with 14 samples in Puebla, 24 in Oaxaca, 8 in Jalisco and 42 in Michoacán.[858] That same year a report was published on the detection of the t-NOS transgenic sequence in 8 of 63 samples in a community in Oaxaca. There were interesting findings: possible scenarios for the origin of the

GMOs were identified and community biosafety strategies were proposed, based on surveys of farmers on socio-cultural aspects.[859]

At this point, it is important to point out that the vast majority of the studies referred to here, with positive results in the detection of GMOs in native corn varieties in Mexico, show the presence of Cry proteins or GMOs that express the production of these insecticidal protoxins. The most recent studies are no longer based only on screening (presence/absence of GMOs) but quantify the presence of GMOs of specific events, especially the most common commercial events worldwide: MON810 and NK603, resistant to insects by the expression of insecticide-acting proteins and tolerant to glyphosate herbicide, respectively.

Lastly, in September 2024, the researchers responsible for a Conahcyt-backed project announced that samples of corn grain, seed and flour had been gathered from collection centers, seed mills and flour mills in 23 Mexican states, based on a nationally representative sampling design. Their analysis revealed the presence of GMOs in 78% of the grain samples collected, 16% of the seed samples and 6% of the flour samples. The highest frequency was in Puebla, Hidalgo, Morelos, Estado de México, Guanajuato and Jalisco, for both grain and seed, and in Oaxaca and the Yucatan Peninsula for flour. Furthermore, so far 39% of all GMO-positive samples have been analyzed for residues of two highly hazardous pesticides, with glyphosate and glufosinate-ammonium detected in 34% and 5%, respectively.[860]

Other recent studies have focused on reviewing and analyzing the biomonitoring studies as a whole coming to the following conclusions: [861,862]

- In Mexico there is an undesired presence of GMOs in different corn varieties; studies have focused mainly on native corn.
- There is a great lack of knowledge about this phenomenon, at the national level, and its dynamics due to the lack of systematic monitoring; to date, representative statistics are available for nine Mexican states.
- It is essential that the biosafety authorities make real efforts to fulfill their monitoring, inspection and surveillance duties in order to mitigate the problem of GMO contamination and its consequent environmental, social, economic and cultural repercussions, as well as to protect the most diverse gene pool of corn in the world for the benefit of all mankind.
- Biosafety strategies must include the full and effective participation of rural communities, especially indigenous peoples and campesino groups who, in

practice, are the people most affected by damage associated with GM corn and its toxic technological package. At the same time, they are the ones who have conserved and generated the biocultural diversity of Mexico's native corn and the genetic richness of its agroecosystems.

The federal government's actions in response to indications of GMO contamination have been analyzed by members of the scientific community.[863,864,865,866] Some authors have pointed out that these actions were characterized as a "performance of seriousness"; in other words, a simulation, deaf to the voices of rural communities and blind to the complexity of genetic landscape functionality: [867,868, 869,870]

- Since they did not respond with effective biosafety strategies, but rather with empty rhetoric focused on reassuring Mexican society: 1) with the development of ad hoc studies, without considering the broad range of detection data or the diversity of actors and knowledge cultures, and 2) with the creation of institutions and norms that pay lip service to international environmental and commercial standards.
- Meanwhile, based on the sophism of the possibility of coexistence, the actions amounted to useless and unfeasible measures to protect native corn, as well as weak and ineffective controls to address the problem of GMO contamination, but favorable for commercial transactions, especially for grain imports from Mexico's northern neighbor.
- They contributed to the imposition of a hegemonic culture that defined itself as "sound science" with metrologies and standards servile to the biotech industry, whose epistemic, ontological and socioecological aspects are discriminatory and hostile to the cultures of Mexico's campesino communities.
- They completely disregarded the voices of rural communities and ignored the dynamic, biocultural and extensive network-like functioning of genetic landscapes, and the fluidity and diversity of corn genomes.

GM corn cannot coexist with native corn, nor with plants that are wild relatives or with hybrid varieties, known as improved varieties.[871,872] GMO contamination is caused by:

1. Corn's reproductive system and the mobility of corn pollen. This refers to the fact that pollen can travel several hundred meters by the action of the wind.

2. The distribution of native breeds in the national territory. Native corn is present in all our country's states, which means it is exposed to GMO contamination.
3. The cultural practices of indigenous peoples and communities, as well as campesinos, involve a dynamic and fluctuating exchange of seeds, entailing their movement in Mexican territory and in other countries. This has historically paved the way for the diversification of corn. If GMO-contaminated seeds are included in the exchange, contamination is likely to spread to other planting sites.
4. The transport of GM seeds for marketing and processing does not guarantee that they will reach their destination without accidental leakage or spillage of this product.
5. Possible illegal planting of GM corn.

In parallel to the study of the presence of GMOs in native corn populations and their wild relatives, during these years hypotheses about the dynamics of corn seeds and the mechanisms of dispersion of foreign genes, such as GMOs, were strengthened: [873, 874, 875, 876, 877]

- The introduction of foreign germplasm into communities is a fluctuating phenomenon that responds to different needs and customs associated with economic and social phenomena and cultural aspects.
- Foreign seeds account for a small fraction of the plots managed by most traditional farmers; seeds with preferential characteristics spread rapidly.
- The small fraction of introduced seeds contributes to variability in the form of diversity of corn types or through introgression into local varieties by hybridization.
- Gene flow between introduced materials and native corn populations are an effective means of disseminating GMOs if they are present in pollen grains.
- There is a high likelihood that the incorporation of foreign seeds and campesino management practices could favor the gene flow of GMOs.
- Seed flow between farmers leads to a much more extensive spread of GMOs than expected from pollen movement alone.
- Theoretical approaches to GMO elimination need to consider ecological complexities, while exploring evolutionary processes and agricultural practices that can help reduce GMO contamination of native corn populations.
- There is a potential negative impact on biological and cultural diversity of the environmental release of GM corn in Mexico.

- The authorities' decisions on the importation and circulation of GMOs in the national territory have a decisive impact on the contamination issue.
- Biosafety regulations must include measures that guarantee the protection of species for which our country is a center of origin and diversification.
- It has been demonstrated that it is impossible for GM corn and Mexican varieties (native, hybrid and their wild relatives) to coexist without GMO contamination.
- Other factors need to be considered in the analyses, such as socioeconomic changes (e.g. migration, trade liberalization and lack of support for Mexican farmers).
- It is necessary to insist on the conclusions and recommendations reported in the CEC report, especially regarding the moratorium and its extension to import restrictions on viable GM corn grain.

Effects on biocultural richness

As we have seen so far, corn in Mexico has great cultural, biological and economic value thanks to its long history of domestication, diversification and cultivation,[878] which dates back to ancient times and is still present today. In addition to being the country's main food source,[879] corn occupies more than half the total area planted in Mexico,[880] as it sustains a way of life hinged between culture and nature.[881]

The cultivation of corn has been preserved over many generations, largely by communities, mostly indigenous, or by small farmers who, through their culture, techniques, technologies, understanding and know-how, have ensured the permanence and improvement of this crop,[882] which dates back approximately 9,000 years[883] maintaining a range of varieties differentiated by their color, texture, flavor and weight. These varieties have adapted to different growing conditions from mesophilic forests in the mountains to the coasts.[884] This means that traditional and indigenous farmers are the custodians of the biodiversity of native corn[885] and that defending this means preserving their own identity.[886]

The cultivation of native corn has survived despite the trend over the last 30 years to abandon traditional lands and practices[887] due to the promotion of hybrid seeds, the projects and consequences of the so-called "Green Revolution" of the 60s and 70s, the effects of the liberalization of yellow corn imports, essentially GM and of low nutritional quality, among other factors.[888,889] More than 40% of the country's total agricultural labor force is devoted to the cultivation of this corn. [890] The existence of

59 native strains in Mexico[891] is due to several factors related to the biocultural importance of corn. There is a culinary preference for native varieties, for their ritual use in various ceremonies and festivals,[892] management techniques, forms of use,[893] as well as their medicinal use,[894] among other things.

Culturally, corn is a foundational element. There are testimonies of its use in archaeological sites and codices such as the Florentine or Mendoza[895] or those from Calakmul[896]. One example of this is the Mayan cultures, where corn is the basis of their social structure and religion and is present in daily life in general. Experts consider that the Mayan calendar developed from corn agriculture[897], which means it is fundamental from the origin myth, the counting of time, to its material production.[898]

Corn as food is prepared fresh as corn on the cob or dried (Table 3). The tortilla is the main form of food processing and is considered the mainstay of 94% of the Mexican population, mainly in rural areas, where its consumption is 328 g per *capita* per day. This makes Mexicans the biggest tortilla eaters in the world, consuming almost 12 million tons of tortillas a year.[899]

With regard to biological aspects, traditional systems such as *milpas* have played a key role in biodiversity conservation, because they conserve local species, native crops and germplasm.[900] Observation, creativity, experimentation and necessity over time have given rise to diverse adaptations of the *milpa* itself and, with it, the care of nature, an example of which is the Milpas Intercalated with Fruit Trees (Milpas Intercaladas con Árboles Frutales or MIAF).[901] These are *milpas* with different levels of animal and plant management,[902] which makes for a synergy of the traditional *milpa* and different biocultural elements.

Understanding, valuing and protecting local food systems, i.e. taking stock of the diversity of actual and potential edible resources available, their nutritional contributions, cultural meanings and the context surrounding ecosystems, the landscapes they form, agroforestry systems, species and varieties used[903] is one of the first steps towards understanding and invigorating food sovereignty.[904]

However, the complexities of global political processes, together with the prioritization of an economic system that tends to devalue or monetarize all these elements, have put agrifood systems at risk, modifying traditional food and replacing it with processed foods that, in most cases, are not made from corn.[905] This state of affairs, along with other factors such as the rural exodus, the loss of biocultural memory and climate change, comprises a risk factor for the conservation of native corn.[906, 907]

In general, available sources of information globalize Mexican white corn as equivalent to corn for human consumption, because that is the international categorization. The fact that corn for human consumption has different colorations (white, creamy, yellow, blue, purple, black, red, mottled, pink, striped, etc.) and textures is overlooked. All these native corns are erroneously globalized statistically as white corn and demerited in price because they are not white.

These native corns are irreplaceable for Mexican multicultural cuisine. No imported corn, white or yellow, can be used to make any of the different corn-based food preparations with the usual and traditional organoleptic quality that exists in Mexico. For example, tortillas in general, tlayudas, the Oaxacan totopo, tamales, pozole, etc., as well as beverages like pozol, tejate, tejuino, tascalate, etc.

Imported GM yellow corn is used for animal feed in Mexico, as well as in the starch, fuel and other industries. However, as mentioned above, research in 2018[908] showed that 82% of corn-derived foods (tortillas, tostadas, flour, cereals and snacks) collected in supermarkets, markets and other retail outlets contain GM corn sequences. In particular, 90.4% of the tortillas studied contained recombinant GM corn sequences, while 60% of those samples had glyphosate residues.

At the same time, there is a close link between environmental harm and adverse effects on the social and economic dynamics of a population. The socioeconomic and cultural consequences of using toxic pesticides such as glyphosate can drastically transform the life of an entire region.[909]

It is important to highlight that the loss or disuse of traditional forms of production and, hence, decreased diversity in production have led to the homogenization of crops that no longer responds to local and national food needs, with production being channeled towards the international market.[910]

Rural communities are directly affected by the use of glyphosate and other pesticides, as well as by low income generation, a shortage of commercial opportunities and scarce sources of employment.[911] Campesino agriculture, with its range of production dynamics, has been negatively impacted for several decades by agribusiness.[912] As a result, traditional practices that allowed the recovery of ecosystem functionality have been lost.[913] Industrialized agriculture therefore puts at risk the biocultural richness and agrobiodiversity of traditional crops in Mexico, causing a reduction in the consumption of various species in many rural areas [914] – foods that have been part of the food base and in other cases, used for medicinal and artisanal purposes for many generations – as the availability of useful wild crops for communities becomes increasingly limited.[915, 916, 917]

Damage and risks to the environment and biodiversity due to the release into the environment of GM corn and the use of glyphosate

Environment

GM corn is produced using monoculture practices that are extensive in terms of land use and require a large amount of resources. The expansion of the industrial agricultural frontier of GM crops has caused environmental degradation, impacting the habitats of various animal and plant species and reducing biodiversity. These practices are also conducive to the emergence of pests and diseases, which increases the risks faced by agriculture. In addition, the production of GM corn generates significant greenhouse gas emissions, contributing to climate change. [918,919]

Furthermore, as mentioned earlier, there has been a significant increase in the use of glyphosate worldwide, associated with the growth of industrial agriculture and the increase of GM crops.[920,921] However, there has been no consistent or systematic monitoring of the environmental effects of GBHs, and accurate data on the quantities of glyphosate-based herbicides sold or applied in agricultural regions around the world are often non-existent.[922]

The increased use of glyphosate in agriculture has therefore also caused environmental problems, with direct and indirect effects substantially impacting biodiversity and ecosystems. In particular, the effects reported involve impacts on the structure and composition of biotic communities, alterations in food webs, as well as different types of damage to the ecological niches and habitats of different species, including some considered of great importance for maintaining the planet's ecological balance, as well as for the functioning of agrifood systems, such as pollinators and insects in general.[923, 924]

Impacts on soil and water

The introduction of herbicide-tolerant GMOs has led to the increased use of glyphosate. This has caused indirect effects on soil ecology. GM crops and their associated technological package, which includes highly hazardous pesticides such as glyphosate, have a negative impact on soil microorganisms (fungi, bacteria, among others), including the beneficial microbiome that breaks organic matter down and helps the decomposition of plant residues. Soil structure and health are altered in the long term.[925, 926, 927, 928, 929]

It has been reported that the persistent presence of Cry protoxins, contained in GM Bt plants, affects interactions with bacteria, fungi and other soil microorganisms that perform a crucial role in the decomposition of organic matter and the availability of nutrients to plants. This has consequences for the structure and functionality of microbial communities.[930, 931] These toxins impact the diversity of organisms in the soil, including some that play important roles in pest regulation, such as predatory nematodes like *Caenorhabditis elegans*, *Eisenia fetida* and soil mites.[932, 933, 934, 935] It has been noted that the *C. elegans* species is sensitive to the Cry1Ab protein of GM Bt corn, which affects growth and reproduction.[936, 937]

Fungi appear to be the organisms most affected by Cry proteins in soil. The loss of symbiont fungi in corn roots affects plant nutrition and makes the plant more susceptible to insect pests because the absence of mycorrhizae diminishes the presence of natural enemies of pests.[938] For example, it has been shown that the GM corn events Bt 11 and Bt 176, and their residues, decreased mycorrhizal colonization and negatively affected the development of these organisms by indigenous endophytes.[939]

In the United States, the effects of Bt corn and its residues were evaluated, in comparison to non-GM corn, on rhizospheric eubacterial communities, the mycorrhizal symbiont *Glomus mosseae* and soil respiration. The experiments showed differences in rhizospheric eubacterial communities associated with the corn lines and a significantly lower level of mycorrhizal colonization on the roots of Bt corn. Greenhouse experiments detected differences between Bt and non-Bt corn plants in rhizospheric eubacterial communities (both total and active), in culturable rhizospheric heterotrophic bacteria and in mycorrhizal colonization. In addition, plant residues from GM plants affected the respiration of soil organisms, bacterial communities and the establishment of mycorrhizae by indigenous endophytes.[940]

At the same time, in contaminated environments there seems to be a synergistic effect from GM corn, as it has been detected that, in cadmium contaminated soils, GM corn responded differently to mycorrhizal fungi inoculation, with a colonization of 14 to 33%, while non-GM corn had a mycorrhizal colonization of 32 to 74%.[941]

As for glyphosate, following its application on crops or weeds, its most frequent degradation pathway is through microorganisms, which transform the glyphosate molecule into AMPA.[942] Different reports have noted that the toxicity associated with AMPA is similar to or greater than that of glyphosate,[943,944, 945, 946] in addition to having greater persistence and mobility in water bodies and soils. This means that it tends to accumulate for a longer time, therefore becoming a source of continuous exposure for organisms in contaminated soil and water. [947,948,949,950]

Also, both glyphosate and AMPA have been found to be widespread environmental contaminants currently found in the atmosphere, soils, various sediments and microbial ecosystems (such as biofilms and microbial mats), in a wide range of surface and groundwater bodies, including drinking water, in urban, peri-urban and agricultural settings, as well as in marine environments.[951,952,953,954,955,956,957] Additionally, accumulation of glyphosate in water sources increases when formulations contain surfactants such as POEA, and numerous studies have also detected this substance in plants, soil and water, along with glyphosate itself and AMPA. [958,959,960,961]

A 2017 study analyzing the presence of glyphosate and AMPA in EU farmland found that glyphosate and AMPA were present in 45% of sampled soils from eleven countries and six cropping systems.[962] Another 2020 study analyzed global environmental risk from glyphosate use and demonstrated that 30% of the world's agricultural area had detectable levels of glyphosate as a persistent contaminant, while AMPA was persistent in 93% of this area[963]. The half-life of glyphosate, before it degrades to AMPA, is between 2 and 215 days; its half-life in soil is 6 to 20 days and in water it is between 2 and 91 days.[964]

Once they have accumulated in soils and water, glyphosate and its metabolite are transported and moved in different ways, thereby spreading environmental contamination, even to regions where these herbicides are not used. Several recent experimental and monitoring studies confirm the movement of glyphosate and AMPA by wind and water.[965,966,967,968] Bento and colleagues demonstrated in a wind tunnel experiment that the presence of AMPA contents and, in particular, glyphosate contents was especially high in finer soil particle fractions, which humans can inhale directly.[969] Differences have been observed, in accordance with cropping systems and soil types, in terms of glyphosate and AMPA transport potentials: non-permanent industrial crops and tubers reveal a higher movement potential through wind erosion while permanent crops and cereals are the most mobile through water erosion.[970]

Regarding the situation on the national level, reports have demonstrated the accumulation of glyphosate in soils and water bodies. For example, a collaborative study by Rendón von Osten and Arellano reported the presence of glyphosate in coastal waters of the Yucatan Peninsula, especially near sites with a higher concentration of agricultural areas.[971] Glyphosate can easily leach into subterranean aquifers. Yucatan in particular is extremely vulnerable because of its soil characteristics and the ring of cenotes interconnected by subterranean channels that transport water reaching the coast of the Gulf of Mexico.[972,973]

In another study by Rendón, glyphosate was detected in groundwater and drinking water in localities in Hopelchén, Campeche.[974] Ruiz Toledo and colleagues found the herbicide in several bodies of water in Chiapas, some of them inside Natural Protected Areas (NPA), as well as in water wells for human consumption.[975]

This accumulation also occurs in the north of the country. Alarmingly, glyphosate has been detected in priority terrestrial regions where there is no agriculture, such as the prairie dog distribution region in the states of Coahuila and Nuevo Leon. Soil samples collected in Nuevo León showed high concentrations of glyphosate, ranging from 5.9 to 13.5 mg/ga; water samples also showed high concentrations, even higher than the level permitted by the US Environmental Protection Agency, established at 700 µg/L for drinking water.[976]

As for the soil, although it was initially argued that glyphosate acted only on plant organisms, a claim that still persists in some academic circles and in certain spheres of the agricultural sector, it is now known that the shikimate pathway is also present in microorganisms. Glyphosate alters microbial growth and activity in susceptible prokaryotic species present in soils, as well as facilitating the accumulation of shikimate at these sites.[977,978,979,980] Glyphosate has been reported to be toxic to bacteria and fungi present in soils, and populations of these microorganisms are reduced by increasing herbicide concentration. [981]

In particular, there is evidence of the negative effects of glyphosate on rhizosphere microbial communities, certain bacteria such as pseudomonads, which are involved in the cycles of soil nutrients such as carbon and nitrogen, as well as in the production of indoleacetic acid, which is one of the most important phytohormones for plant development.[982] Other effects on rhizosphere bacteria have been documented in the acidobacteria group, which become relatively scarcer in environments with excessive use of glyphosate, for example, in HT GM corn and soybean crops. This reduction leads to a decrease in biogeochemical processes important for plant growth.[983]

Other bacteria of great importance in agriculture are those included in the group called rhizobia, which includes more than 35 species in Mexico, mainly of the genera *Rhizobium*, *Bradyrhizobium and Mesorhizobium*. Their importance lies in their ability to fix atmospheric nitrogen when they establish symbiotic relationships with leguminous plants and form nodules in their roots. [984] A recent study found that glyphosate inhibits rhizobia growth in root nodules of GM soybean plants. Resistant and glyphosate-treated soybeans were found to have lower chlorophyll content, root mass, nodule mass, total plant nitrogen and nitrogenase enzyme activity.[985]

Biodiversity

The widespread adoption of GM corn may lead to the loss of biodiversity in agroecosystems. This could have long-term implications for the ability of crops to survive diseases, as well as their adaptability.[986] In this respect, it has been observed that plants treated with glyphosate were more susceptible to pathogens.[987] The introduction of HT-type GM crops causes changes in the abundance of wild crops, which in turn affects the availability of seeds, an important food source for farmland birds.[988] Bees are also affected by the decrease in wild crop diversity due to the diminishing richness and abundance of floral resources, such as nectar and pollen from these plants.[989]

Mass planting of GM monocultures involves gene flow between GM crops, non-GM crops and wild relatives, the development of insect resistance and affectations to soil fauna and non-target organisms.[990] In particular, there is concern about the flow of genetic information that can affect biodiversity.[991] Additionally, the introduction of GM corn may alter the ecological balance in agroecosystems by affecting non-target organisms.[992,993,994,995]

The accumulation and persistence of glyphosate, with the consequent production of AMPA, can alter the structure and composition of aquatic and terrestrial communities, which entails a significant ecological risk that can seriously affect biota inhabiting the ecosystems.[996] During the review required to renew the registration of glyphosate-based herbicides, performed every 15 years by the EPA, it was found that 93% of plant and animal species (fish, amphibians, reptiles, birds and mammals) included in that country's Species at Risk Act (ESA), as well as 96% of their habitats, are at risk because of the use of the glyphosate herbicide in that country, even when it is used in accordance with the recommendations on the label and in the relevant regulations.[997] The EPA's report implies that the survival of 1,800 plant and animal species is compromised by current use of this herbicide which, for decades, was considered environment-friendly by the EPA.

Multiple scientific investigations have shown how the use of glyphosate on different crops around the world has direct and indirect effects on populations of various non-target organisms, from microorganisms such as algae and protozoa, as well as beneficial fungi and bacteria, to complex organisms such as plants and animals, including both invertebrates (e.g. insects, micro-arthropods and arachnids) and vertebrates (e.g. fish, amphibians and mammals). [998,999]

Effects on aquatic biota

Aquatic ecosystems are strongly connected to the surrounding agricultural landscapes, in which chemical compounds such as pesticides are used often affecting non-target groups of organisms. GMO planting associated with the use of highly hazardous pesticides has been extensive; however, there are few risk assessments that consider invertebrate aquatic organisms. According to a meta-analysis conducted in 2018, there are 39 publications dealing with GMOs and their impact on the aquatic environment. Available information is limited to a few agricultural varieties, events and trial organisms. Analyses of studies on the fate of leached toxins, degradation of plant material and distribution of crop residues in the aquatic habitat have not been sufficiently investigated.[1000]

Subsequent studies suggest that the transfer of GM corn by-products from agricultural fields to nearby watercourses after harvesting is significant and persists more than one year after planting. Tissues such as leaves, stalks and corn cobs become a detrital food source for organisms such as shredders in the river ecosystem, for example the river crayfish, *Faxonius rusticus,* young specimens of which, after being fed with Bt corn varieties, showed lower growth than those fed with negative controls or glyphosate-resistant corn.[1001]

Recent evidence points to the serious effects of glyphosate, POEA, AMPA and commercial HBG formulations on marine ecosystems.[1002] In one study, these compounds were found to cause primary DNA damage in zebrafish larvae and RTG-

2 cells. All of these compounds are genotoxic to zebrafish larvae, and it is glyphosate that causes the greatest DNA damage.[1003] The genotoxicity of this compound and of commercial products containing it, such as *Roundup Transorb*®, was also reported in fish gill cells and erythrocytes.[1004] DNA damage and chromosomal alterations have also been reported in numerous fish and insect species.[1005]

It has been found that continuous exposure among rainbow trout larvae to glyphosate at existing environmental concentrations poses a potential risk during the early life stages of the fish because it induced erratic swimming behaviors. This could have detrimental consequences on the escape response of different species to predators or other hazards. Furthermore, this same model reported cytotoxic and oxidative DNA damage effects of different magnitudes in *in vivo* and *in vitro* studies.[1006]

The adverse effects of *Roundup*® herbicide also impact the viability of hemocytes, cells that are essential for the immune response in marine invertebrates.[1007] It has also been observed that, following *in vitro* exposure to sublethal concentrations of HBGs, the phagocytic capacity of some bivalves and marine crabs diminishes.[1008] In these marine animals, exposure to glyphosate has been reported to cause membrane destabilization and alterations of the lysosomal system, which affects their ability to feed and to filter contaminants.[1009]

Glyphosate is also associated with direct effects on microorganism communities in freshwater bodies. According to different studies, this herbicide is a common contaminant in freshwater bodies and is linked to modifications in the abundance and diversity of both autotrophic and heterotrophic species of plankton and epilithic organisms.[1010,1011,1012] In turn, the modification in plankton and picoplankton may be related to alterations in food webs in aquatic ecosystems. [1013] Attention in monitoring and studying this phenomenon is especially urgent, since glyphosate was considered to have a relatively short half-life in water, so its ecological impact was assumed to be minimal. New evidence shows, as mentioned above, that accumulation can be persistent and therefore the effects far more substantial.

In addition to the alterations observed in microorganism communities, glyphosate, together with other pesticides such as glufosinate, endosulfan and 2,4-D, is considered to be one of the main factors in the decline of numerous amphibian populations.[1014] A study in South America that evaluated the survival and mobility of toad populations in ponds contaminated by herbicides concluded that glyphosate and other herbicides were causing higher mortality rates in populations of these amphibians.[1015] Exposure of amphibians (*Scinax nasicus* and *Elachistocleis bicolor*) to the commercial formulation of the herbicide dicamba causes biochemical alterations and lesions in liver tissues and cell function. The formulation showed high biotoxicity in the two amphibian species after short-term exposure.[1016]

Another recent meta-analysis, with data from 21 countries, evaluated real concentration toxicity of glyphosate in freshwater ecosystem organisms. The effects were evaluated in organisms of different trophic levels, represented by an algae species, a crustacean and a fish. The findings reveal that algae and fish were more sensitive to glyphosate when the median lethal dose (LD_{50}) was used as a parameter. The authors conclude that concentrations higher than LD_{50} in surface waters affect primary producers and therefore endanger the food web.[1017] Furthermore, an ecotoxicological study showed damage to certain species of marine bacteria; simultaneously, glyphosate proved to be moderately toxic to freshwater crustaceans.[1018]

Effects on various insects

Insects and other arthropods play a crucial role in the ecological balance and the stability and resilience of ecosystems by virtue of their contribution to food webs. The overwhelming scientific evidence demonstrating the effects on insects caused by the use of various pesticides is highly significant and requires urgent attention by the states, the academic community and the different sectors of the population, since recent studies have reported an alarming decrease in populations of various insect species worldwide, which could entail the alteration or total disappearance of ecological processes and ecosystem services that insects form part of.[1019,1020,1021,1022] In the particular case of Mexico, this process could have a direct impact on the resilience and stability of most ecosystems, given that official data show that our country is home to around 48,000 insect species, making us one of the most diverse countries in terms of this group. [1023]

It has already been shown that the most widely used GM crops worldwide have two main traits: herbicide tolerance – mainly to glyphosate – and insect resistance with endogenous production of toxic proteins. These properties can be included separately or together in transgenic events.

Bt GMOs act after being modified by the insertion of genes from the bacterium *Bacillus thuringiensis* to produce protoxins that operate in the midgut of insects, resulting in the formation of pores in the intestinal membrane and intoxication, leading to septicemia and death.[1024,1025, 1026, 1027] There are several types of Cry proteins (such as Cry1A, Cry1B, Cry1J and Cry2Eb) which are active against Lepidoptera (butterflies and moths), Coleoptera (beetles), Diptera (flies, mosquitoes and others), Hemiptera (cicadas, aphids, bedbugs and others) and Hymenoptera (bees, ants, wasps and others).[1028,1029]

Crops expressing these insecticidal toxins can affect organisms considered pests, but also beneficial species, including entomophagous insects (parasitoids or predators), i.e. insects that parasitize or prey on insects that feed on crops, and other arthropods that act as natural enemies of pests.[1030,1031,1032]

Parasitoid wasps are natural enemies of major agricultural pests and arthropod-borne diseases. The parasitoid wasp *Macrocentrus cingulum* has been widely used to control the insect pests *Ostrinia furnacalis* (Asian corn borer) and *O. nubilalis* (European corn borer).[1033] The latter species is common in Mexico. Another endoparasitic wasp used for pest control is *Tetrastichus howardi*, which is used to control the codling moth *Spodoptera frugiperda*, a major corn pest in Mexico.[1034] According to a 2009 meta-analysis, pest parasitoids are more susceptible to Cry toxins than pest predators. These findings were made by comparing laboratory analyses of 27 predator and 21 parasitoid species.[1035]

A study evaluated the toxic effects of proteins produced by *Bacillus thuringiensis* on two generations of *T. howardi* parasitizing Bt-resistant *S. frugiperda*. Survival of wasps developed on *S. frugiperda* larvae of both sexes was affected. The organisms that managed to survive showed negative effects such as altered host-seeking behavior, stunted development and lower reproductive rates.[1036]

Another study using GM Bt corn (events 176 and Bt11) showed seasonal abundance affectations to the specialist parasitoid *Macrocentrus cingulum*, endoparasite of the European hornworm. The abundance of *M. cingulum* was 29-60% lower in Bt GM corn compared to non-GM corn.[1037] Predatory *Chrysoperla carnea* larvae prefer prey fed on non-GM corn instead of Bt corn, thus reducing their exposure.[1038]

Another wasp species widely used for controlling the *O. nubilalis* worm is *Trichogramma chilonis*. Bt toxins cause acute toxicity in *T. chilonis,* as well as alterations in longevity and residence time in host eggs, when *T. chilonis* females are fed with sublethal doses of Bt bioinsecticide spores.[1039]

Hymenopteran parasitoids often displayed negative effects when parasitizing hosts that fed on GM Bt plants, due to poor host quality.[1040] In other cases, survival, developmental period and cocoon weight were negatively affected in parasitoids such as *Cotesia marginiventris*, *Aphidius* and *Parallorhogas pyralophagus*, when their hosts were fed with genetically modified Cry1Ab, Cry3A and Cry9C cultures.[1041,1042,1043]

It has also been noted that the abundance of certain hymenopteran parasitoids decreased in fields growing Bt cotton.[1044] The presence of Cry1Ac protein in cotton had a delaying effect on the development of hymenopteran parasitoids reared on their hosts, possibly due to sublethal effects on the latter.[1045] In China, a parasitoid crucial for controlling the cotton bollworm *Microplitis mediator* experienced decreased survival and stunted growth when its prey, *Helicoverpa armigera*, was fed with Bt cotton leaf powder containing Cry1Ac.[1046]

With regard to predatory insects, one study found that *Chrysopa pallen* ladybug pupae feeding on aphids in Bt GK12 cotton were larger and produced more eggs than those fed on NuCOTN 99B Bt cotton, possibly due to differences in the nutritional quality of the prey.[1047] At the same time, it has been shown that *Adalia bipunctata* ladybugs exposed to different concentrations of activated Cry1Ab and Cry3Bbb toxins registered mortality of larvae and pupae, as well as affectations in the time of development and the accumulation of general body mass, even at the lowest concentrations. The foregoing demonstrates that the mode of action of these proteins is not specific to the insect pests they are intended to control. The supposed specificity and mode of action of these proteins have implications for populations of this species and their biological pest control functions in GM crop ecosystems.[1048]

In Bt cotton fields, an increase in the overall diversity of pest subcommunities was observed, but so was a decrease in the diversity of natural enemy subcommunities.[1049] These changes in natural enemy density were often associated with prey dynamics or indirect plant-mediated causes. The longevity of the predators *Orius tristicolor* and *Geocoris punctipes*, reared on Bt cotton-fed prey, dropped significantly by 27-28%.[1050]

There are also the effects of glyphosate associated with HT GMOs. Several studies have shown the herbicide's direct sublethal effects on mite and insect communities and also how glyphosate causes major indirect effects by eliminating plant species related to the life cycle of insects and microarthropods, for example food sources, mating sites or oviposition sites.[1051,1052,1053]

Researchers from the Swiss Federal Institute of Technology and the German Agency for Nature Conservation conducted tests on lacewing larvae *Chrysoperla carnea*, which were fed doses of *Roundup® HBG WeatherMax* at concentrations lower than doses used on crops. The results showed that the arthropods suffered developmental damage, impaired cocoon formation and massive lethal malformations. This reveals the severe direct toxicity of glyphosate for non-target arthropods.[1054]

Melanin plays an important role in the immune system of insects. Recently glyphosate was shown to inhibit melanin production in insects from two phylogenetically distant species, a dipteran *(Anopheles gambiae*) and a lepidopteran *(Galleria mellonella*). This impact is detrimental to health in both species studied and suggests the same effect on a broader spectrum of insect species, making them more susceptible to microbial pathogens.[1055]

In other organisms, such as the potato beetle *(Leptinotarsa decemlineata*), environmental and direct HBG concentrations increase the likelihood of larval mortality. It is concluded that, even at lower glyphosate concentrations than the ones tested, the survival of herbivorous insect larvae may be undermined.[1056]

Emphasis on detrimental effects on pollinating insects

Despite the major importance of pollinators and, in particular, bees, many anthropogenic activities are threatening their survival. A 2016 study indicates ten areas that governments should focus on to protect pollinators and secure pollination services, one of which points to GM crops as potential risks to pollinating insects.[1057]

In the same year, the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) published an assessment report on pollinators, pollination and food production. This report points out that the main potential risks to pollinators include several practices inherent to intensive agriculture, such as the use of insecticides and the planting of GM crops.[1058] Bee visitation to fields grown with HT-modified canola are reportedly declining.[1059, 1060]

Newer approaches to analyzing the effects of Cry proteins propose realistic scenarios that include interactions between Cry proteins and pesticides normally present in the field.[1061] As this field of research grows, it becomes possible to better understand the effects on pollinators, either in isolation or interacting with other agents present in agriculture such as pesticides.

In Brazil it was found that the larvae of the *Melipona quiadrifasciata* species suffered stunted development when ingesting food containing Cry proteins. The effects intensified to lethal levels when larvae were exposed to Cry proteins together with glyphosate.[1062]

Negative effects were also found in *Bombus terrestris* bumblebees when worker bees were fed syrups containing Bt formulations that included Cry1Ab proteins, at concentrations recommended for field use. Mortality was observed among the bees along with other negative effects such as a decreased reproduction rate.[1063]

The use of glyphosate and other highly hazardous pesticides is related to the disappearance of plants that are essential for various insect species. This has seriously affected pollinators. Due to their toxicity, pesticides pose a risk to bees that varies depending on the biology of the species and their ability to metabolize toxins.[1064] Experts worldwide have identified pesticide poisoning as one of the main factors contributing to the decline of bee populations.[1065,1066]

Pollination is essential for regulating and maintaining the balance of terrestrial ecosystems, for human agri-food systems and for life on our planet in general. The bee group is comprised by approximately 20,000 species, most of which are efficient pollinators. Some studies have revealed that 70% of the 124 major cultivated plant species in the world rely on insect pollination.[1067,1068]

In particular, herbicides used in agriculture pose an indirect threat to native bees and other pollinators because they reduce the abundance and diversity of flowering plants associated with crops that provide pollen and nectar for pollinators.[1069] The use of herbicides therefore leads to the reduction and elimination of these species that are crucial both ecologically and economically.[1070]

Evidence shows that the indirect effects of glyphosate negatively impacts pollinators by damaging the flora in the agricultural environment.[1071] However, even more alarming than these indirect effects is the direct damage to the bees' gut microbiota and their increased susceptibility to pathogens or malnutrition. The effects on the gut microbiota of bees have been widely documented and are based on the fact that the metabolic pathway that is affected by glyphosate (known as the shikimate pathway) is shared with most of the bacteria in the microbiota.[1072]

In 2018, it was shown that exposure of honeybees to glyphosate concentrations in agricultural environments decreased the abundance of the main bacterial species found in the bee gut, thereby increasing mortality among bees subsequently exposed to a frequent pathogen.[1073] Other studies have reported that the development of honeybee larvae fed with trace amounts of glyphosate stunted growth and resulted in lower weights compared to those fed without glyphosate.[1074]

During foraging, bees may be exposed to glyphosate in pollen, nectar, water or dust, and then pass this contaminant on to the hive. This could affect mortality rates or reduce bee productivity,[1075,1076, 1077] as well as causing significant alterations to honey quality. In one study, the presence of glyphosate residues was detected in 27% of honey samples taken directly from the hive and in 33% of samples from places where it is marketed.[1078]

Glyphosate is known to affect bees transversally, causing alterations to embryonic development that manifest in adulthood as well as affecting orientation and navigation.[1079] Other studies report that pesticide exposure affects the coordination of collective activities in the comb and the ability to associate flower scent with sugar (nectar) collection.[1080] It has also been observed that glyphosate causes alterations to the metabolism and gut microbiota of bees, decreasing larvae weight and survival rates, and increasing their susceptibility to pathogens.[1081,1082,1083,1084]

Another recently reported and important effect concerns sleep in bees. It was found that the ingestion of a sugar solution added with glyphosate caused alterations in the sleep patterns of bees, which can cause erratic behavior and chaotic movements that limit their ability to find flowers and collect pollen.[1085]

Then there is thermoregulation, an important process for pupal development. A recent study demonstrated the effects of glyphosate on bumblebees fed realistic field doses. No effects were detected on an individual level, but at the colony level there was a decrease in the bumblebees' ability to maintain the necessary temperatures in the hive. Temperatures dropped by 25%. This decrease affects the proper development of bumblebee pupae. The authors conclude that these effects may lead to the decline of these organisms.[1086]

With regard to pathogens, there is evidence that interaction between glyphosate and common *A. mellifera* pathogens, such as *Nosema microsporidia*, significantly reduces bee survival rates. Evidence indicates that glyphosate used on a large scale in intensive systems may compromise the survival of *A. mellifera*.[1087]

The studies referred to above have tested the effects of a single compound – glyphosate – on bees. However, some studies have tested interactions between glyphosate and other common agricultural pesticides, such as cypermethrin (insecticide) and difenoconazole (fungicide). Results have shown lethal and synergistic effects between these toxic agents. In agricultural settings, bee populations are exposed to multiple pesticides, so testing for combined or synergistic effects offers a more realistic approach.[1088]

The special case of the monarch butterfly

There is evidence of the effects of GM Bt corn crops on butterflies caused by the activity of Cry proteins.[1089,1090] Scientific studies have shown that pollen has been a route of exposure for non-target insects and its consumption affects them.[1091] In particular, the case of the monarch butterfly, *Danaus plexippus*, has sparked intense debate about the risk that the planting of GM corn and other GM crops poses for this emblematic pollinator.[1092]

This species, whose populations migrate annually between Canada, the United States and Mexico, has an important ecological, social and cultural value in our country. However, its population has been significantly depleted in the last decade.[1093,1094] A decline of about 58% in milkweed *(Asclepias spp.*) on which the lepidopteran feeds was observed in the US over the penultimate decade; this loss coincides with a rise in the use of glyphosate-based herbicides along with an increase in the area used for planting glyphosate-tolerant GM corn and soybeans. The disappearance of the common milkweed, along with other factors, led to an 81% reduction in the monarch butterfly population in Mexican temperate forests during the overwintering season.[1095]

Evidence shows that the survival of monarch butterfly larvae into adulthood is undermined by exposure to GM Bt corn pollen.[1096] Simultaneous exposure to both Bt corn pollen and anthers had an additional impact resulting in decreased monarch butterfly larvae survival rates.[1097] This same effect was observed in a type of beetle, *Propylea japonica*, when fed with Bt rice pollen.[1098]

Another study shows that monarch butterfly larvae fed on milkweed *(Asclepias curassavica* Griseb.) impregnated with Bt corn pollen ingest less food, grow more slowly and experience higher mortality rates compared to larvae fed on leaves impregnated with non-GM corn pollen or on milkweed leaves without pollen.[1099]

Glyphosate is also a risk factor for monarch butterflies. The use of this highly hazardous pesticide has been identified as one of the main variables responsible for the decline in the species' population. This factor is even more significant than the loss of forest area or the use of certain insecticides.[1100]

The indirect effects of this pesticide may be a determining factor in the species' demise. Glyphosate and HBG kill milkweed. They also contaminate milkweed nectar and damage monarch overwintering sites; consequently, the species is undermined and its population falls.[1101,1102]

Negative effects of glyphosate at the genetic level have been identified in other butterflies. An assay conducted on the species *Lycaena dispar* noted damage caused by an increase in cell micronuclei, indicative of genomic instability that may diminish the butterflies' vitality and increase the risk of local population extinction.[1103]

Other environmental effects: generation of "super pests" and "superweeds"

"Super pests"

A major challenge arose with the widespread adoption of Bt-type GM corn and its continued use in agricultural fields: the possibility of insect pest populations developing resistance to Bt proteins. The development of pest resistance to Cry toxins is one of the greatest threats posed by the prolonged use of GM crops.[1104]

When pest populations become resistant to Bt proteins, it means that these proteins are no longer effective in controlling them, hence the term "super pests". They can survive and reproduce causing severe crop damage and economic losses for farmers. In addition, this insect resistance to Bt proteins can be inherited, thereby increasing its prevalence in the field.

For more than 10 years there has been evidence that several pests, for which GM Bt corn was used as a means of control, have developed tolerance to Cry toxins (Cry1F, Cry1Ab, Cry3Bb1, Cry1A.105, Cry2Ab2, Cry3Bb1, Vip3Aa20 and mCry3A). An experimental study conducted in the United States found that Cry toxins had no effect on the pest species *Helicoverpa zea,* in terms of larval weight reduction, the number of insects entering the pupal stage, pupal weight, time to hatching and the number of pupae able to successfully hatch to adulthood.[1105]

The same phenomenon has occurred in tropical countries, in this case with the *Spodoptera frugiperda* species (corn earworm), which has shown resistance to the transgenic event TC1507 which produces the Cry1F protein supposedly to protect this type of pests, in addition to other lepidopterans. Controlled laboratory evaluations found that *S. frugiperda* collected from GM Bt corn plots were less sensitive to the Cry1F protein than populations from other regions. In response to this resistance event, technology suppliers have suspended commercial sales of TC1507 corn in Puerto Rico, pending a possible reversion to susceptibility.[1106]

In Colombia, resistance to GM corn varieties that release the Cry1F toxin has also been found among *Spodoptera frugiperda*. One study deduced that the endotoxin did not exert total control over populations, suggesting that these insects could develop high resistance to plants with Cry1F endotoxin.[1107]

The following list presents findings from other scientific research showing resistance to Cry proteins present in GM crops and in different insects, now considered "super pests":

- Seven species of Lepidoptera and one of Coleoptera have developed resistance to GM plants that produce insecticidal Bt proteins.[1108]
- The corn earworm, *Spodoptera frugiperda*, has developed resistance mechanisms to organophosphate, pyrethroid and diamide insecticides, as well as to the Cry1F protein;[1109] cross-resistance, selected with GM corn, to Cry2Ab2, causing resistance to Bt crops expressing similar proteins;[1110] resistance to Cry1Fa and Cry1A proteins in populations in Puerto Rico, the United States and Brazil;[1111] resistance to Cry1 insecticidal proteins in South America;[1112] resistance to Vip3Aa20 protein from Bt corn in South America;[1113] resistance to the Cry1F protein from GM corn event TC1507 with some strains showing high levels of cross-resistance to Cry1A.105 and Cry1Ab;[1114] also, resistance to the Cry1Fa2 protein, as well as cross-resistance to other Cry1A proteins in Puerto Rico, United States (Rico, Florida and North Carolina), Brazil and Argentina.[1115, 1116, 1117, 1118]

- The African corn stem borer has developed resistance, particularly in GM Bt corn expressing the Cry1Ab protein.[1119] In addition, the European corn borer, *Ostrinia nubilalis*, is resistant to Bt proteins.[1120,1121]
- Resistance of *Diabrotica barberi* to corn expressing the Cry3Bb and Cry34/35Ab1 proteins in North America.[1122] Field populations of *Diabrotica virgifera virgifera* have resistance to the Cry3Bb1 and mCry3A proteins of GM corn, and cross-resistance between these two types of Bt corn. Also, resistance to eCry3.1Ab corn and cross-resistance between Cry3Bb1, mCry3A and eCry3.1Ab.[1123]
- The bollworm, *Helicoverpa zea*, has shown to be resistant to Cry1 and Cry2 proteins, with different levels of dominance and recessiveness depending on protein concentration.[1124] The same species has developed resistance to Bt proteins, with up to 1,000-fold levels of resistance to Cry1Ac;[1125] dominant resistance to Cry1Ac and minimal cross-resistance to Cry2Ab;[1126] resistance to Cry1A.105 and Cry2Ab2 among 22 field populations collected on Bt corn;[1127] high levels of resistance to the Vip3Aa protein used in GM Bt corn and cotton, pyramided with Cry1 and Cry2 proteins, in the United States;[1128] and developed resistance to the Vip3Aa protein.[1129] In Australian populations of *Helicoverpa punctigera*, developed resistance to Bt proteins has been observed, specifically to the Cry2Ab protein of Bollgard II cotton;[1130] resistance to Vip3 proteins has been observed in Australian populations of *H. armigera* and *H. punctigera*. [1131]
- The pink bollworm, *Pectinophora gossypiella,* has developed resistance to the Cry2Ab toxin, associated with mutations in the ABCA2 gene; [1132] and resistance to Cry1Ac and Cry2Ab2 proteins from GM cotton in India.[1133, 1134]
- There is resistance to Cry1Ac in *Trichoplusia sp.* due to multigene mutations.[1135]

<u>"Super weeds"</u>

The spread of herbicide-tolerant GM crops has also caused imbalances; weeds have evolved to develop high tolerance to herbicides that are part of the transgenic technological package.[1136,1137] The intensive agricultural model with high doses of herbicides and the expansion of the agricultural frontier have caused high selection pressure for weeds, which have become "superweeds" or "aggressive weeds". [1138] In turn, the appearance of these plant varieties has resulted in an increase in the dose of glyphosate applied throughout the agricultural cycle, as well as the use of combinations with other herbicides that are more toxic, leading to more harmful effects for human health and the environment in an unsustainable vicious circle with high health, ecological, economic and social costs.[1139]

This resistance has been attributed to the use of glyphosate in no-till, small- and large-scale agriculture, and intensive monoculture farming of both GM crops and conventional varieties.[1140, 1141] Some resistant varieties have emerged as a result of a gradual evolution of herbicide-exposed weed species, while others have emerged due to gene flow from glyphosate-tolerant GM crops to their wild relatives. [1142] The platform of the International Survey of Herbicide Resistant Weeds recently reported 317 cases of 47 species with glyphosate resistance in 29 countries, and the list shows a growing trend with new resistant species every year.[1143, 1144, 1145, 1146]

In the case of GM corn, engineered to be resistant to specific herbicides, there is a risk that the selection of weeds resistant to these herbicides will accelerate.[1147] The main ones include: *Amaranthus tuberculatus, Amaranthus spinosus, Amaranthus rudis, Amaranthus palmeri, Ambrosia artemisiifolia, Ambrosia trifida, Bassia scoparia, Chloris virgata*, *Chloris truncata*, *Conyza bonariensis, Digitaria insularis*, *Echinochloa colona*, *Eleusine indica, Erigeron canadensis, Lolium multiflorum, Lolium rigidum, Poa annua* and *Sorghum halepense*. [1148,1149,1150,1151,1152,1153,1154,1155,1156,1157,1158,1159,1160,1161,1162,1163,1164] In Mexico, some species that have developed resistance have been reported: *Leptochloa virgata, Bidens pilosa*, *Steinchisma laxum, Aster squamatus* and *Amaranthus palmeri.* [1165]

It should be noted that weeds are wild plants that grow inside and on the edges of plots and are considered as "bad plants" under the industrial agriculture model, contrary to the integral vision of traditional agriculture and agroecology that considers them beneficial as they are a source of additional nutrients when decomposing and help maintain soil moisture, in addition to having various agricultural, medicinal, food (as quelites for humans or fodder for animals), ceremonial, ornamental and handicraft uses.

3.3 Evidence of agricultural affectations and other socio-economic considerations related to GM crops and glyphosate

Industrialized agricultural food production systems have been shown to have several negative effects. This form of agriculture is one of the human activities that have most transformed the biosphere, causing soil degradation, deforestation, depletion of water resources and chemical contamination; in addition, due to its poor ecological and genetic diversity, it is extremely vulnerable to pests, diseases and climate change. Because of all these characteristics, industrialized agriculture poses one of the greatest risks to human and environmental health.[1166]

As shown in previous chapters, GMO monocultures are detrimental for the environment and biodiversity and can affect the pest composition of a region. These types of alterations in ecosystem dynamics also have economic impacts. In a study conducted on soybean and corn crops in the United States, where more than 90% are transgenic events, it was observed that the composition of beneficial insects had fallen 24%, incurring costs for producers of approximately 58 million dollars a year, due to declining yields and increased use of pesticides.[1167]

Regardless of the yields of industrialized agriculture, it should be noted that the inherent characteristics of industrialized agricultural systems have negative economic impacts. The loss and alteration of biodiversity due to monocultures, the impact on health caused by the use of agrochemicals, the low availability of nutrients and the development of resistant weeds and pests amount to a very high economic risk because the environmental and health damage they cause can cost up to billions of dollars per year.[1168]

This system is therefore not profitable not only in terms of human, animal and environmental health, but also in economic terms. The continued acceptance of industrialized agricultural systems based on glyphosate and GMOs entails being in a state of high vulnerability and social uncertainty in the long term. The people who suffer most from the social impact of these unsustainable food systems are the most vulnerable social groups who live in the most precarious and impoverished conditions, and are undernourished, often because they do not have access to food despite its availability.[1169]

The COVID-19 pandemic has further highlighted the global risks and damage caused by industrialized food systems.[1170] In view of this, there is a greater need to promote agroecological food systems that foment the health of the population, since they produce healthy, diversified and agrotoxin-free food. In addition, these food systems come with greater ecological resilience because they favor the biological interactions of ecosystems by relying on polyculture systems and strengthen the independence of farmers so they have no need for agro-inputs marketed by oligopolies.[1171]

A study showed that, during the first few months of the pandemic, agroecological systems in different regions of Latin America were essential to cope positively with the impact on food production and consumption, thanks to their dynamism, adaptability and biodiversity.[1172] For example, in Argentina, the network of agroecological actors enjoyed significant growth during the pandemic, highlighting practices such as compost production, seed exchange and the integration of government programs.[1173] Similarly, in Guatemala, engagement with farmer organizations and agroecological practices contributed to the resilience of rural communities in the face of the economic impact caused by the pandemic.[1174] In the case of Uruguay, agroecological practices and possible transition paths for family livestock farmers were promoted.[1175]

The reliance on industrialized agriculture for food production, with a strong dependence on chemical inputs, is very risky in economic terms, but, because of its strong impact, it is also risky in environmental, social and cultural terms.[1176] In particular, the environmental and health risks associated with the use of glyphosate have raised growing international concern about evaluating alternatives to its use. The French government, for example, has evaluated alternatives for weed management based on physical or mechanical methods such as tillage, cultural planting practices, as well as recommending research into the development of mechanical weed control systems and bioherbicides.[1177]

In addition, groups of scientists and agronomists have modeled different cultivation scenarios in herbicide-limited environments. In Australia a model was used to perform 10-year simulations to assess weed management, productivity and profitability with and without glyphosate. The model showed that yields can be maintained without the use of glyphosate during early planting. Furthermore, emphasis has been placed on the importance of promoting multidisciplinary research into the devising of strategies with limited use of pesticides and lower environmental impact.[1178]

Economic impact of controlling “superweeds"

As mentioned above, weeds have been studied and found to have developed resistance to herbicides of different types of action, such as glyphosate, which poses a major threat to crop productivity, health, quality and profitability.[1179] It has been extensively documented that glyphosate and GBHs lose effectiveness because the plants they are intended to control develop resistance to them.[1180] This increases the need to use larger amounts of herbicide to achieve the objective, a fact that has been pointed out, for example, for glyphosate-resistant soybean producers in the

US, who use 28% more herbicide.[1181] Herbicide resistance is one of the most significant economic risks faced by herbicide-based agriculture.[1182,1183]

The issue of weeds developing resistance to glyphosate has emerged as a critical new front in debates about sustainability in agricultural production and has affected hundreds of millions of hectares of cropland in different regions of the world.[1184] The economic impact of weed resistance management ties up with the need to raise production costs with more herbicides, as well as crop yield losses caused by competition.[1185] It is difficult to estimate the total economic impact of herbicide resistance on agriculture.[1186]

It has also been shown that a highly effective way to prevent the development of herbicide resistance has been the use of cultural cultivation practices, manual weeding, crop rotation and conventional tillage systems. Glyphosate-resistant farming systems in the US, Brazil and Argentina, where integrated agricultural management practices are not used, have witnessed the evolution of herbicide-resistant weeds.[1187]

Impact on non-GM crops

Another argument that has been used historically to justify the use of glyphosate is that it strengthens conservation agriculture and does not harm other types of agriculture. However, a pioneering study published in January 2021 showed that agrochemical-based agriculture (ABA) affects the surrounding areas where agroecological agriculture is performed due to the mobility of pesticides in the soil. Samples of 19 herbicides were taken, including three derived metabolites. In 90% of the samples glyphosate and AMPA were found in the soils where ABA is performed; alarmingly, in 32% of the lands where agroecological agriculture is carried out, these compounds were also found at a distance of 300m from the boundary with the ABA fields. This demonstrates that pesticides reach and contaminate the agroecological system studied, both because of its proximity to the conventional system, but also because it is located in a region dominated by agrochemical-based agriculture. [1188]

Economic impact on fisheries

Any herbicide used in agriculture can be washed into aquatic ecosystems through water runoff and soil erosion, from croplands to adjacent locations, contaminating rivers, streams, lakes and coastal areas. This situation jeopardizes the population that relies economically on these ecosystems in fishing areas.[1189, 1190]

Once in the aquatic ecosystem, pesticides cause losses to fisheries in several ways. At high concentrations in water, they kill fish directly; at low doses they can kill highly susceptible fish fry or eliminate essential fish food, such as insects and other invertebrates. Moreover, because safety restrictions prohibit the capture or sale of fish contaminated with pesticide residues, such fish cannot be marketed so there are also economic losses for the population.[1191,1192] Fisheries are valuable resources, as fish provide food services for humans. They also provide benefits to the population through direct financial earnings or recreational enjoyment, providing jobs for commercial and retail fishermen.[1193]

Economic impact on beekeeping

Honeybees and wild bees are essential for the pollination of fruits, vegetables and other crops, as well as crucial for the production of about one-third of the world's crops. The negative economic impact of the aforementioned demise of pollinators due to the use of glyphosate and other pesticides is beyond calculation. The economic benefits provided by pollinators for EU agriculture alone are estimated to be around $40 billion a year.[1194] Given the decline in bee populations, farmers have had to resort to renting bee hives to pollinate their crops, thereby increasing production costs. In different regions of Mexico, pesticides have been detected in honey, which has a negative socioeconomic impact on rural producers, leading to marketing issues, subsequent bankruptcy and loss of jobs for many beekeepers, given that one of the main limitations for the marketing of honey is the presence of chemical residues.[1195]

Economic impact of drinking water contamination

Lastly, it is important to highlight the costs of contamination of domestic water derived from the use of glyphosate and other pesticides. Groundwater contamination by pesticides is a major concern since around half the human population obtains domestic drinking water from wells and once groundwater is contaminated, pesticide residues remain for long periods of time.[1196] Apart from the substantial risks to human health, the contamination of water for domestic use with pesticides entails a high level of government spending on sampling and monitoring, estimated at around $1,100 per year per well.[1197,1198, 1199] In addition, there is the high cost of cleaning and treating the water to remove pesticides and make it suitable for human use, which is beyond the public sector's financial capabilities. [1200, 1201]

Rationale and motive

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