Food safety of genetically modified crops
Today, the subject of genetically modified crops continues to be one of great public controversy. The debate started in 1986 when the first field trials with genetically modified (GM) crops were organized. The discussion re-started in the mid 1990s when the first ships carrying genetically modified soy docked in Europe. Hungarian scientist, Arpad Pusztai added fuel to the fire by stating on primetime television that GM technology was unsafe. Gilles-Eric Séralini’s more recent rat study renewed the discussion regarding the food safety of GM crops. Even though scientists throughout the world have shown that Pusztai and Séralini’s interpretations are not correct, the debate regarding GM crops rages as never before.

In this VIB Fact Series issue, we discuss current scientific understanding regarding the food safety of GM crops. Just as there are scientists who deny global warming or who disregard the effectiveness of vaccines, there will always be people, even from the scientific community, who state that GM technology in itself poses a threat to public health. However no single scientific argument can be found to doubt the safety of GM technology. Food safety institutions, companies, research institutes and universities have conducted large-scale tests and studies on GM crops over the past thirty years. The significant scientific consensus about the safety of GM technology is based on this. However, it must be clear that the applications of GM technology must be evaluated case by case before a crop can be authorized for cultivation and/or food and feed use by local governments.

Hundreds of studies, strict risk analyses, stringent authorization procedures and continuous follow-up show that the currently authorized GM crops are at least as safe as their non-GM counterparts. GM crops have already been part of the food consumed by us or by farmed animals for twenty years and on an increasingly larger scale. There is no evidence of a single human or animal case in which consuming food containing elements of the currently commercialized GM crops has had an adverse effect. GM crops have an unprecedented track record of safe use.
Facts and figures

No single crop can be said to be 100% safe. Food toxicity depends in the first instance on the amount of the product that is consumed.

It is estimated that we consume five to ten thousand different natural toxins per day and ingest approximately 1.5 g of natural toxins per person per day.

Conventional crops are considered safe because, historically, they have presented no risk in normal use.

No single crop has been tested more than a GM crop.

Whereas traditional plant breeding mixes as much genetic information as possible to create new strains, GM technology has been developed to introduce only one or a limited number of changes.

DNA rearrangements generally occur in nature and in themselves do not pose a threat to public health.

The process of genetic modification itself does not lead to toxicity or allergic reactions. Until now, no single food product originating from commercially cultivated GM plants has been found to result in toxic symptoms or new allergies.

Food safety is determined by the characteristics of a crop and not by the technology that was used to obtain these properties.

Dozens of analyses and many years of experience in practice demonstrate that the production of Bt proteins in insect resistant GM crops has no adverse effects on the health of humans or animals.

Because of the significant reduction in insect damage, insect resistant Bt crops are less vulnerable to fungal infections, producing Bt harvests that contain fewer fungal toxins and that are thus healthier.

For each study that claims to find harmful effects caused by GM crops, there is an abundance of studies that find no adverse effects caused by GM crops compared to their non-GM equivalent.

In contrast to the current controversy, GM crops were originally greeted positively in Europe.

The succession of European food safety crises at the end of the 1990s stimulated anxiety and suspicion regarding GM crops.

Introduction

1983 was a remarkable year for plant scientists. Firstly, Barbara McClintock was awarded the Nobel Prize for Physiology for her genetic work with maize. Secondly, four scientific articles were published that demonstrated for the first time the insertion of a specific DNA fragment in plants without the use of crossing. New farming applications were self-evident, but with this new development also came resistance to the technology.
A new era

The 20th century was the century of technological progress. Scientific knowledge, the motor of technological development and vice versa, grew rapidly. New disciplines appeared, such as biotechnology, and mankind gained insight into the working, function and impact of DNA, the genetic material of all living beings. One of the things that became clear was that physical characteristics are determined to a significant extent by the composition of DNA. The reverse is also true: altering DNA can result in new characteristics. Mankind has attempted to modify crops and animals to meet its needs since the origin of agriculture: improved harvests, greater disease resistance, more meat and more milk. Our ancestors achieved this goal without having knowledge of the science behind their actions. Centuries of human effort evolved into tomato, into high-yield maize whereas all kinds of cabbage, from brocoli to sprouts, were selected from a single wild cabbage variety. The same strategy was taken in the animal production. Farmed animals were bred for more meat and/or milk.

In 1983 the basis was laid for the targeted modification of plants through the direct introduction of genetic information. These plants were named genetically modified plants. In the first place, the technology to adjust the genetic information of plants meant a revolution for scientific research regarding plant growth and development. Scientists were offered the opportunity to switch specific genes on or off, which led to a better characterization of the function of these genes. Secondly, GM technology created added value for plant breeding. Instead of crossing plant genes. Secondly, GM technology created added better characterization of the function of these switch specific genes on or off, which led to a revolution for scientific research regarding plant growth and development. Researchers were offered the opportunity to switch specific genes on or off, which led to a better characterization of the function of these genes. Secondly, GM technology created added value for plant breeding. Instead of crossing plant genes.

The FLAVR SAVR tomato was available in the shop. Instead of avoiding attention, the media was intentionally urged to report on the product. The British citizens were thus well informed and customers were always offered non-GM alternatives. The British really liked the GM tomato puree and 1.8 million tins were sold in three years. The FLAVR SAVR tomato was given a second lease of life, this time in Europe. In 1996 the British company, Zeneca, took a license on the GM tomato. The Californian tomatoes were processed into tomato puree and the product was introduced onto the British market in an extremely responsible and transparent way by supermarket chains J. Sainsbury and Safeway Stores. The tins carried a clear GM label (which was not compulsory in Europe at the time), there was always a non-GM alternative available on the shelves and accompanying information about the FLAVR SAVR tomato was available in the shop. Instead of avoiding attention, the media was intentionally urged to report on the product. The British citizens were thus well informed and customers were always offered non-GM alternatives. The British really liked the GM tomato puree and 1.8 million tins were sold in three years.

Besides consumption, cultivation of GM crops also started in Europe. In 1998 Europe approved the cultivation of insect-resistant GM maize. The maize is resistant to the European stem borer, a feared insect in Mediterranean maize production. Spain, Portugal, Romania, the Czech Republic and Slovakia still cultivate the maize today, collectively on some 150,000 hectares. The cultivation of insect-resistant GM maize has led to a reduction in insecticide use in maize cultivation. The environmental impact of GM maize cultivation in Spain between 1998 and 2013 is 20% lower than that of non-GM maize. Globally a reduction of 50% can be reported.
The rise of the GM debate

Two years after the introduction of the first GM tomato, agrochemical company, Monsanto, started the first commercial cultivation of GM soybeans in the United States. In the autumn of 1996 the first ships with GM soy docked in Europe. The mood in Europe regarding GM crops changed entirely. Environmental organization Greenpeace organized a campaign against GMOs and tried to stop ships from berthing in Antwerp and Ghent. The slogans, images and concepts such as Frankenfood were lapped up by the media and public and the impact was tremendous. Suddenly there was no longer talk of genetically modified crops but genetically manipulated, which clearly has a more negative connotation.

In this same period, consumer confidence in the food industry fell significantly. Food crises such as swine fever (1997), mad cow disease (1997) and later the dioxin affair (1999) mounted up. At the time that the population and government had serious doubts about food safety in general, Dr. Arpad Pusztai announced in 1998 during a television show that he had scientific proof that showed that all GM products were harmful to public health. A later analysis of his data indicated clearly that his conclusions were incorrect (see chapter 4); but the words of the Scottish Rowett Institute researcher had tremendous impact. A government response was requested and from 1998 several member states blocked the further admission of GM crops. In anticipation of stricter European regulations, a de facto moratorium on GM crops took hold in Europe.

Under the influence of a large-scale anti-campaign by Friends of the Earth, consumers and distributors turned en masse against GM foods. 

The sale of the once popular FLAVR SAVR tomato puree collapsed and the tins were removed from the shelves. The year was 1999.

What is the GMO debate actually about?

The debate regarding genetically modified crops has lasted for more than 30 years. That there is opposition to a technology is far from unusual. Almost every important technological development - certainly developments that had enormous impact on society - has been a target of fierce criticism. However, what is striking is that over a period of 30 years there was a perceptible evolution in the kind of arguments used against GM technology. Where previously safety for public health and the environment were targeted, now mainly socio-economic arguments dominate the debate. The arguments and concerns can be divided into two categories and actually belong to two different debates:

Biological issues

Are GM crops harmful to public health? Are there risks for our environment? What is the effect of GM crops on the diversity of plants, insects and microorganisms in nature, also known as biodiversity? How can GM crops help promote sustainable farming?

These questions continue to be posed, probably because they were not answered precisely enough or because GM technology is developing further and is used on an ever-increasing scale. Indeed, the hectarage of GM crops increased a 100-fold in less than 20 years. In 2014, 181.5 million hectares of GM crops were cultivated across the world.
the world, which is comparable to a surface area equal to five times that of Germany.

Social issues

Besides biological issues, there are also social concerns. What effect does the growing presence of multinationals in the seed sector have on GM crops? What about patents and intellectual property protection of crops? How important is freedom of choice for consumer and farmer and how can this be met? Who gains most from cultivating GM crops?

The socio-economic issues are part of a larger social debate about the direction in which we as society would like our farming and our food production to evolve. Notwithstanding the fact that these questions are relevant and extremely interesting, they are separate from any type of breeding technology. They apply to both crops that were bred in a classical way as well as to those developed via GM technology.

This issue of VIB Fact Series only covers food safety of genetically modified crops. We will address concerns based on recent scientific literature. In order to position this correctly we will first focus on the safety of traditional crops.

**Safety of traditional crops**

Providing evidence that a food crop is not harmful to humans is not simple. Harmful substances can be found in every plant and more importantly this harmfulness depends on the amount of the product that is consumed and how the food is prepared. Food safety for us means, in the first instance, a tradition of safe use.
Food safety of genetically modified crops

Learning through trying
Since the beginning of mankind, we have eaten what nature offers. Plants have always been an extremely important source of nutrition. We have learned through trial and error which plants can be eaten safely and which plants are best to avoid. Of the quarter of a million of plants, the first people probably had to try thousands. Today only around a hundred crops are cultivated intensively and the products of only a handful of crops are present in almost everything we eat. Good cooking methods also needed to be found. Some plants or fruits can after all only be eaten safely after they have been boiled or treated in another way. The gathered knowledge was passed on from generation to generation until it became reaped wisdom. A clear example of this is the use of the potato. Shortly after the potato arrived in Europe, people thought that the berries were the edible part. Many people died after eating these poisonous berries. Later people realized that the tuber was tasty after cooking and could be eaten safely. Now the potato is one of the most important suppliers of carbohydrates across the world.

Toxicity of our traditional food
The possible toxicity of our food has always been a point of attention, and rightly so. We are even trained evolutionarily in this. Few people will drink milk that has gone off and we generally identify contaminated foodstuffs from smell alone, after which a feeling of disgust dominates.

Harmfulness depends in the first instance on the amount of the product that is consumed.
The scientific and technological progress of the 20th century has enabled us to use chemical analyses and toxicity tests to investigate which harmful components are present in our food. A large number of our crops that we consume daily contain toxic substances. After all, plants did not originate to serve as food for mankind and animals. Plants produce toxic substances as part of a defense mechanism against insects and plant eaters; including us. For example, cabbage produces up to fifty different natural pesticides including cyanides, glucosinolates and phenols. Luckily a large proportion of these substances that are harmful to us are degraded and made harmless through certain preparation methods. Potatoes, for example, are peeled because the largest concentration of solanine is found just below the skin whereas kidney beans need to be boiled for long enough to break down the phytohaemagglutinin that is naturally present. But even then, we still collectively consume an estimated five to ten thousand different natural toxins per day and approximately 1.5 g of natural toxins per day per person. The consumption of plant toxins obviously depends on the diet. As toxins are a part of a plant’s defense mechanism the concentration depends on the age of the plant and the environmental growth conditions. The concentration can also easily increase by a factor of ten as a response to damage by plant eaters. Table 1 gives an overview of the most prevalent natural toxins in a selection of common crops.

Designing toxicity studies is not simple, purely because harmfulness depends in the first instance on the amount of the product that is consumed. It can generally be stated that 100% safe food does not exist and has never existed. This doesn’t mean that our food is harmful, but rather that it contains substances that in large quantities are toxic or carcinogenic. Ames et al. (1990) stated that of the 52 tested natural plant toxins, 27 had carcinogenic properties. Even health-promoting food ingredients can have harmful effects depending on the amount. For instance, drinking a lot of water (e.g. 6 liters) in a short period (for example 3 hours) can be fatal, because the kidneys are unable to process the excess water.

Food allergies and intolerances
As well as toxic substances, our traditional food also contains products that can spark allergies and intolerances. Over the last decade, food allergies and food intolerances have gained more attention. We all know someone who is allergic to milk, seafood, nuts or kiwi or is gluten or lactose intolerant. A food allergy is defined as a rapid immune response to specific food ingredients that are generally considered to be safe. It is often the result of a genetically determined fault in the immune system of the consumer. In contrast, food intolerance is a non-immunological response, in which the symptoms appear after a much longer delay. In both cases the result is that the body responds to the presence of certain substances in the ingested food.

A possible cause of new food allergies and intolerances is attributed to novel foods. This can mean foods that are new for certain populations. The introduction of the kiwi is such an example.
supermarkets and consumers for new products have created the risk that certain proteins appear in our diets that we never faced before. These can have health-promoting effects, but just as easily could trigger allergies or intolerances. The kiwi was introduced on a large scale in 1970 in the United States and Europe. Ten years later it became clear that certain people are allergic to kiwi.\textsuperscript{15} Novel food can also relate to food that is produced using new technology. These new technologies are in the first instance intended to reduce possible allergic reactions. In this way heat treatment can be used to alter the structure (denaturing) of the primary apple allergen (Mald1).\textsuperscript{14} But heat treatment can also ensure that certain non-noxious allergens can suddenly cause an allergic response. Fish allergies are an example of this.\textsuperscript{14} The advantages and disadvantages of certain technologies must thus be evaluated per case.

**CASE STUDY: THE POTATO**

Many plants produce substances to protect themselves from insect damage, including the potato. Solanine and chaconine are present in the tuber. These are alkaloids and are poisonous to humans and animals. Typical symptoms of poisoning are headache, vomiting, and stomach ache but there are also known cases of fever and high blood pressure that resulted in coma, and even death. From the available data concerning humans it appears that 1 to 5 mg of alkaloids (solanine and chaconine) per kg body weight is sufficient to cause mild to severe symptoms of poisoning. Ingestion of 3 to 6 mg per kg body weight can be fatal.\textsuperscript{11} The maximum permitted dose of alkaloids in potato is around 200 mg per kg fresh weight.\textsuperscript{11} All authorized potato varieties, however, have a lower alkaloid concentration. A potato tuber contains between 10 and 150 alkaloids per kg.\textsuperscript{11}

Someone weighing 50 kg can thus consume 300 g to 5 kg of unpeeled potatoes, depending on the variety, without suffering problems. Boiling does not breakdown the alkaloids, but some do leach into the cooking water. Only deep-frying seems to reduce alkaloid levels.\textsuperscript{12} As 30 to 80% of the alkaloids are found beneath the skin, the most effective way of reducing alkaloids is to peel the potatoes before eating.\textsuperscript{11} The risk of alkaloid poisoning, however, increases when potatoes have been exposed to sunlight for long periods. The alkaloid levels then increase spectacularly, which means eating green potatoes is extremely unhealthy. It cannot be said that any single crop - even the daily consumed potato - is 100% safe. Everything depends on the quantity that is consumed. Conventional crops are considered safe because, historically, they have presented an insignificant risk in normal use.

Plants did not originate to serve as food for humans and animals. That is why they defend themselves against plant eaters.
New varieties, new risks

Mankind has attempted to modify crops to meet its needs since the origin of farming, for example higher yields or improved taste. Lack of knowledge and technology meant that these attempts did not go much further than selecting new, spontaneously-developed characteristics or than accidentally-created crossing products. The way of plant breeding changed drastically in the 20th century. A series of different methods was developed to induce DNA changes in plants, such as targetted cross-fertilization within but also outside plant species, the use of radiation and/or chemicals to introduce random and unknown changes in DNA (mutation breeding) or the modification of the number of chromosomes during in vitro fertilization using cell division toxins. These interventions add, delete, change or rearrange DNA and the activities of certain genes can change which can create new proteins or influence the production of existing proteins. Thousands of plant varieties with new characteristics were introduced to the market in this way. The pink pineapple for example was developed following mutation breeding and a variety-crossing hybrid of wheat and rye - was obtained using in vitro fertilization and accompanying chemical treatments. Nonetheless, these genetically changed crops do not fall under the name GM crops. They are the products of traditional plant breeding.

It goes without saying that such traditional interventions can have an effect on the level of natural toxins and/or allergens in plants. Generally, through recent selection and breeding efforts, this level is lower than the level of harmful substances in the wild varieties of our crops. However, the reverse sometimes also occurs. An insect-resistant celery variety was placed on the market in 1984 but was removed following a torrent of complaints after farmers who came into contact with it developed skin rashes and burns. It subsequently appeared that the new celery contained almost eight times the amount of psoralen compared to traditional celery. As well as carcinogenic properties, psoralen makes the skin extra-sensitive to ultra violet radiation. This meant that skin tissue that came into contact with the celery and was then exposed to the sun, burned immediately. A new potato variety - Lenepe - was also removed from the market in 1974, because the first consumers became nauseous after eating it. The potato contained too high concentrations of solanine and chaconine, natural poisons that reduce nerve activity and generate a poisonous reaction. The potato breeder had crossed a popular potato variety, Delta Gold, with a wild potato from Peru to transfer the insect and disease resistance of the latter to the cultured potato. But he unwittingly also transferred the toxin genes. After all, such surprises with classical breeding methods cannot be avoided in advance.

Safety analyses of traditional crops

Wheat, potato, sprouts, kwi ... they all contain substances that can be harmful in high doses or substances to which certain people are allergic. It can never thus be said that traditional crops are 100% safe. Using experimental science it is not possible to demonstrate the absence of a risk. It cannot be proven that something does not exist. The definition of safe food is therefore based on experience. Very little traditional food has ever been tested to toxicological and nutritional analysis. If a crop or food product has formed a permanent part of our diet for a longer period without negative effects, it is generally considered to be safe. The United States Food and Drug Administration refers to crops as being 'generally recognized as safe' or GRAS if, seen historically, the crop and derived food product present no risk in normal use.

Food safety in practice means, in the first instance, a tradition of safe use

When new varieties are developed, in most cases these do not have to undergo food safety testing. That is why accidents occur, as with the traditionally bred celery and potato (see above). In some countries additional tests are required for some crops. In the Netherlands, the US and Sweden, for example, new potato varieties need to be tested for glycoalkaloids to be certain that these remain below a certain level.

For risk analysis of novel foods attempts are made to find an existing product on the market that is equivalent to the new product and with which it can be compared. In such a case the term substantial equivalent is used. If the new food is a substantial equivalent to a product that is considered safe, this new food can also be considered safe. To estimate the risk of possible allergy, it is investigated whether known food allergens are present in the new product.

Horizontal gene transfer

Besides the presence of proteins and secondary metabolites, some people are also concerned about the presence of DNA in our foods. In contrast to what 60% of interviewees in an American survey thinks, all our food contains DNA and we eat an estimated gram of DNA per day. A certain proportion of the population is convinced that DNA from ingested food will integrate in our own DNA or in that of our intestinal bacteria; a process known as horizontal gene transfer. Vertical gene transfer is the passing on of DNA from parent to child; horizontal gene transfer is the passing on of DNA without sexual reproduction.

What happens exactly with the DNA that we absorb via food? Once eaten, DNA - from whatever origin - will be broken down in the digestive tract into the four building blocks: adenosine (A), thymine (T), guanine (G) and cytosine (C). This process already starts in the mouth. Saliva contains deoxyribonuclease, an enzyme that decomposes deoxyribonucleic acid (or DNA). More of this enzyme is produced in the pancreas and in the small intestine. Additionally, the acid environment of the stomach attacks nucleotides A and G so that the DNA molecule disintegrates further. The further the DNA passes through the gastro-intestinal system, the more it degrades. The degradation of DNA in animals can differ from species to species and also depends to a large extent on the form in which the food is consumed. For instance, among birds, digestion already starts in the gizzard, and among ruminants with several stomachs, the consumed DNA is fully broken down in the stomachs. In this case, the part of the intestines that absorbs nutrients and the intestinal bacteria that are present do not come into contact with DNA from feed. In sheep a difference was noted, depending on the feed formulation: DNA eaten in plastic from feed. In sheep a difference was noted, depending on the feed formulation: DNA eaten in plastic from feed. In sheep a difference was noted, depending on the feed formulation: DNA eaten in plastic from feed. In sheep a difference was noted, depending on the feed formulation: DNA eaten in plastic from feed. In sheep a difference was noted, depending on the feed formulation: DNA eaten in plastic from feed.
Food safety of genetically modified crops

Recently, scientists investigated the extent to which miRNAs from plants can be absorbed by mammals via food and the extent to which these can regulate the activity of their DNA. The studies showed that certain plant miRNAs - which were added in high concentrations to the diet of mice - could be detected in the blood and urine of laboratory animals. By adding to the feed certain miRNAs that have a suppressive effect on cancer, the development of tumors could be reduced in mice. However, the studies used a 1,000x higher concentration of plant miRNAs than can be found in our food. To investigate whether miRNAs present in food can also be absorbed, a food experiment was conducted with rice. Test subjects were requested to eat two bowls of cooked rice on an empty stomach after which the presence of rice miRNAs were tested in the blood. After the rice diet, no rice miRNAs could be detected in the blood either among test subjects or in mice. The absorption of plant miRNAs administered in large quantities orally opens a new approach for treating illnesses including cancer, but appears to be of little relevance regarding food safety.

Safeguarding food safety for the crops that we use as food is one thing. However, since time immemorial, many products have been used during the production of our food to allow crops to grow better (fertilizer) and to protect them against disease and pests (pesticides or plant protection products). Globally 2.4 million tons of pesticides were used in 2007. It is evident that the safety of these products must also be included in a global food safety analysis. The main questions here are whether and for how long pesticides remain on the crops (the so-called "residuals") and whether and for how long food can be absorbed by the consumer as a result of pesticide residues that are present in the food. The most important aspects to consider are whether pesticidal molecules or their residues can be absorbed, whether the food from which the pesticides are taken can be absorbed and whether that food has ever been incorporated in our own DNA.

The central mechanism in molecular biology is that the genetic information stored in DNA is first transcribed into RNA after which it is translated into a protein. However, there are also RNA molecules that do not lead to production of protein. Some of these are called microRNAs (miRNAs) and can influence the activity of certain genes and/or proteins. MicroRNAs from plants are different from those from animals. The third final nucleotide of plant miRNAs is methylated on the 2’ place of the ribose. This means they are protected against degradation in the digestive systems of animals.

The degradation of DNA and possible horizontal gene transfer has been researched among people too. Ileostomy patients and test subjects with entire gastro-intestinal systems were given a meal comprising a soya burger and soya milkshake. Among the ileostomy patients it could be demonstrated that a small fraction of the consumed DNA was still detectable at the end of the small intestine (with a maximum of 3.7% of the monitored genes). But these fragments no longer had the function of the original DNA molecule. With the degradation of DNA, the DNA also loses its function and is transformed from an ingenious information storage form into a source of nutrients. However, DNA fragments could reach the large intestine and could in theory be absorbed into the bloodstream and/or by intestinal bacteria. Among test subjects with entire gastro-intestinal systems, the DNA was fully broken down and was no longer traceable in the stools.

Similar studies were conducted with mice. Each day we eat an estimated 1 gram of DNA from animals, plants, bacteria, fungi and viruses has been eaten. Our digestive tracts have been exposed to all kinds of DNA fragments for some tens of thousands of years. Using current powerful DNA analysis technologies, human DNA can be investigated for DNA fragments originating from other species. Plant, animal, bacteria, virus and other DNA can after all be distinguished from our own DNA. Virus genes appear to be present in our DNA, but did not arrive via our food. Viruses have inserted their DNA themselves, during viral infections. We have also apparently received many genes via horizontal transfer from bacteria. However, plant genes are not found in our DNA. The last horizontal gene transfer in man appears to date from the time of the common ancestor of man and ape. Thus, in spite of the theoretical possibility, it is extremely unlikely that DNA from our food has ever been incorporated within our own DNA.

Each day we eat an estimated 1 gram of DNA
When there is a large diversity in the use of herbicides and sufficient variation of products, the probability that a certain product will be present in the food chain in large quantities is very small. The situation can change when one product is so successful that farmers switch to this en masse. This is for example the case for glyphosate and glyphosate-tolerant plants. Such a “success product” results in a shift in herbicide use. Where previously many herbicides were used, in soya and maize cultivation a huge uniformity has now been created, both concerning use in the field as well as residues in the food derived from herbicide-tolerant soya and maize. Glyphosate tolerance is, moreover, present in various food crops (soy, maize, and sugar beet), which means that the same residues reach us or our farmed animals from different food products. As stated earlier, the safety of a product depends on the quantities that are consumed. After twenty years of using glyphosate-tolerant plants, not one food safety problem has been reported. In Belgium, the Federal Agency for the Safety of the Food Chain (FAVV) is monitoring the situation. In recent years, during sampling no glyphosate residues could be detected or the quantities of glyphosate were lower than the permitted residue quantities. However, it should be clear that pesticide residues in all crops must continue to be monitored by the food safety agencies in order to prevent the predetermined limits being transgressed. This applies to rimsulfuron in potatoes as to imidazolinones in non-GM imidazolinone-tolerant maize as well as glyphosate in GM-glyphosate-tolerant soya.

**HERBICIDE-TOLERANT CROPS**

Concerning food safety, it should be taken into account that crop protection products can remain on or in the crop. This also applies to herbicides or weed killers. Weed control is one of the most difficult tasks for a farmer. When the farmer decides to use herbicides, he must use a mix of herbicides that destroys the weeds in the field but that the crop is capable of withstanding. After all, the crop should not be damaged by the herbicides and must thus be tolerant. In this way grasses can be removed in a potato field using specific herbicides (e.g. the active substances propaquizafop and rimsulfuron) because the potato tolerates these products. But crops (in this case potatoes) that are tolerant to a certain herbicide do absorb the product, which means that the herbicide or its degradation products remain present in the plant for a certain time. Sensible use of pesticides and controls on pesticide residues through government regulations are then also essential to safeguard food safety.

In an attempt to improve weed control, plants that are tolerant to a broad spectrum of herbicides have been searched for since the 1970s and thus prior to the initial development of genetically modified crops. Such herbicides destroy most of the plants in the field. In order to be able to use these herbicides, the crop must thus first be made tolerant. Maize, wheat, rice, oil seed rape and sunflower have been made tolerant to imidazolones via classical plant breeding. These have been commercialized under the name Clearfield since 1992. Oil seed rape has also been made tolerant to triazine and soya to metribuzin. With the rise of GM technology, herbicide tolerance could be introduced in plants in a much more efficient way. The most well-known and successful examples are glyphosate and glufosinate tolerance, respectively commercialized under the name, RoundupReady and LibertyLink. Soy has also been made tolerant to imidazolones via GM technology. From 2015, new herbicide tolerances developed by GM technology such as 2,4-D and dicamba tolerance will be available to farmers. Herbicide tolerance thus mainly answers the needs of farmers and is separate from any breeding method.
Safety of genetically modified crops

New technologies and new products bring new concerns. Twenty years after the introduction of GM crops in agriculture, a thorough analysis can be made of their impact on food safety. This chapter explains how GM crops differ from traditional crops and how their safety is assured.

A safe technology

A logical consequence of introducing extra DNA in plant DNA is that DNA rearrangement occurs. Is such a rearrangement specific to GM crops and are there risks associated with this? In both cases we can answer ‘no’. DNA rearrangements generally occur in nature and are actually the source of genetic diversity. DNA rearrangements during the formation of reproductive cells are partly responsible for the fact that children from the same parents differ genetically. Separate from crossing, rearrangements also occur many times during classical plant breeding. As discussed previously on page 16, many of our commercially available plant varieties were developed using extreme techniques such as mutation breeding, chromosome doubling, in vitro fertilization of plant embryos and interspecies crossings. Varieties developed in this way - from pink grapefruit to triticale - have undergone drastic DNA rearrangements. And yet we and our farmed animals have consumed these for decades illustrating a history of safe use for these breeding techniques. Rearrangements in DNA are in themselves thus not harmful. Even when these occur using GM technology. Moreover, when plants are modified genetically via GM technology, the DNA rearrangements are much more limited and subtle.

DNA rearrangements generally occur in nature and in themselves do not pose a threat to public health.

A much-heard criticism from the anti-GMO corner is the lack of scientific consensus about the food safety of GM crops. The safety of food is however in the first instance determined by the characteristics of a crop and to a much lesser extent by the technology that was used to obtain these characteristics. Just as there are scientists who deny global warming or who disregard the effectiveness of vaccines, there will always be people, even from the scientific community, who state that GM technology in itself poses a threat to public health. However no single scientific argument can be found to doubt the safety of GM technology. Food safety institutions, companies, research institutes and universities have conducted large-scale tests and studies of GM crops over the past thirty years. These form the basis for a significant scientific consensus about the safety of GM technology. This conclusion was recently underlined by a study that demonstrates that sweet potato - an important food crop for millions of people in Africa and Asia - has fragments of DNA from the soil bacteria Agrobacterium, and that...
The DNA is inserted via Agrobacterium’s natural DNA transfer mechanism on which the GM technology is based.41 The sweet potato is thus actually a product of GM technology, although it was created in nature and not in the laboratory. What’s more all 291 tested sweet potato varieties contain these bacterial genes.41 In other words, certain population groups have already eaten products from genetic modification for thousands of years. This indicates that there is no single argument to submit a GM crop to an increased risk analysis just because it was developed using GM technology.

The applications of GM technology - in other words: the genetic characteristics that were added to the plant - must however, be evaluated case by case before a crop can be authorized by the competent authorities for cultivation and/or food feed use (see below).

**Food safety analysis in Europe**

Following the development of a GM plant that, according to the producer, meets all safety and market conditions, a market approval must be requested at European level. For countries within the European Union, this application procedure is centralized. The first step is to submit an application dossier to EFSA, the European Food Safety Authority. EFSA will use the documented results to formulate an opinion on the basis of which the European Commission will draw up a proposal for a decision to permit the specific GM crop for use in food and feed or not. The analyses themselves must be supplied by the applicant/company and conducted by an accredited laboratory within or outside the company. The kinds of analyses that this dossier must contain are detailed below, mostly with technical details.42

**Molecular characterization of the GM plant**

This section must contain detailed information about the method used to genetically transform the plant: using Agrobacterium or mechanical DNA transfer via ‘particle bombardment’ (for more information see Facts series Virus resistant papaya in Hawaii43 page 17-18). All necessary information must be given about the DNA fragment that was inserted into the plant (sequence, relationship to toxins if an extra protein is produced, anti-nutrients and allergens) and about the transformation vectors that were used to transfer the DNA fragment into the plant.

The insertion site and the direct environment in which the DNA is inserted must be described in detail. Once DNA is introduced in a plant cell, it can be inserted in plant DNA in a stable way via natural DNA breaks and successive repair mechanisms. Given that using the current technology the position of insertion is not known in advance and given that this differs for each transformation process, the risk assessment investigates whether the insertion has caused unintended changes. Besides the desired situation in which the insertion has caused no additional effect, alternative situations may occur: 1) the foreign DNA can be inserted in a plant gene, which partially or entirely switches off that gene (the function in itself or its activation or repression role), 2) a new gene is formed, for example, because of DNA repair actions, 3) the regulation of other genes can change. How are the alternative scenarios avoided in practice? When a gene of interest is inserted into a plant using GM technology, not only one but hundreds of GM plants are produced and analyzed. Then the search starts for the one plant in which the extra DNA is functional and in which the insertion of DNA has not caused any unintended irregularities. For the risk assessment, the company must investigate where and how the extra DNA is inserted and whether there are side effects. This means that DNA fragments on the right and left of the insertion must be determined. Bioinformatics are then used to investigate where the gene is inserted and whether, theoretically, a new gene can be formed. Potential new gene sequences are checked in a toxicity database.

Finally, detailed information is required about the GM plant itself. How many times the DNA fragment was inserted, the exact site of the insertion(s), the expression level of the added genes depending on different environmental conditions, the stability of the incorporated DNA fragment over five generations, are just a few examples.
In order to ascertain whether the newly produced protein is safe, the protein is purified from the GM plant or produced separately and subjected to various tests. The first step is a molecular and biochemical characterization of the new protein (amino acid sequence, molecular weight, post-translational modifications, function description, temperature and pH conditions for enzyme activity, substrate specificity) and a homology analysis with harmful proteins, such as toxins. A second step is a laboratory analysis in which the stability of the protein is investigated depending on temperature and acidity (pH value) and in the presence of proteolytic enzymes (e.g. pepsin). Proteins are best broken down as soon as possible after absorption via food. If they stay intact in the body for too long, there is an increased risk that they will react with other molecules and in this way cause allergic or harmful effects. The stability of the newly produced protein during the passage through the digestive system must thus be as small as possible.

As a final step, a toxicity study must be implemented with repeated doses; a study over several days in which laboratory animals consume the to be tested product via their feed. If there is no reason to expand this test, a standard 28-day feed study is conducted with rats according to strictly imposed rules and guidelines, in which the protein to be analyzed is added to the feed.

**Comparative analyses between the GM and the corresponding non-GM plant**

The GM plant is compared with the corresponding non-GM plant regarding composition (protein, macro and micro nutrients, anti-nutrients, natural toxins, allergens, relevant vitamins and minerals, fatty acid profile for oil-rich plants, amino acid profile for protein sources) and farming attributes (e.g. yield, morphology, flowering period, maturation period, pollen viability, sensitivity to pathogens, insects and drought). This step investigates whether the characteristics of the GM plant fall within the normal variation of the control plants. Demonstrating that GM plants are safe thus entails an analysis to demonstrate that a GM plant matches the conventional crop. In the simplest case, there is no difference between the GM and the non-GM plant, except for the added trait. However, if there are additional differences, further research will be undertaken to determine whether these differences could be harmful.

**Toxicity study of the newly-produced protein**

From a historical viewpoint, all traditionally bred crops are considered safe for public health. If the above mentioned comparative study demonstrates that the GM plant does not differ from the non-GM plant except for the new trait, then the GM plant is declared substantially equivalent and therefore the GM plant is classified as being as safe as the non-GM plant. If the GM crop produces a new protein, the safety of the newly produced protein must of course be investigated.

**Allergenicity study of the newly-produced protein**

Allergy is a pathological aberration of the immune response to a certain substance. It is not the allergen itself, but the abnormal reaction of the immune system that can cause serious health effects for certain people. It is an individual reaction that is very difficult to predict.

If it is reported that certain patients respond allergically to a certain protein, then the GM crops that produce this protein will not be authorized. However, it is difficult to determine in advance whether humans and animals will have allergic responses to a certain component or not. The allergenicity studies are thus mainly based on structure analysis of the new protein and the possible similarity with existing allergens. If an 80 amino acid long fragment of the new protein demonstrates a 35% similarity or more with a known allergen concerning sequence or structure, then in vitro binding assays are conducted using the new protein and antibodies from the serum of patients who are allergic to the potentially similar allergens. A stability test must also be conducted as a good indicator for potential allergens. Resistance of the new protein to degradation during pepsin treatment and low pH after all indicates a delayed degradation and points indirectly to a potential health risk.

An allergenicity study with the complete plant must only be conducted when the crop in question (thus the non-GM variety that was used for genetic improvement) is known to induce allergic reactions, for example, peanuts, kiwis and strawberries. This is to determine whether or not the genetic modification strengthens the existing allergenicity.

**Nutritional analysis**

If the molecular characterization and/or the comparative analysis with the non-GM plant demonstrate unexplainable differences, additional experiments must be performed to test the nutritional value of the crop. Depending on the product, specific feed studies must be conducted with rats, poultry or cattle. Even when the GM crop is declared substantially equivalent and additional nutritional analyses are thus not required, the applicant still often conducts a test (and adds this to the dossier) to compare the nutritional value of the GM plant with that of the non-GM plant. This is mainly done for crops that are to be used as animal feed. In most cases this concerns chicken feed studies (broilers that grow into chickens in a short space of time).

If the GM plant has an intentionally altered nutritional composition (for example production of a certain vitamin), the biological availability of these specific nutrients must also be investigated. Take golden rice, for example, the GM rice that produces pro-vitamin A in the rice grain (for more information see ‘The GMO revolution’). Biological availability refers to the fact that humans (or animals) can absorb the produced pro-vitamin A. It must thus be investigated whether there is a noticeable difference in vitamin A levels in the blood of persons (or animals) eating the GM plant (with pro-vitamin A) or the non-GM plant (without pro-vitamin A).
Food study with the complete crop

When the molecular characterization, comparative studies and composition analyses show that apart from the new trait there is no difference between the GM plant and the non-GM control, scientifically there is no added value in conducting a 90-day feeding test with rats using the complete GM plant as feed.6 Until 2013 this was only required by EFSA when there were unexplainable differences between the GM and non-GM crop. However, in the current regulations, the European policymakers disregard the EFSA advice and for each application a 90-day feeding study must be conducted6 even when this, according to EFSA, is scientifically unnecessary and superfluous.

Post-market monitoring or follow-up of commercial crops

If a GM crop is declared as safe as the non-GM equivalent, EFSA will award a positive advice to the European Commission. With such advice the European policymakers can authorize a GM crop for the market. Even then, however, Europe remains careful. Besides the risk assessment that precedes the market authorization the authorized product itself must be monitored. This is mainly done to ascertain whether the product is used as assumed, to confirm the expected effects of the product (although declared safe) and/or to ascertain whether there are unexpected side effects. For a GM crop authorized for feed and food use, monitoring after commercialization is for example needed for products that have altered nutritional composition and/or specific health claims.

Twenty years of safe use

The risk assessments that are carried out by governments before a GM crop is authorized for the market, appear to be more than sufficient. GM crops have already been part of the food consumed by us or by farmed animals for twenty years and on an increasingly larger scale. There is no evidence of a single human or animal case in which consuming food containing elements of GM crops has had an adverse effect. GM crops have a convincing history of safe use.

A recent study took advantage of this long period to investigate the effect of GM feed on farm animals in the US.30 During 2000-2011 an estimated 100 billion animals were slaughtered (chickens, turkeys, cows and pigs) of which 95% were fed on a GM diet.47 All animals that are slaughtered undergo rigorous checks prior to and after slaughter in which the growth and development of the animals and their general health is checked to the level of organs. Should GM crops cause unexpected effects, then it may be assumed that this would have become clear from analysis from abattoir data over the years.

The period between 2000 and 2011 was compared with the period between 1983 and 1994, during which no GM crops were processed in feed.54,55 As far as slaughtered cows are concerned the researchers found that the number of cases of mammary gland and udder infection fell by 30% between 1995 and 2011.52 Of the 770,000 cows that were rejected between 2003 and 2007, just 12% came from the group of cows that had been fed GM maize and soya for a longer period, while this group formed the majority (82%) of the total number of cows in the study. In other words, there is no single indication that GM feed has negatively influenced the health of cows in the US over the past twenty years.56

Also for slaughtered chickens, a reduced percentage of rejected animals was observed during this period, with 2011 having the lowest number of rejected chickens.47 The health and development of broilers appears to be a good indicator of feed quality. In their short existence of 42 to 49 days, their body size increases sixty-fold which means that they are very sensitive to an unbalanced diet.47 Yet the study showed that there were no negative effects on the growth, development and health of chickens that were fed GM crops. The abattoir data from the period 2000-2011 can be seen as one large and powerful dataset with practical data from more than 94 billion chickens over 24 successive generations that were bred in real conditions with GM feed.47

The results of this meta-analysis are confirmed by countless other studies. In recent years it has been demonstrated many times that the current generation of GM crops is equivalent, as far as composition is concerned, to non-GM crops,57-62 and that there are no significant differences regarding food digestibility and the health and performance of animals fed on GM feed.52 What’s more there are no observed differences in the nutritional value and nutritional profiles of products originating from animals that are or are not fed with GM feed.63-65 Even with the most sensitive detection methods, neither proteins nor DNA originating from GM crops could be detected in animal products.65 From the viewpoint of food safety there is thus no single argument to label animal products (meat, milk, eggs) from animals that were fed with GM feed.
Desperately searching for a difference

Once in awhile studies are published that report differences between GM and non-GM crops or between laboratory animals that have or have not been fed with GM feed. The studies however never go further than demonstrating differences. An important characteristic of good scientific research is demonstrating a mechanism responsible for the differences. An observed difference does, after all not need to be the consequence of using GM technology. What's more, a difference does not always need to have biological relevance. If the GM technology or a certain GM crop was effectively harmful then it must be possible within a foreseeable time to demonstrate in which way the technology or the product causes the harm. In the studies that report differences, a clarification of the cause behind these differences is consistently missing. Also such 'difference studies' are not followed up, most probably because the observations cannot be repeated. Instead, new studies are published that again report another difference without any explanation being given or making connections to GM technology. These often-sensational studies have no scientific value, but quickly find their way to a series of anti-GMO websites after which they refuel many GM discussions.

For every study that supposedly finds harmful effects of GM crops - two of these are discussed extensively in the following chapters of this report - there are hundreds of studies that find no difference. As previously indicated, we will not discuss the food safety issues of plant protection products in this report. However, we do devote attention to the insect-resistant GM crops, because in this case the plant protection product is produced by the crop itself in a way that is only possible via GM technology. The additional genetic information with which insect-resistant plants can protect themselves originates from the bacterium Bacillus thuringiensis, (abbreviation Bt) which does not form part of our traditional diet. All these analyses and new studies are published that again report an observed difference without any explanation being given or making connections to GM technology. The environmental impact of cotton and maize cultivation over these 18 years has lowered by some 30% and 50% respectively.

As previously indicated, there is a world of difference between a crop that produces bacterial proteins and, for example, a GM tomato that produces a protein of sweet pepper. In this latter case, after all, nothing new is added to our diet. The only difference is the plant from which it comes.

Since Bt proteins form an integral part of the Bt plant - and in contrast to sprayed insecticides cannot be washed off - these must be tested in vivo (in animals) that Bt proteins have a very specific mode of action (for more information see Fact series ‘Bt cotton in India’). Once ingested by an insect a Bt protein is recognized in the insect’s intestines by specific intestinal wall receptors, a type of antennae that initiates a reaction when they perceive a signal. Bt is only active against specific insect families that have the right ‘antennae’. Neither humans nor animals have these receptors which means that Bt proteins cannot attach to our intestinal wall and are thus safe to eat. Numerous studies have shown experimentally that Bt proteins have no effect on animal systems. One of the most extensive studies dates from 1995. Scientists demonstrated both in vitro (in the laboratory) as well as in vivo (in animals) that Bt proteins cannot attach to the gastro-intestinal tissue of mammals. For the in vivo experiments, the re-
searchers used Bt tomatoes that were fed to rats with a Bt quantity that, when converted, would be equivalent to a human consumption of 2,000 kg Bt tomatoes per day. Even with such gigantic doses, no binding could be established.70 Moreover, the digestive system of insects and vertebrates is different. Both in vitro as well as in vivo experiments show that Bt proteins in vertebrates are broken down early in digestion (through acidity and enzyme activity). No functional Bt proteins could be found in the gut, the site of action for Bt proteins.70 Besides these detailed experiments, the harmlessness of Bt proteins for humans and animals was repeatedly and independently demonstrated. Numerous food studies with rats, mice, rabbits, cattle and pigs were carried out and demonstrated that the studied Bt crops were just as safe for public health as the non-GM varieties.70-72 A European consortium of public research institutions that have no contact with the crop-breeding companies examined insect-resistant Bt maize, particularly the MON810 maize that has been cultivated in Europe since 1998 and is used in animal feed. The researchers conducted their research contracted by the European Commission, which in the framework of the stirred-up GM debate in 2012 (see chapter 5) assigned a new food safety analysis on MON810. Researchers from the GRACE project (GMO Risk Assessment and Communication of Evidence) conducted two 90-day feeding studies in which rats were fed with two different MON810 maize varieties, the corresponding non-GM maize varieties or four additional conventional maize varieties.73 Following analysis of a large number of clinical and pathological parameters no relevant toxicological differences could be noted between the rats eating GM feed and those not, even when 33% of the diet comprised Bt maize. Fully in line with the transparent character of the GRACE project, the raw data from all analyses have been made available publicly.73 Finally, two additional rigorous studies in which both cows as well as pigs were fed with MON810 GM maize for a longer period (up to 25 months) deserve a special mention. The results obtained from these studies led to more than ten publications.71,72,74-79 The studies were designed according to the rules of the art with correct and sufficient checks and with sufficient laboratory animals. The researchers analyzed a large number of growth and health parameters and examined the extent to which DNA fragments from the GM maize and the Bt protein were present in meat and milk. All these analyses showed that there was no difference between animals fed on GM maize or non-GM maize. Neither DNA nor protein originating from GM crops could be detected in the animals’ tissues or in the products.

**Between 1996 and 2013 insect-resistant maize cultivation had 50% less impact on the environment compared to non-GM maize.**

**USE ORGANIC KNOWLEDGE TO PROTECT CROPS**

At the start of the 20th Century, it was discovered that the bacterium Bacillus thuringiensis produces proteins that are harmful to some caterpillars of moths and butterflies or to larvae of other insects. Farmers - including organic farmers - have used the Bt bacteria to protect their crops against specific insects since as early as 1920. In most cases, the surface of plants was sprayed with traces (survival structures) of the Bacillus bacteria. However, insects that pave a way through stems and leaves are protected against insecticides that are administered from outside. By having plants produce Bt proteins, crops can defend themselves from within.
Food safety of genetically modified crops

In contrast to conventional maize (right), insect resistant maize is protected against maize borers. In Europe, maize is used in the first instance as animal feed but even then mycotoxin contaminated feed poses a serious risk to human health. Once consumed by milk-producing animals, aflatoxin B1 is partially broken down to aflatoxin M1. This substance is excreted via milk and is still carcinogenic and poisonous to the consumer of the milk. In Europe, food agencies monitor mycotoxin levels in animal feed and human food to ensure that these do not exceed specific limits.

The cultivation of insect-resistant Bt crops has resulted in a drastic insecticide use reduction. Besides this direct environmental (and food safety) benefit, Bt crops also seem to have indirect food safety advantages. As Bt crops suffer less insect damage, fungi have less chance of infecting the plants. For feed crops such as maize this is a welcome advantage since fungi such as Aspergillus and Fusarium produce toxins (respectively aflatoxins and fumonisins) that remain in the harvested end product (for example maize kernels). Different analyses show that the harvest of genetically modified, insect-resistant maize contains less fungal toxins compared to classical, non-insect resistant maize. In addition, the problem of fungal toxins should not be underestimated. Fungal toxins, often called mycotoxins, can act as carcinogens in animals and humans in the long term and increase the predisposition to liver cancer. Globally approximately 25% of all crops are said to be infected with mycotoxins, which forms a real threat to public health, particularly in developing countries. It is estimated that approximately 4.5 billion people in developing countries are chronically exposed to uncontrolled quantities of mycotoxins via their food. One of the most serious outbreaks of aflatoxicosis was reported in Kenya in both 2004 and 2005. More than 150 people lost their lives by eating poorly stored, home-produced maize.

Reduce fungal toxins

In developing countries, aflatoxicosis leads to liver cancer. Even a one-time exposure can result in aflatoxicosis, a serious, acute liver poisoning. Globally approximately 25% of all crops are said to be infected with mycotoxins, which forms a real threat to public health, particularly in developing countries. It is estimated that approximately 4.5 billion people in developing countries are chronically exposed to uncontrolled quantities of mycotoxins via their food. One of the most serious outbreaks of aflatoxicosis was reported in Kenya in both 2004 and 2005. More than 150 people lost their lives by eating poorly stored, home-produced maize.

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In March 2013, this efficiently-operating European control system detected high levels of aflatoxins in animal feed and milk in various places in Germany and the Netherlands. The source of the aflatoxin contamination appeared to be a batch of maize originating in Serbia. Both the animal feed and the milk were destroyed. This recent situation is not an isolated event. Between 1996 and 2014 the European Union’s Rapid Alert System for Food and Feed identified 89 harvest condemnations because of high mycotoxin levels in conventionally cultivated harvests. This amounts to 1 threshold exceedance per 122,000 hectares per year. Organic cultivation, which does not use synthetic pesticides, has extremely limited possibilities to prevent fungal growth. This means that mycotoxin levels are exceeded much more often, namely once per 1,250 hectares per year. This is almost a hundred times more than in conventional cultivation. This trend is confirmed in a study investigating the presence of Fusarium toxins in conventional and organic grain-based products. Fungal toxins were found in 11% of organic products compared to 3.5% in conventional products. The real risk regarding the presence of mycotoxins in conventional and organic products are in stark contrast to the European harvests of insect-resistant GM maize. Over 17 years, no single European GM harvest has exceeded the maximum permitted mycotoxin levels.

Insect-resistant maize is, however, not a miracle solution in preventing fungal toxins in food. Maize fungal infection does not only happen during cultivation but also after harvesting. High humidity and temperature and poor ventilation during vegetable product storage stimulates the growth of fungi on the harvest. Moreover aflatoxin infection prior to harvesting is not only caused by crop damage from insects but also when the crop is weakened by stress, such as drought. Nevertheless, insect-resistant GM maize appears, in many cases, to be less affected by fungi, which means that lower levels of toxins are present in the harvest, enabling these crops to contribute to safe food production.

Antibiotic resistance genes

Since the beginning of the era of genetic modification, one set of genes has received additional attention: antibiotic resistance genes. Particularly in the early days of GM technology, these genes were commonly used to identify plant cells that had received additional DNA after modification, in other words, to distinguish the genetically modified cells from the non-modified ones. Other selection systems increasingly started to be used, such as herbicide-tolerance or fluorescence (see further). The more that crops were introduced onto the market with antibiotic resistance genes in their DNA, the more questions were posed as to whether eating these genes would lead to a reduced antibiotic effectiveness in medicine. The fear is that antibiotic resistance genes could be transferred via horizontal gene transfer (see page 13) to soil bacteria or to our intestinal bacteria, possibly making these resistant to certain antibiotics.
Hypothetically, this resistance could then be transferred to pathogenic bacteria that could infect us.

The risk does exist in theory but the fear is ungrounded for various reasons:

• First and foremost, antibiotic resistance genes (present in the cells of the crop) must be able to withstand processing into food. After all, few existing GM crops are eaten raw and unprocessed. The genes must also be able to withstand the DNA-degrading effect of saliva enzymes and the acid environment of the stomach (see page 18). This is all extremely unlikely.

• Then our intestinal bacteria will need to take up the DNA and without first breaking it down, introduce this into their DNA in such a way that the antibiotic resistance genes’ functionality is retained. However, bacteria handle their genes very efficiently and if new genetic information has no added value, this is not retained in the DNA.

• In the final step, a second horizontal gene transfer would need to take place from intestinal bacteria to bacteria that hold a completely different lifestyle, namely to infect our bodies and cause illness. The bacteria must also be able to have physical contact. For example bacteria that cause respiratory infections do not come into contact with intestinal bacteria.

• As well as the unlikelihood of the previous steps the type of antibiotic resistance genes is also extremely important. The probability of horizontal gene transfer is after all of limited relevance for the risk assessment. The question must always be: If it does happen, what could the consequences be? In other words, are specific antibiotic resistance genes already abundantly present in nature? Is the distribution of these resistance genes in nature a danger to public health or not? Are the specific antibiotics still used in human or veterinary medicine? For the risk assessment, antibiotic resistance genes are classified into three different groups, depending on the medicinal importance of the corresponding antibiotics:

  - Group 1 comprises antibiotic resistance genes that are generally present in nature as well as in intestinal bacteria (e.g. the nptII resistance genes against kanamycin) and act against antibiotics that are not or no longer used in medicine and that have limited use in veterinary medicine (e.g. hygromycin). The European Food Safety Authority, EFSA, authorizes the use of these resistance genes for both experimental work as well as for commercial applications. The antibiotic resistance genes are after all widely distributed in nature. Should resistance genes of GM crops end up in nature via horizontal gene transfer, there is a very small chance that this will increase the resistance already present.

  - Group 2 comprises antibiotic-resistance genes that are already present in nature and act against antibiotics that are used in medicine and/or veterinary medicine in specific cases, e.g. chloramphenicol, ampicillin, spectinomycin and streptomycin. These resistance genes may be used in laboratory conditions, but not for commercial applications.

  - The situation is different for group 3: antibiotic genes against antibiotics that are relevant for human medicine may not be used as selection markers.

Although multiple studies already demonstrated that there is intrinsically no danger hidden in the use of certain antibiotic resistance genes, these days other selection tags are used or the antibiotic resistance genes are removed during the GM plant development process because of the negative perception. An alternative to antibiotic resistance is for example the phosphomannose-isomerase gene. When this gene is introduced into a plant cell, it allows the cell to use mannose as sole carbon source. After the genetic modification process all plant cells are grown in a carbon-poor environment with mannose as the only important energy source. In these conditions only the transformed cells can grow into a GM plant. Another selection method uses the GFP gene; GFP stands for green fluorescent protein. Cells in which the GFP gene has been introduced, emit a green fluorescence (under UV light) which enables the genetically modified cells to be distinguished visually.

To wrap up the discussion regarding antibiotic resistance it should also be noted that the historic use of antibiotic resistance in commercial cultivation is not general. Herbicide-tolerant crops – in 2014 these amounted to 100 of the 181.5 million ha of GM crops – do not possess antibiotic resistance genes. This is because the herbicide tolerance characteristic is used during the selection of the genetically modified plants in the laboratory.
A gene switch from a plant virus

To convert genetic information incorporated in a gene to the production of a protein that has a specific function, that gene must first be activated. When and how strongly the gene is switched on is determined by the promoter, a DNA fragment that is located in front of the gene. The mechanism of switching on and translating the DNA message into a protein differs between bacteria and plants. In other words, introducing a bacterial gene in a plant (such as Bt for insect resistance) requires a switch that is active in plants to switch on the gene. This switch was found in a plant virus, specifically the cauliflower mosaic virus (CaMV). The CaMV 35S promoter is used extensively for experimental work as well as for commercial production of GM crops, because it allows a gene to get expressed in a plant in an efficient and stable way.

In 2012 two EFSA researchers published an article entitled “Possible consequences of the overlap between the CaMV 35S promoter regions in the plant transformation vectors used and the viral gene VI in transgenic plants” in which they stated that certain variants of the CaMV 35S promoter also contained the code (gene) for a protein of the plant virus.96 Based on the title, a story quickly spread that the presence of virus genes in GM crops had been missed and that public health was being threatened by harmful viral proteins in our food.97 The virus gene in question, however, is part of the 35S promoter of the cauliflower mosaic virus and thus originates from a plant virus that can infect neither people nor animals.96 Moreover the viral promoter of the cauliflower mosaic virus with its DNA and viral proteins for a very long time because many fruits and crops that we eat are infected with and thus contain the virus. The amount of viral DNA consumed in this way is much higher than that which enters our diet via GM crops. Consequently the virus has a history of safe use.

Reducing food allergies

As mentioned in chapter 2, certain food components can trigger allergic reactions in some people. Although, each product has the capacity to provoke an allergic reaction, approximately 90% of all food allergies are caused by just eight food products: peanuts, nuts, milk, eggs, wheat, soya, fish and seafood.99 It is obvious that if someone is allergic to soya, that person would also be allergic to GM soya in which these same allergens are present. It is also more than logical to assume that, should an allergen from a peanut be produced in maize, for example, those people with peanut allergies would react after eating that specific GM maize. The process of genetic modification, however, does not develop new allergic reactions. And until today no single food product originating from GM plants has been identified that leads to new allergies.100 101

One reason why GM crops are often unjustly connected with allergies is probably a situation in Brazil dating from 1996. Scientists had introduced a gene from a Brazil nut into soybeans.102 This resulted in the production of a methionine-rich protein, the aim of which was to improve the amino acid composition (and thus the nutritional value) of soya. Soya is a great source of protein, but lacks essential sulphur-retaining amino acids such as methionine. This means that extra methionine needs to be added to animal feed (which contains mainly soya). The initial tests on serum originating from persons allergic to Brazil nuts showed immediately that the modified soybeans would also generate allergic reactions in people with a Brazil nut allergy.103 The research was stopped and this specific GM soy was never commercialized.104 This story actually mainly demonstrates that the detection process for potential allergies works very well for genetically modified crops, a process that is entirely absent in classical crop breeding.

In contrast to what many people fear, adapting genetic information using GM technology can even lead to reduced allergenicity. Allergens from nuts, soya, wheat and other crops can be detected and disabled in the DNA of these plants. In the laboratory the most important soya allergens can be switched off using GM technology without altering the nutritional value of GM soya.105 Also extremely complex problems that are difficult to resolve via classical crop breeding, such as gluten allergy, can be addressed. Thanks to plant biotechnology, celiac disease patients can look forward to wheat that no longer causes gluten allergy.106
The Pusztai case

In the summer of 1998, plant scientist Arpad Pusztai spread the news that rats fed on GM potatoes incurred digestive tract abnormalities. The media and environmental organizations jumped on this story and the scientific world shook to its foundations. Although Pusztai’s conclusions appeared to be premature and not founded on scientific data, the first doubts regarding GM plant food safety were sown.

A lectin-producing potato

In 1995, the Scottish department for agriculture, environment and fisheries funded a three-year research project on insect-resistant GM crops. The most important goal of this study was to investigate whether specific genes that offer resistance to insects (and nematodes) had unwanted effects on the environment and public health. GM potatoes were selected for the project. Hungarian biochemist, Dr. Pusztai, employed in the Scottish Rowett Research Institute, conducted a chemical analysis of GM potatoes and designed feed experiments on rats. The plant material itself was supplied by Durham University and the Scottish Crop Research Institute.

The GM potatoes concerned produced lectin originating from snowdrops (Galanthus nivalis). Lectins are carbohydrate-binding proteins that have a role in plant immune systems. They do this mainly by causing disturbances in the digestive tracts of plant eaters or attackers. Some lectins, such as those in beans, are even entirely toxic for humans and animals, which is why some beans need to be boiled before we can eat them.

The rats in the Pusztai laboratory were divided into groups of six animals in which each group was fed a certain diet for 10 days: lectin-producing GM potatoes, non-GM potatoes or non-GM potatoes in which lectin was added as powder. After 10 days, the test animals were investigated for different clinical and pathological parameters. The researchers determined that the rats fed on the snowdrop lectin demonstrated gastric mucosa abnormalities. This was the case for both the raw as well as cooked potatoes and in both the non-GM potatoes to which lectin was added as well as the lectin-producing GM potatoes. However, this effect was to be expected. A previous study had already demonstrated that lectin-affected rats’ gastric mucosa. These irregularities were thus caused by the snowdrop lectin itself and not by the GMO technology. This immediately indicates that when a genetically modified plant is developed, it is more important to know the details of the GM application involved (e.g. lectin production), rather than which technology is used to introduce the lectin-producing gene.

The rats that were fed on raw GM potatoes, however, showed additional abnormalities of the small intestine. These effects were not noted in rats fed on non-GM potatoes with or without added lectin, or in the rats fed on cooked GM potatoes. The rats fed on cooked GM potatoes had further abnormalities in the appendix, while these effects were not noted in the rats fed on raw GM potatoes or in rats fed on non-GM with or without added lectin. Based on these results, Pusztai decided that the unexpected effects in the intestines were not attributable to lectin and that the reason was rather to be found in the genetic construct, the process of genetic modification, the insertion site of the lectin-producing gene or through a combination of these possible causes. Later it appeared that these conclusions were incorrect (see further).

The communication blunder

In the summer of 1998, Pusztai was guest on the television program World in Action. During a short interview he was asked his opinion about the long-term effects of eating GM crops. Pusztai alluded casually to ongoing experiments in his laboratory and indicated that he was concerned about the results that his laboratory had obtained. Without

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4 Food safety of genetically modified crops

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* The gene that is introduced into the plant DNA requires a number of additional DNA fragments to be functional in the plant such as the switch or promoter to switch on the gene’s expression. All the DNA fragments that are introduced are together called the genetic construct.
knowing the real reason for the health problems in his GM-fed rats, he was seduced into making general conclusions about GM crops. He announced that "he would not eat the GM potatoes were he given the choice" and "that it is very unfair to use our fellow citizens as guinea pigs".

A media storm engulfed Pusztai and the Rowett Research Institute. Because of the lack of clarity about which experiments were precisely involved and because of the communication errors that followed on from this, the Rowett Research Institute took the decision to dismiss Pusztai. Additionally, his laboratory and research results were subjected to a thorough audit. The scientific community condemned Pusztai because he had announced partial results without knowing the full facts. His research was still too premature. The conclusions that Pusztai drew were at most a hypothesis that should have been further investigated in follow-up experiments. These were however not conducted. Part of Dr. Pusztai's research was ultimately published in The Lancet entitled; "Effect of diet containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine".

The true facts

The Pusztai laboratory experiments showed that in some cases, the lectin-producing GM potatoes caused disturbances in the digestive tracts of laboratory animals that could not be explained by the presence of lectin. Pusztai simply ascribed this to genetic modification; a non-validated conclusion. Pusztai's speculation could count on little support from the scientific community. The Pusztai article was criticized by many because it used too few laboratory animals per treatment, a lack of varying doses of GM potatoes in the diet and the fact that potatoes, both raw and cooked, are not a suitable diet for rats. It is indeed remarkable and impossible to explain why the GM potatoes in raw form could generate an effect in the small intestine of the laboratory animals but not in cooked form, while this was the opposite in the appendix. The crucial argument for stating that the article conclusions are not reliable is the lack of good controls. An experiment succeeds or fails with controls. If the effect of a GM potato is to be investigated this must be compared with a potato that is identical, except for the additional attribute. If the potatoes differ in multiple areas, it is after all impossible to determine in hindsight the reason for the differences between the laboratory animals. The experiments conducted prior to the laboratory animal study, indicated that the GM potatoes differed in various characteristics from the reference non-GM potatoes. Chemical analysis demonstrated that both the protein level as well as the levels of starch, lectin, trypsin and chymotrypsin inhibitors differed. On the other hand, based on the published data, the level of the most obvious components with a known effect on the digestive tract (such as solanine, see page 15) was not determined. Therefore the reason for the published differences in the laboratory animals could not be ascertained and because the plant material differed so strongly from each other there was no point in conducting laboratory animal tests after all.

Pusztai's research group started the animal testing with wrong plant material and with an incorrect study design. When chemical analyses showed that the GM potatoes differed very strongly from the control group of potatoes, a control line without the genetic construction should have been generated from the GM potatoes via crossing, or more controls should have been included in the experiment in order to take the additional variation into account.

Food safety of genetically modified crops

In spite of the overwhelming criticism of the experiments and especially Pusztai's incorrect conclusions, the Pusztai story has caused immense and probably irreversible damage to society's debate about GMO technology. Nevertheless, Pusztai's experiments have taught us that the current safety analyses detect potential dangers. Each GMO intended for commercial use is subject to a series of tests, including chemical analyses (see page 24). If something changes in the DNA of a crop - whether through genetic modification or not - which means that the chemical composition of the crop changes detrimentally, then alarm bells sound. Finally, it is important to report that the lectin-producing potatoes were developed as part of a research project and that it was never the intention to commercialize these potatoes.
The Seralini case

Gilles-Eric Séralini and a team of colleagues from the universities of Caen and Verona wanted to research whether the long-term consumption of a commercially available genetically modified maize variety (NK603*) and the herbicide Roundup was harmful to health. To answer this question, they designed a two-year feeding study with rats. Two years is the approximate lifetime of a rat. Séralini conducted the following test diets on the laboratory animals:

1. A diet that partly comprised the genetically modified NK603 maize in which three different ratios were tested: 11%, 22% and 33% genetically modified maize. The other 89%, 78% and 67% of the diet was a standard, commercially available laboratory rat feed.
2. A diet that, like the first diet, was composed partly from genetically modified NK603 maize, but in which the maize was treated in the field with the herbicide Roundup. Again these were tested in three ratios - 11%, 22% and 33% NK603 maize. The levels of Roundup residues in the maize were however not measured.
3. A diet without GM maize, but in which the rats had free access to drinking water in which Roundup was added in a concentration of 0.5%, 0.09% or 0.000000011%.
4. A diet with 33% non-genetically modified maize that had a genetic background that looked a lot like the NK603 maize. The other 67% comprised commercially available laboratory rat feed.

Séralini and his colleagues fed each test diet to a group of male and female animals. In this way a total of ten different diets were tested, of which one was the control. Per diet, Séralini and colleagues tested ten animals of each sex, thus 200 rats in total.

Séralini et al. used ‘Sprague-Dawley’ rats in their study. This is a laboratory strain that is often used to test the safety of chemical substances. Such testing is normally conducted on the basis of a 90-day toxicological study. To draw conclusions from such a food study, certain guidelines must be followed. These guidelines are internationally recognized and indicated that in a 90-day study, at least ten animals per sex and per treatment must be used. However, Séralini carried out a two-year long study. It is known that the ‘Sprague-Dawley’ rats spontaneously develop tumors from an age of approximately 90 days. The older they become, the more ill they become. When these rats can eat without any restrictions - as in the Séralini study - the numbers of tumors are highest. The rats can be used for a two-year study on the condition that other guidelines are followed. At the end of the study, the research must actually be able to make a distinction between tumors that occurred spontaneously and tumors that were possibly caused by diet. To be able to make this distinction, the guidelines prescribe that in such a study 50 animals per sex per treatment should be used, and if not, no conclusions can be drawn. Séralini used only 10 animals per sex per treatment. With such a limited number of animals per group, no correct conclusions can be drawn because the chance is high that the results can be attributed to coincidence (see box ‘Why numbers are important’). This is the first fundamental fault in the study design by Séralini et al. Too few animals were used per treatment group.

Incorrect study design

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* NK603 is a genetically modified maize variety from Monsanto that is resistant against the effect of the herbicide Roundup. Roundup is a so-called broad spectrum herbicide that in principle destroys all plants. The genetically modified NK603 maize is tolerant to Roundup, which means that Roundup can be used in a maize field to control all weeds without harming the maize.
WHY NUMBERS ARE IMPORTANT

What does it mean that only 10 animals were tested per treatment in the Séralini study? The smaller number of animals that are used, the greater the chance that the result can only be attributed to coincidence. This can be illustrated well by the following example. Suppose a researcher wishes to investigate the difference between the Dutch and Belgians when it comes to blue eyes. The researcher selects ten random Belgians and ten random Dutch and observes objectively that among the Belgians three people have blue eyes and among the Dutch, seven. He then draws the conclusion that the Dutch are more than twice as likely to have blue eyes as Belgians. But does this conclusion reflect reality? A second researcher puts this to the test and repeats the test with 10 different Dutch and 10 different Belgians. In the group there are six Belgians with blue eyes and four Dutch. What does this mean? This means that researcher 1 has done insufficient observations to obtain a repeatable and thus relevant result and that researcher 1’s conclusion was premature. If the test is repeated with a larger group of people, the results of both researchers will start to come closer together. The larger the group, the more the observed and thus relevant result and that researcher 1’s conclusion was premature. If the test is repeated with a larger group of people, the results of both researchers will start to come closer together. The larger the group, the more similar the results of the researchers will be. Group size and the number of repetitions form the basis of scientific research. If too few observations are done, no statements can be made and the research is worthless. This is the reason why in each scientific experiment a statistical foundation of the selected group size, and parameters such as variance and standard deviation should always be reported. The study by Séralini and colleagues failed completely in this.

Incorrect conclusions

The conclusions that the researchers drew are a fine example of “cherry-picking”, a subjective approach in which only those results are used that fit with a presupposed hypothesis. Such a method is of course against scientific deontology and integrity. Séralini concluded that, among the female animals two to three times more animals died in the group that was fed the GM maize NK603 in comparison with female animals that were fed the non-GM maize. The data from the study, however, also show that the mortality in male animals in which a third of the diet comprised GM maize was three times less than among the animals fed on non-GM maize. Using the same logic as Séralini, this would indicate a health promoting effect of NK603 GM maize, but these data were never commented on in the article. These strange and conflicting results should have alarmed the researchers (and the reviewers of the scientific journal) that there was something fundamentally incorrect regarding the test design.

An additional red flag should have been the lack of a dose-related effect. When a harmful substance is added to a diet, it is expected that a higher dose would have a stronger or at least equal effect compared to a lower dose. No such relationship can be found throughout the study. Frequency of death, disease symptoms and number of tumors were rather randomly distributed across the different groups; repeatedly the animals that were fed the largest quantities of GM maize were shown to have fewer disease symptoms than the animals that were fed less GM maize.

Because of the significant noise in the limited data set due to the small number of animals used and the absence of sufficient controls, Séralini et al. went out of their way to search for explanations for their findings and did not use standard statistical methods. They ignored the most obvious explanation, namely that the observed variability in the data is not counterbalanced by a correct experimental design, which makes correct interpretation of the data impossible.

Misleading representation of the results

Furthermore, besides an incorrect experimental design and scientifically irresponsible conclusions, the authors of the rat study were also guilty of a misleading representation of the results. As stated previously, the laboratory animals used spontaneously develop tumors. The reader, however, is never informed in the article about this huge sensitivity, or about how the researchers took this into account in the experimental design. Even worse, the photo collage that spread across the world only showed those animals that had eaten GM maize and/or herbicide. The photos showed rats that had developed tumors. But a photograph of a control rat that was fed a non-GM diet is missing, while it is known - based on previous publications but also from data from the Séralini study - that the rats in the control group also developed similar tumors.

To illustrate the detailed analysis of the different organs, photographs of healthy organs were selected from the control group, while from the treated group, photographs were selected from affected organs. This misleading representation of data illustrates once more the subjective undertone of the article and entirely undermines its creditability.
Trust arrives on foot but leaves on horseback

The European Food Safety Authority (EFSA) but also the food safety agencies of the individual EU member states analyzed the data put forward in the Séralini study. They all came to the conclusion that the study fell short in the area of experimental design, analysis and interpretation of the results. It may then also be clear that the article should never have passed the quality assurance of a well-known scientific journal. Nevertheless, the Séralini article caused a tsunami at policy level. There was a lot of discussion about the use and need of laboratory animal studies before the Séralini story was published, but the commotion that Séralini caused, has sharpened the discussion further. As a consequence of this, the European regulations regarding the risk assessment of GM crops was further tightened (see above).

Also the journal Food and Chemical Toxicology that published the Séralini article, concluded that the Séralini conclusions were not founded. The publisher decided to retract the article. This is a normal procedure when it appears that there are serious substantive problems with a scientific article. In the meantime, the authors were given the opportunity to republish their article unchanged and without independent data control in another journal. This is very regrettable as it supports the use of deliberate misinformation produced under the guise of scientific research to continue to muddy the GMO debate.

Conclusion

Food safety is a fundamental right that must be strictly controlled by society. It goes without saying that all initiatives to guarantee food safety must be encouraged. Alertness for new products and new production technologies is an essential component of this procedure. However, it is essential that the discussion about food safety is conducted in accordance with scientific facts.

In the GMO debate we note that certain NGOs and campaign groups manipulate the emotion and intuition of the wider public and thus also the policy. Even science is misused to strengthen an anti-GMO feeling using poorly conducted studies. The scientific facts regarding food safety are, however, overwhelming. Hundreds of studies, strict risk analyses, stringent authorization procedures and continuous follow-up show that GM technology is safe and that the currently authorized GM crops are just as safe as their non-GM counterparts. GM crops have an unprecedented track record in the area of food safety.
Food safety of genetically modified crops

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112 www.harlanbio.com see life span and disease


120 EFSA (2012). Final review of the Séralini et al. (2012a) publication on a 2-year rodent feeding study with glyphosate formulations and GM maize NK603 as published online on 19 September 2012 in Food and Chemical Toxicology. EFSA Journal 10.


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