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Cultivation of genetically engineered maize: Risks not under control

Overview: Why the EU should not allow the cultivation of transgenic maize engineered to produce insecticidal toxins

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Content

Summary

The EU Commission wants to make a decision on the cultivation of genetically engineered maize before the growing season 2017 starts. Three variants of transgenic maize producing insecticidal toxins (registered as MON810, Bt11 and Maize 1507) are being considered. Two of them are resistant to the herbicide glufosinate (Bt11 und Maize 1507).

Releases of genetically engineered organisms and, in particular, large-scale cultivation introduce new biological functions and compounds into the environment that have not been tested or evolved in evolutionary processes. The cultivation of genetically engineered maize, therefore, carries substantial risks and potential hazards for humans and the environment, and for this very reason EU Directive 2001/18 requests comprehensive risk assessment.

This current review shows that risk assessment carried out by the European Food Safety Authority (EFSA) does not meet the standards set out in the EU regulation and has serious gaps. These gaps are related to the:

- interaction between plant genomes and the environment,
- data on risks of protected species, pollinators, predators and wild life species such as mammals and birds,
- impact on the microbiome,
- combinatorial effects that enhance toxicity of Bt toxins,
- uncontrolled spread of the transgenes in the environment.

There are further reasons for the EU not to allow the cultivation of the genetically engineered maize:

- pest insects will adapt to the insecticides produced in the maize if grown on larger scale,
- the requirements for monitoring unintended effects on human health and the environment set out in EU regulations are not being met.

In view of these findings, Testbiotech recommends the EU Commission and the EU Member States do not authorise the cultivation of the genetically engineered maize.

Introduction

The EU is currently discussing whether to allow three variants of transgenic maize producing insecticidal toxins (registered as MON810, Bt11 and Maize 1507) to be grown in 2017. Two of them are resistant to the herbicide glufosinate (Bt11 und Maize 1507). According to internal documents made available by the EU Commission, a decision will also be made on a further genetically engineered glyphosate-resistant maize produced by Syngenta (GA21) after the decision has been made for the current three applications.

So far, only one transgenic maize, MON810, is allowed for cultivation in the EU. At the present time, an application has been filed for the renewal of the EU authorisation for cultivation of MON810. Most EU Member States have already prohibited its cultivation on their territory by referring to EU Regulation 2015/412. In Spain, however, the maize is grown on around 100.000 hectares of agricultural land. It is also cultivated in the Czech Republic, Slovakia, Portugal and Romania albeit on much smaller areas. According to information from industry, in 2015, there was a reduction in the areas used for the cultivation of genetically maize both in the EU and the US.[1]

The EU Commission is not expecting Member States already prohibiting the cultivation of the genetically engineered maize on their territory to vote against the authorisation for cultivation. They argue that these countries will not be impacted by the EU decision. However, the single market will allow the harvest of the maize to be sold in the whole

[1] www.isaaa.org/resources/publications/briefs/51/toptenfacts/default.asp

EU. Moreover, these national prohibitions have not yet been confirmed in any court decision. Thus the upcoming decision is relevant for all EU Member States.

Whatever the case, it is still necessary to carry out risk assessment as requested in EU regulations (Directive 2001/18). To meet the standards in the EU regulation, environmental risk assessment must be carried out with the following objective:

> *"to identify and evaluate potential adverse effects of the GMO, either direct and indirect, immediate or delayed, on human health and the environment which the deliberate release or the placing on the market of GMOs may have."*

Currently, this objective is not being met in risk assessment carried out by EFSA.

In the light of these findings, it is important to call to attention the fact that at the beginning of October, the EU Parliament passed a resolution against authorisation for the cultivation of the genetically engineered maize.

The following brief summary outlines some of the risks known so far.

1. Interactions with the environment

It is known that genetically engineered plants can react to stressful conditions in different and surprising ways compared to conventionally bred plants (see, for example, Meyer et al., 1992; Matthews et al., 2005; Zeller et al., 2010). Further, it is known, that Bt gene expression is dependent on environmental conditions (Then & Lorch, 2008) as well as on varietal background (Adamczyk & Meredith, 2004). Results from very specific experiments show that gene expression in genetically engineered maize is not predictable if climate change conditions act as stressors on the plants (Trtikova et al., 2015).

To assess the risks of cultivating genetically engineered plants, the interactions between the newly introduced DNA, the plant genome and the environment have to be assessed. In order to gather sufficient data, the whole period of vegetation, several generations and a broad range of defined environmental stressors should thereby be taken into account.

So far, there are no reliable data on how much Bt toxin is produced by the genetically engineered maize varieties grown in Spain, taking into account changing environmental conditions. Therefore, little is known about how far the environment is exposed due to pollen, root exudation and parts of the plants. As yet, there are not even any sufficiently reliable methods to determine the Bt content in the plants (Székács et al., 2011).

In regard to the genetic stability of these maize plants, it is further problematic that, according to the data from the applications, several DNA sequences were inserted unintentionally due to the method of genetic engineering. These DNA sequences can also impact the reaction of the plants to environmental stressors.

Without sufficiently reliable date on genetic stability, no conclusions can be drawn on the impact the plants have on ecosystems, the susceptibility of the plants to specific pests or the emergence of unintended compounds in the plants with immunogenic, anti-nutritive or toxic properties. At the same time, there has been no systematic investigation of the interaction between the plant genome and the environment.

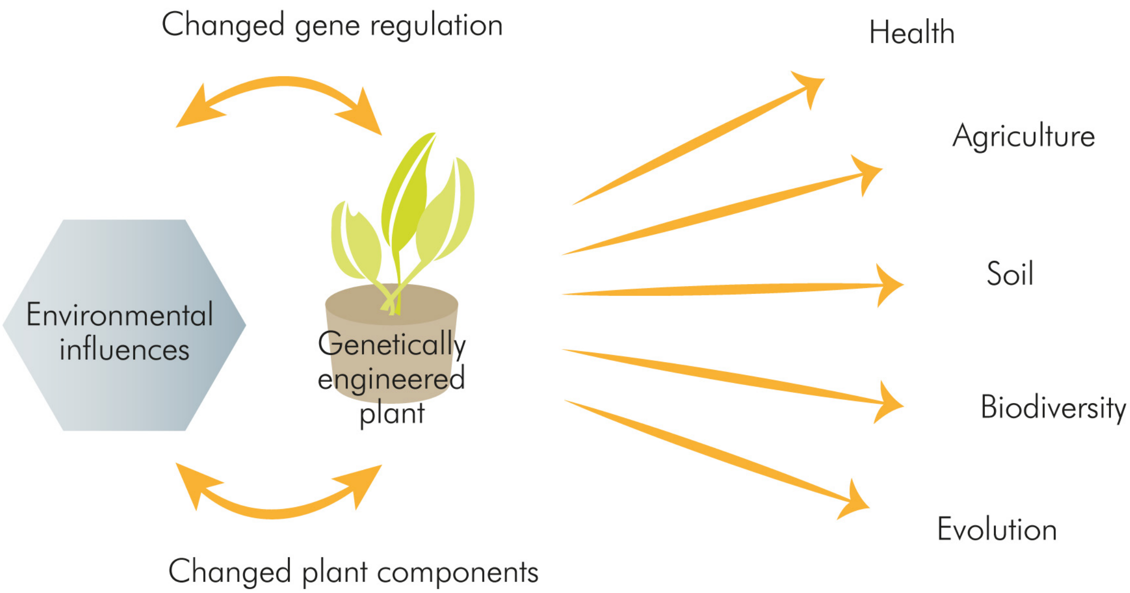

Figure 1: Schematic overview of some relevant risks taking into account interactions with the environment.

2. The impact on fauna

Bt toxins are supposedly designed to be toxic only to specific insect species. This is the premise for them to be regarded as generally safe for other animals and humans. However, the mode of action of the toxins is not fully understood. There are even contradictions in the scientific explanations of how these toxins interact with cells and organisms (Then, 2010; Hilbeck & Otto, 2015, Melo et al., 2014). In conclusion, the specificity of the Bt toxin is not a sufficiently known or established fact. On the contrary, there is evidence showing that the range of susceptible species is much broader than originally thought (van Frankenhuyzen, 2009; Lövei et al., 2009; Hilbeck & Otto, 2015).

Risk assessment is further complicated because the Bt toxins as produced in the plants are changed in their structure to enhance efficacy compared to the native variants. These structural changes can also extend their toxicity to non-target organisms (Hilbeck & Otto, 2015).

Therefore, risks for humans and animals have to be tested empirically and cannot be excluded just by making general assumptions. However, the relevant data is missing for many species such as butterflies, pollinators, predators as well as wild birds and mammals.

For example, EFSA does not have any reliable data on the susceptibility of European butterfly species in regard to the Bt toxin produced in Maize 1507 (Then & Bauer-Panskus, 2014). EFSA was, in fact, aware of missing data and consequently developed a simple strategy: They opted to use computer-based modelling developed by their own experts to determine a safety distance between the fields and protected areas (Perry et al., 2012). As a result, EFSA concluded that just a few metres are sufficient to safeguard protected species, while in reality, the pollen from maize plants can travel for kilometers (Hofmann et al., 2014, 2016).

Because this modelling system is by no means based on empirical data but mostly on assumptions, there is substantial scientific controversy on the approach taken by EFSA (Lang et al., 2011; Holst et al., 2012; Camastra et al., 2013). Certainly, this kind of modelling cannot be used to reliably assess the real environmental risks.

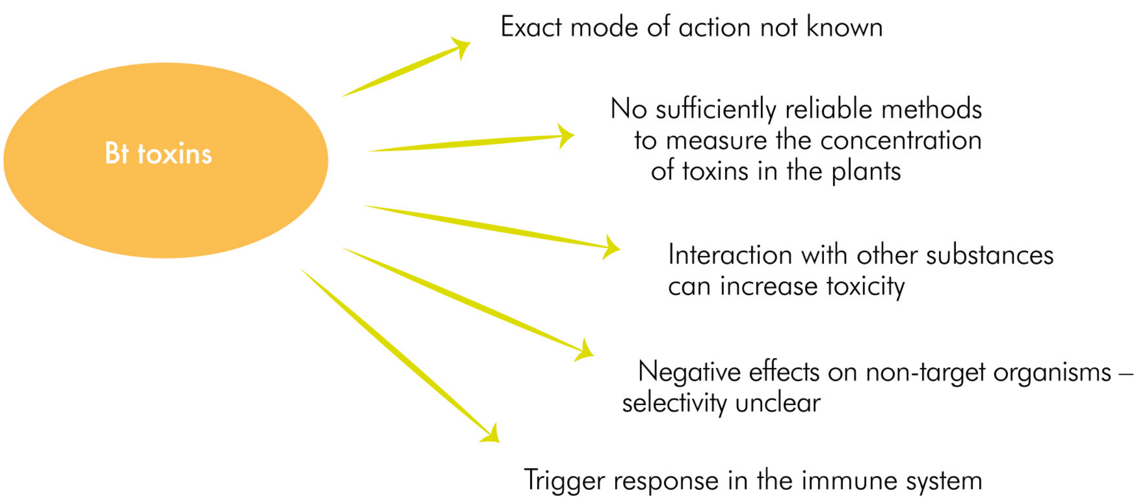

Figure 2: Schematic overview of some problems in risk assessment of Bt plants.

3. The impact on plant microbiomes

There is a close symbiosis between plants and their microbiomes. The plant microbiomes are made up of microorganisms such as bacteria, archaea and fungi, which are especially active in the rhizosphere and on the upper parts of the plants. This symbiotic community is specific for each plant species.

Interaction between the plant microbiome and the plants is orchestrated by various chemical and biochemical substances and signals. Depending on the circumstances and the conditions for the plant (growth, temperature, water supply), specific organisms will be attracted which can help the plant to overcome plant diseases and pest organisms (Bakker et al., 2013).

Research on microbiomes has attracted more and more attention in the last few years. Terms such as ‘holobionts’ and ‘superorganism’ have been introduced to describe just how closely related plants and animals are with their microbiome. Holobionts are units, developed by co-evolution and their joint genome is also called as 'hologenome' (Rosenberg & Zilber-Rosemberg, 2016).

Only a small number of the organisms forming the microbiome, especially in the plant rhizosphere, are known so far. Most of these cannot be isolated and cultivated in the laboratory. And most of the knowledge we have so far has been gained from DNA sequencing and screening of metabolites. However, these data only partially allow conclusions to be drawn on the biological characteristics of the species and their interactions.

As yet, the risk assessment of genetically engineered plants does not systematically take the plant microbiome into account. From the point of view of industry and authorities, it might simply be regarded as too complex to assess if the concept of holobionts and hologenoms is taken into account.

However, from the perspective of environmental protection, the assessment of these risks is essential. Large-scale cultivation of genetically engineered plants can deeply impact soil ecology. The consequences can affect soil fertility, plant health and the functioning of the agro-ecological systems in addition to food security: The microbiomes of plants, animals and humans are in permanent exchange mode.

In trials with genetically engineered plants, differences were identified in the composition of the microbiome in comparison to conventional plants (see, for example, Cheeke et al., 2010). Some of these were not consistent and seem to be dependent on various factors. A review of relevant publications regarding the impact of genetically engineered plants on the rhizosphere is provided by Singh & Dubey (2016). The authors suggest carrying out more detailed investigations taking additional environmental stressors into account.

Da Silva et al. (2016) carried out the first investigations into changes in the microbiome of above ground parts of the plants. They found changes in the endophytic fungal community in the leaves of Maize 1507 as well as in stacked maize containing this event in comparison to its conventional counterparts. The relevance of these findings for risk assessment is huge: For example, further investigations are needed to find out whether these changes in the fungal community can endanger food safety. Further, the interaction of the plants with biotic or abiotic stressors can be severely impacted. Da Silva et al themselves emphasise the need for further investigation:

> “This research study is the first to evaluate the symbiosis of microbial foliar

> communities between maize hybrids or landraces. It showed that the genetic composition of the maize populations is the determining factor for the changes detected in the endophytic fungal community of maize leaves. Further studies are required to verify if such changes can affect the plant response to biotic and abiotic factors of the crop environment, and consequently, compromise agrobiodiversity conservation."

It has to be stated that EFSA has neither requested systematic data on changes in plant microbiomes nor targeted investigations into plant responses to defined biotic or abiotic stressors (see above). In fact, EFSA entirely failed to assess the data from the da Silva et al. (2016) study, even though they are especially relevant for the cultivation of Maize 1507.

It is quite plausible that many more specific differences will be found if plants producing Bt toxins are systematically examined: For example, Bt toxins exudate into the soil via the roots and can remain bound to particles for months (Saxena et al., 2002). Further, there might be other unintended changes in the signalling of the plants and their biological compounds that might also impact the plant microbiome. For example, Vidal et al. (2015) found changes in the proteome of Bt maize related to plant defence mechanisms. If the relevant differences have not been detected and understood, the likelihood is that there are as yet no adequate methods for doing so (for more on the development of new methods see for example: Oburger & Schmidt, 2016).

As long as the impact of genetically engineered plants on the plant microbiome and the associated ecosystems above and below ground have not been assessed in more detail, commercial large-scale cultivation is not acceptable from a precautionary point of view.

4. Combinatorial and accumulated effects

There are combinatorial effects that can significantly enhance the toxicity of Bt toxins (Then, 2010). Such effects have been, for example, described in snails and daphnia, which are regarded as relevant model organisms (Kramarz et al., 2007, Bohn et al., 2016).

Risks due to combinatorial effects can emerge in the fields as well as in mixtures of food and feed. Relevant compounds are, for example, allergens, toxicants and residues from spraying with herbicides. However, combinatorial effects were completely ignored during risk assessment of the Bt maize, despite EU Directive 2001/18 requirements to take cumulative effects into account.

Table 1: Overview of some combinatorial effects that can enhance the toxicity of Bt toxins

Some co-factors that can enhance toxicity	Some relevant publications
Presence of bacteria in the gut	Broderick et al., (2009)
Impact of other toxic stressors	Kramarz et al. (2007); Bohn et al. (2016);
Delayed degradation of Bt toxins by plant enzymes (protease inhibitors)	Pardo Lopez et al. (2009)
Other Bt toxins	Sharma et al. (2004); Tabashnik et al. (2013); Bohn et al. (2016)

One example of how EFSA is dealing with these risks: In 2016, Norwegian scientists described unexpected combinatorial effects between Bt toxins and glyphosate in Daphnia (Bohn et al., 2016). Daphnia were generally not thought to be susceptible to these Bt toxins. However, significant negative impacts were observed in this important model organism. These effects were dose-dependent. However, EFSA (2016 a) did not assess the results in detail and simply dismissed the findings on the grounds of methodology and uncertainties. During the assessment, EFSA did not even try to contact the scientists to resolve any of the open questions.

From a scientific point of view, the approach EFSA is taking is highly questionable. So far, hardly any investigations into the combinatorial effects between Bt toxins and herbicides have been carried out. EFSA should have beyond doubt requested more investigations instead of dismissing the study simply due to uncertainties.

In this context, Testbiotech is also very much concerned about conflicts of interest at EFSA: The expert leading the EFSA assessment, Yann Devos, is also actively involved in the International Society for Biosafety Research (ISBR), an organisation which is at least partially funded by industry.[2]

5. Spread of transgenes

Teosinte is reported to have been spreading in France since 1990, and in Spain since 2009. Maize and teosinte together can produce viable offspring. The risk: By crossing with transgenic maize, teosinte can become a new superweed, producing insecticides and becoming resistant to herbicides.[3]

Spain is the region where most genetically engineered maize plants are grown in the EU. In 2015, there was official confirmation that around 750 hectares of maize fields in several regions were affected by teosinte, but it is likely that many fields remained undetected. Some of the teosinte plants were found in the fields where transgenic maize is grown. It is not known, whether transgenic hybrids of teosinte already exist, but their occurrence only seems to be a matter of time. Via teosinte, the transgenes could also be

[2] www.testbiotech.org/en/node/1666

[3] www.testbiotech.org/en/press-superweed-genetically-engineered-maize

passed to other fields cultivated with conventional maize, where they could persist and spread further.

After NGOs alerted the EU Commission, EFSA was requested to carry out an assessment. However, as the EFSA opinion (2016b) shows, crucial data for risk assessment are largely missing:

- Some of the various species and subspecies of teosinte produce many more hybrids with maize than others, thus increasing the risk of gene flow. However, it is not known which species and subspecies are spreading in the fields.
- The biological activity of the transgenes is dependent on the plants overall genome. Consequently, hybrids from maize and teosinte could, for example, produce a lot more insecticidal toxins in comparison to the original maize plants. But this risk was never investigated.
- The assumptions made by EFSA on how to control teosinte are not sufficiently based on data: The data available show a strong increase in the number of fields affected from 2014 and 2015, despite control measures already being in place.

Therefore, much more data would be needed before any conclusions can be drawn on the actual risks. But instead of requesting more data, EFSA simply concluded safety. Apparently, there was some undue time pressure created by the EU Commission, which wanted to make a decision on the applications for cultivation before the end of 2016.

Tellingly, the lead author of the EFSA opinion was Yann Devos, who is known to have a parallel leading position in an organisation called the “International Society for Biosafety Research” (ISBR), which is largely funded by industry (see above).

Fig 3: Regional government of Aragon, Spain, provided information about the problem with teosinte[4]

[4] www.aragon.es/estaticos/GobiernoAragon/Departamentos/AgriculturaGanaderiaMedioAmbiente/TEMAS_AGRICULTURA_GANADERIA/Areas/03_Sanidad_Vegetal/PUBLICACIONES_CSCV/I_F_TEOSINTE.pdf

In a recent monitoring report, Monsanto (2016) for the first time admitted they had not investigated the spread of teosinte. The reasoning behind this lack of monitoring is clearly in conflict with the basic principles of environmental risk assessment:

> *Monsanto has not conducted monitoring activities specifically addressing the presence of teosinte detections in Spain. (…) Monsanto is of the opinion that the presence of teosinte in Spain cannot be classified as information that regards the risk of MON 810 to human health or the environment, nor can it be regarded as information that influences the evaluation of the safety in the use of food or feed.*

Both Directive 2001/18 and EFSA guidance require that potential gene flow is assessed as one of the crucial elements of environmental risk assessment. For example, AnnexII of Directive 2001/18 calls for the assessment of:

> *Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species.*

Thus, Monsanto is without doubt contravening EU regulations.

6. Resistance in pest insects

This is an increasing problem in countries such as the US, where Bt plants are grown on millions of hectares of land. Pest insects do indeed adapt to the cultivation of Bt cotton as well as Bt maize. There are already resistances to the Bt toxins as well as shifts in the populations of pest insects - so-called pest replacement (Tabashnik et al., 2013).

In the US, the corn rootworm (*Diabrotica virgifera*), in particular, is becoming resistant to the toxin produced in the Bt maize (Gassmann et al., 2011). More and more resistances to the Bt toxin produced in Maize 1507, which is classified as Cry1F, are also being observed: For example, Huang et al. (2014) are warning that pest insects with an acquired resistance to Cry1F in Puerto Rico, are moving into the south of the US. In 2016, Cry1F was reported to be no longer effective against the Western Bean Cutworm which is spreading throughout the corn belt in the US (Ostrem et al., 2016).[5]

So far, there have been no observations of resistances developing in the fields where maize is grown in the EU. One reason for this could be that in comparison the areas of cultivation are much smaller than those in the US. If the areas in the EU increase, the pressure on pest insects to develop new biological strategies to survive will also increase.

[5] www.dtnpf.com/agriculture/web/ag/news/crops/article/2016/10/05/herculex-trait-fails-western-bean-4

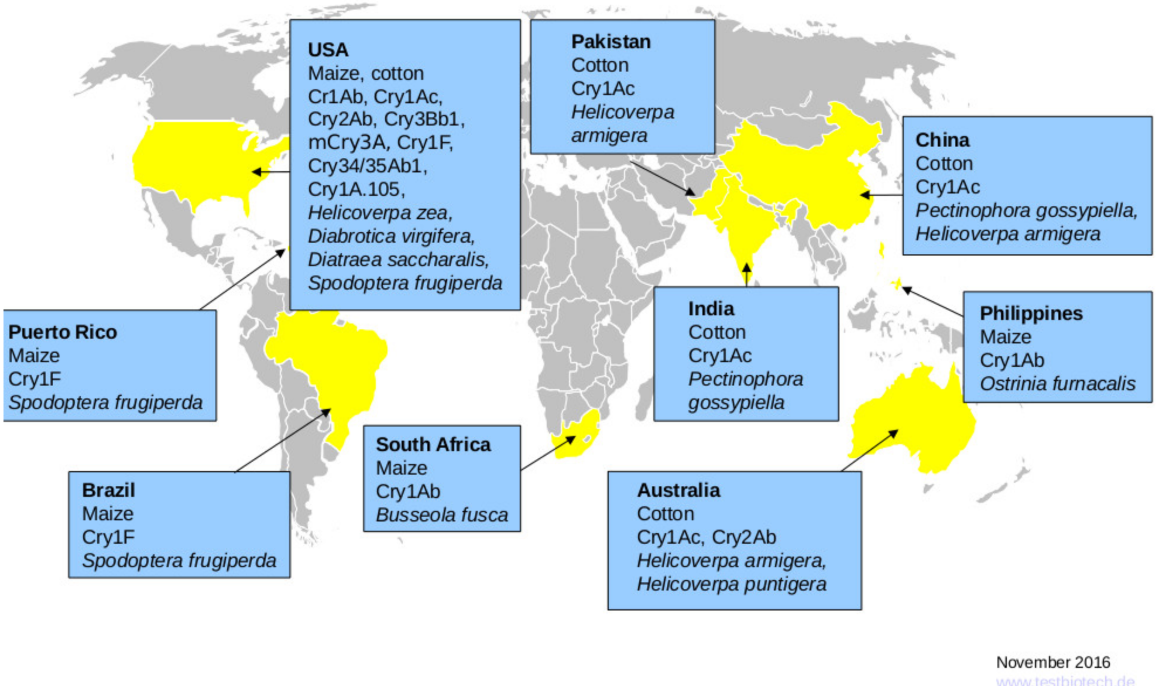

Figure 4: Increasing resistance in pest insects to Bt plants.

7. Monitoring does not comply with EU regulations

Directive 2001/18 requires post-marketing monitoring:

> *"in order to trace and identify any direct or indirect, immediate, delayed or unforeseen effects on human health or the environment of GMOS as or in products after they have been placed on the market."*

The current system of monitoring as applied by Monsanto in the cultivation of MON810, mostly relies on questionnaires being filled in by the farmers. However, any real observation of the effects on human health and the environment cannot be organised with this method.

This is also evidenced in the case of teosinte: These plants have been known to be spreading in Spain since 2009, something which Monsanto completely failed to report for several years. It took Monsanto until 2016 to report findings on tesosinte. This report (Monsanto, 2016) is in itself astonishing: While Monsanto complains about EU monitoring regulations being too strict, and also claims it is voluntarily providing more information than needed, it is still forced to admit that no investigation was carried out on the spread of teosinte (see above).

This clearly shows that Monsanto is not willing or not able to comply with EU standards. Also, according to the EFSA opinion, several previous reports prepared by Monsanto were insufficient.[6]

Furthermore, the patent on MON810 expired in 2012. In response, Monsanto started to question the whole system of monitoring: As the company told the EU Commission in writing, other companies could bring MON810 seed onto the market, without informing Monsanto. Thus, Monsanto could lose oversight of the field sites where MON810 is actually grown. As far as is known by Testbiotech, the patents on Bt11 and Maize 1507 have also expired.

As long as the conditions for an effective monitoring are not met, no permission can be given for the cultivation of genetically engineered plants.

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[6] www.testbiotech.org/node/1224

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