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MON88017 'Roundup Rootworm Maize' - ready for cultivation?

Testbiotech comment on EFSA Scientific Opinion on application (EFSA-GMO-CZ-2008-54) for placing on the market of genetically modified insect resistant and herbicide tolerant maize MON 88017 for cultivation

Andreas Bauer-Panskus & Christoph Then

Content

Abstract

Genetically engineered maize MON88017 produces the Bt protein Cry3Bb1, which makes the plants toxic for the larvae of corn rootworms (*Diabrotica* spp.). MON88017 is also tolerant to pesticides containing glyphosate. In 2008, Monsanto filed an application to cultivate this product in the EU. The European Food Safety Agency (EFSA) published a positive opinion on the safety of MON88017. However, analyses show that the safety of this maize line cannot be inferred from the available data. The reasons include:

- significant differences in plant compounds and differences in phenotype were not investigated sufficiently, instead references were made to questionable 'historical' data from industry.
- many data concerning risks for non target organisms were derived from other genetically engineered events.
- there are several studies showing that the Bt toxin in MON88017 poses risks in non-target organisms.
- new data show that large-scale cultivation of MON88017 could enhance the spread of the corn rootworm.
- large-scale cultivation of herbicide-resistant plants can reduce populations of protected species such as butterflies.
- risks emerging from residues of spraying with herbicides were not assessed.
- possible synergistic effects were not assessed.
- EFSA does not give sufficient weight to the effects of glyphosate on plant diseases and microbial communities in soil.
- the negative impact on sustainable agriculture was not assessed in a realistic manner.
- monitoring measures as proposed by EFSA do not allow the identification of delayed, unexpected or accumulated effects as required by EU regulation.

Therefore, the EFSA opinion on MON88017 should be rejected.

Introduction

'Roundup Rootworm Maize' MON88017 is genetically engineered to produce insecticidal proteins that are toxic for the larvae of corn rootworms (*Diabrotica* spp.). It expresses the Bt gene Cry3Bb1. Furthermore, MON88017 is engineered to be tolerant to pesticides containing glyphosate as an active ingredient.

MON88017 was approved for cultivation in the USA (2005), Canada (2006), Brazil (2010) and Argentina (2010). In the EU, MON88017 was approved in 2009 as an import product. Monsanto filed an application for cultivation of this maize line in 2008. In 2011, EFSA published a positive opinion on the cultivation of MON88017. The risk assessment was finalised under the new EFSA guidelines for Environmental Risk Assessment that came into force in 2011.
In its opinion, EFSA states that cultivation was unlikely to raise safety concerns, but identifies several risks that might arise from MON88017 maize, such as the emergence of resistance in target insects or risks associated with the usage of glyphosate. As the following outline shows, this EFSA conclusion is not sufficiently supported by scientific findings and should be rejected.

Molecular data

There is a complete lack of metabolomic data as well as data showing to which extent the gene activity of plant genes is affected by the artificial introduction of gene constructs.
These data would be highly relevant, since it cannot be denied that there are significant unintended changes in the composition of components (such as Vitamin B1, fatty acids, amino acids, zinc and lignin) and significant unexpected differences in phenotype (such as height, seedling vigour and yield).

Comparative assessment

The comparative assessment is flawed because of biased interpretation of the existing data. There were significant differences in plant components (such as Vitamin B1, fatty acids, amino acids, zinc and lignin). These differences were not investigated further. Instead, EFSA made references to unspecific and questionable 'historical' data unrelated to the actual field trials.
Differences in plant compounds might indicate unintended and unexpected changes in plant metabolism and plant composition in comparison with the isogenic lines. Given these findings, a detailed study of changes in gene activity and plant metabolism should be performed under various and defined environmental stress factors to examine genetic stability of the plants. This is not only relevant for the expression data of the newly introduced gene constructs, but also for investigations to which extent unintended compounds can emerge in the plant tissue or, for example, if the plants are more vulnerable to certain plant diseases.

The EFSA opinion stating that the changes in plant composition are within the range of historical data is not sufficient indication of the safety of these crops. To avoid major uncertainties, there must be more investigation into why there are significant differences in plant composition in comparison to the isogenic lines. (Hilbeck et al 2011). Only after further detailed examination can these data be interpreted regarding potential risks. It also has to be stated that there is no reference to the historical data as mentioned by EFSA. It is likely that EFSA is referring to the ILSI database. As a statement made by Joe Perry, a Member of the EFSA GMO panel shows, this database is not reliable and cannot be used to demonstrate substantial equivalence:
*"I think we're in a situation where we would be unwise at the present time (maybe in the future this will be different), but at the present time we can't trust the ILSI database. There is not sufficient environmental information from where these trials were done and that's why we insist that the commercial reference variety should be planted simultaneously with the GM and the non-GM. Otherwise I think we are in an unsafe situation and I would worry that the limits would be too wide."(*Observations of Mr. Joseph Perry, Vice-Chair, at EFSA's consultative workshop on its draft guidance for the selection of Genetically Modified (GM) plant comparators, held in Brussels on 31 March 2011).

Risk assessment of Bt toxin

The EFSA opinion on MON88017 maize is based on assumptions about the mode of action of Bt toxins that are not sufficiently based on scientific evidence. There are several modes of action described and not just one theory about how these toxins function. Some of these publications show that selectivity cannot be assumed without detailed testing. Others show that synergistic interactivity has to be taken into account. Most of the literature concerning the mode of action of Bt toxins as quoted by EFSA is about Cry1Ab and cannot be regarded as adequate for the assessment

of specific Cry3Bb1 effects.

In general, the mode of action of Bt toxins is not fully understood. This is even a matter of controversial debate (Pigott & Ellar, 2007). Strict selectivity of the Bt toxins is not shown by empirical evidence but deduced from its mode of action as described previously. More recent research (Soberon et al., 2009) shows that there are mechanisms that might cause toxicity in other species and even in mammals. As Pardo Lopez et al. (2009) and Pigott et al. (2008) show, synthetically derived and modified Bt toxins can have a much higher toxicity than native proteins. Even small changes in the structure of the proteins can cause huge changes in toxicity. Thus, risks for human health cannot be excluded by assumptions or considerations but only by empirical testing before market authorisation.

EFSA did not elaborate on these partially contradictory theories on the mode of action of Bt toxins. For example, no detailed study was performed on the potential impact of Cry3Bb1 on mammalian cells to find out if these toxins actually do not have any impact.

Standardised methods for determining gene expression are missing

In general, basic prerequisites have to be met to enable proper risk assessment. If these data are not available, hardly any feeding trial or other toxicological test can be designed, performed and interpreted in a meaningful way.

One of these prerequisites is sufficient data on the expression of the newly expressed proteins. But in the case of Bt toxins, standardised protocols to achieve results that can be reproduced by other laboratories are largely missing (Székács et al., 2011). Further, it is not clear how these plants and the expression rate of the newly introduced proteins will be influenced by more extreme weather conditions such as drought or other environmental factors. There are also no data on gene expression in volunteers that can remain after cultivation. Further, the impact from the genetic background of certain varieties has to be taken into account. Several investigations show that genetically engineered plants can exhibit unexpected reactions under stress conditions (see for example: Matthews et al., 2005). This can also impact the Bt content in the plants (Then& Lorch, 2008). In the case of MON88017, significant differences were observed in the level of gene expression in plants cultivated in the US and Europe. While it is true that these differences do not pose a risk per se, the relevant impact factors should be investigated to gain sufficient insight into the functional stability of the inserted gene.

Risk assessment of the residues from herbicides

Another basic prerequisite for risk assessment in this context are reliable data on residue loads from spraying with glyphosate formulations. The amount of these residues depends on the specific agronomic management used in the cultivation of the herbicide resistant plants. The fact is that reliable data covering the actual range of residue load in the plants are not available (Kleter et al., 2011; Then 2011, EFSA 2011b). There are several reasons why risk assessment of genetically engineered plants with herbicide tolerance cannot leave aside the issue of residues from spraying:

- Commercial large-scale cultivation of these plants means there is a selective pressure on resistant weeds, thus increasing the amount of sprayed herbicides and the load of residues. Under these circumstances, the complementary herbicides are likely to be sprayed several

times, thus the pattern of usage and the level of residues can be significantly higher compared to other plants.

- Herbicide tolerant plants are meant to survive the application of the complementary herbicide while most other plants will be killed after short time. Thus, metabolites and the resulting residues can be rather specific.
- The residues are inevitable constituents of plant composition leading to a very specific exposure of the food chain.
- In the case of stacked events, a combination of specific plant constituents is fixed in the genetically engineered plants. The combination of the residues from spraying and of insecticidal proteins (as it is the case for example in MON87701 x MON89788) causes a unique and unavoidable exposure of the feed and food chain with very specific residues. Possible interactions have to be investigated in detail.

For these reasons, residues from complementary herbicides have to be considered during the risk assessment of genetically engineered plants. They are an inevitable element of plant constituents to which the food and feed chain will be exposed. Data on the actual load of residues in the plants resulting from varying agricultural practices have to be made available by the applicant. The data on residues are also relevant for the assessment of combinatorial effects.

The toxicity of glyphosate is currently under revision by the EU. Several experts are warning that toxicity could be higher than expected (Antoniou, et al., 2010; Benachour et al., 2007; Paganelli et al., 2010; PAN AP, 2009). Since the revision of glyphosate under pesticide legislation has not been finalised, cultivation of these plants cannot be allowed. In this context, the additive POEA also has to be taken into account because it is even more toxic than glyphosate (BVL, 2010).

Assessment of synergistic effects

Neither EFSA nor Monsanto have presented an assessment of synergies and accumulated effects. The only synergy discussed is the one between the enzyme EPSPS that confers resistance to glyphosate and the Cry3Bb1 toxin. But from the perspective of toxicology, potential synergies between the Cry3Bb1 toxin and the formulations (and metabolites) of glyphosate used for spraying the plants are much more relevant. There were no tests carried out to examine potential synergies. Synergistic effects can become highly problematic for non- target organisms. Interaction of the toxins with each other or with other compounds can cause higher toxicity and lower selectivity (Then, 2010). These effects may impact human and animal health as well as the protection of the ecosystems. Some plant enzymes that diminish the digestion of proteins (protease inhibitors) can strongly enhance the toxicity of Bt toxins (Pardo Lopez et al., 2009). Even the presence of very low levels of protease inhibitors can multiply the insecticidal activity of some Cry toxins. It is known that maize produces such inhibitors (Shulmina et al., 1985). In this case, resistance to glyphosate is combined with the insecticide. This leads to a combination of potentially hazardous residues from spraying.

The plants will go into feed and might, therefore, be mixed with other genetically engineered plants. Tests need to be carried out to determine potential accumulative or combinatorial effects. But no assessment of combinatorial effects with other genetically engineered plants used in food and feed were requested. No investigations were conducted to assess the impact of a permanent ingestion of these plants on the intestinal microbial composition in human and animals.

Further tests have to be performed to find potential combinatorial or accumulated effects. Residues from spraying and from insecticidal toxins can result in permanent long term exposure of humans and animals and, therefore, relevant studies to examine chronic effects have to be performed. This has become especially relevant because MON863, which also produces the toxin Cry3Bb1, has since shown several significant effects in animal feeding trials that were classified as signs of toxicity (Seralini et al., 2007).

All in all, this product has a substantial range of risks and there is a high level of uncertainty concerning its safety for feed and food.

Allergenicity

There are several proteins in maize that can cause allergic reactions. The newly introduced gene construct might, for example, enhance an immune response to endogenous plant protein(s).Targeted studies on potential impact on the immune system are necessary to exclude risks for animals, farmers and consumers as it is known that some Bt proteins react with the immune system.

Potential allergenicity was assessed by applying a pepsin digestion assay. The result was that the Cry protein was thought to degrade quickly in the gastrointestinal tract. However, new evidence published by Walsh et al. (2011) shows that the protein Cry1Ab can be found in the colon of pigs with an 80% success rate. It appears that the Cry proteins can have a much higher stability in monogastric species than predicted by current in vitro digestion experiments. These findings require further assessment by EFSA, such as digestibility tests in vivo.

Environmental Risk Assessment

a) Bt toxins

As far as the risk assessment of the Bt toxins, their mode of action and their potential synergies are concerned, there are already many details that are also relevant for environmental risk assessment.

EFSA did not request a systematic overview of the potential impact of these toxins on various non-target organisms. Some studies on non- target organisms have been cited, but a more systematic screening of relevant organisms, including wild life species, is necessary to assess potential impacts on non-target organisms. It should also should not be left just to the applicant to choose the most relevant organisms related to the ecosystems in various geo-climatic regions.

Synergistic effects can become highly problematic for non-target organisms. Interaction of the toxins with each other or with other compounds can cause higher toxicity and lower selectivity (Then, 2010). These effects may impact the ecosystems on various levels. For example, it has been shown that slugs incorporate the Cry3Bb1 toxins. It is also known that co-stressors such as cadmium and nematodes can cause toxicity of Cry toxins in slugs (Kramarz etl al., 2007, Kramarz et al., 2009). Nevertheless, this issue was not included in risk assessment. In general, there should be systematic screening of synergistic or accumulated effects on a sufficiently broad range of organisms. This should also include the cultivation of other genetically engineered crops.

b) Long term and large-scale effects

The cultivation of these plants will lead to a long term and large-scale exposure of various organisms and therefore requires suitable studies to examine long chronic effects. In the case of MON88107 most studies were only performed for one year.

Detailed empirical investigations of the organisms in the receiving environments must be conducted and include several tiers of the food web. Bt toxin can accumulate in the food web, reaching higher levels of content than in the genetically engineered plants. But even the risks for most relevant non-target organisms (*Coleoptera*) were mostly assessed by modelling and not by empirical investigations. The tiered approach as applied in risk assessment is too narrow to really exclude risks for ecosystems. For example, risks for wildlife species were not included in risk assessment. The impact on rodents, birds and other animal species should be assessed carefully. Large-scale cultivation will bring many wildlife species into contact with these plants.

c) Risks for non-target insects not adequately assessed

Further, most studies were not performed on MON88107 but on other genetically engineered plants that also produce Cry3Bb1. EFSA considered these tests as being comparable because of the nearly identical structures of the insecticidal proteins. However, as Saeglitz et al (2006) show, Bt toxins with identical structures but derived from differing sources can vary extensively in their toxicity. Therefore, major uncertainties remain about whether data derived from traits such as MON863 or MON853 can really be used in the risk assessment of MON88017.

Since many studies that were presented in Monsanto's original application stem from other maize events producing Cry3Bb1, e.g. MON863, Monsanto was asked by Member States and EFSA to submit additional data on the effects on non-target organisms (as reported by COGEM, 2011). Following this request, the company delivered one study on honeybees and one study on the effects of MON88017 on the Colorado potato beetle (*Leptinotarsa decemlineata*), a pest insect. However, in reference to the honeybee study, it was remarked that Monsanto failed to prove that active Cry3Bb1 protein is present in the pollen (COGEM, 2011). Therefore, no conclusions can be drawn from the honeybee study regarding the effect of MON88017 pollen on honeybees.
In the second study with Colorado potato beetles, Monsanto tried to show equivalence of the two variants of Cry3Bb1 as produced in MON88017 and MON863 by providing data on the LC50 values of the E. coli produced Cry3Bb1 proteins from both MON88017 and MON863. According to the study, there is a high degree of overlap between these values, and it can be concluded that the two variants of Cry3Bb1 are functionally equivalent. However, a high degree of overlap between the LC50 values of these E. coli produced proteins for a target insect is not sufficient proof of corresponding effects on non-target insects (COGEM, 2011). Further, Monsanto presented a literature review to show that no negative effects on non-target insects are expected. This review is flawed because in many cases other genetically engineered maize events producing Cry3Bb1 (like MON863) are included in the assessment. If all data on these other maize lines is excluded, there is an evident data gap for the functional group of ground beetles (*Coleoptera: Carabidae*) (COGEM, 2011).

As regards the functional group of *Chrysomelidae*, Monsanto has presented a model to show that no harm is expected from maize MON88017. However, a very recent study investigating the sensitivity

of the cereal leaf beetle, *Oulema melanopus* (*Coleoptera: Chrysomelidae*), to MON88017 shows that larval survival was reduced when larvae were fed with this maize line in comparison to larvae fed with conventional varieties (Meissle et al., 2012).

EFSA (2011) has, for methodical reasons, dismissed the Schmidt et al (2009) study on Cry3Bb1, which shows the impact on non-target organisms. But a recent study by (Hilbeck et al., 2012) confirmed the findings of the Schmidt et al., (2009) study. Further, Waltz (2009) has reported further adverse findings in the larvae of ladybirds. These findings are being held back and have not been published by industry.

In conclusion, much more investigation on non-target organisms will be necessary before any conclusion can be drawn on the safety of MON88017.

d) Plants expressing Cry3Bb1 might help a new "super root worm" population to spread

The emergence of restistance in rootworm populations is identified as a potential risk in EFSA's opinion on MON88017:

> *"The possible resistance evolution to the Cry3Bb1 protein in coleopteran target pests is identified by the EFSA GMO Panel as a concern associated with the cultivation of maize MON 88017, as resistance evolution may lead to altered pest control practices that may cause adverse environmental effects."*

The introduction of these plants is likely to foster the spread of rootworm in maize growing areas,as these plants do not produce enough toxin in their roots to kill the pest insects with a >99% likelihood. Instead, around 4% of the pest insects can be expected to survive. Survival rates of 1.5% were recently confirmed in a study by Clark et al. (2012).

Several studies have shown that incomplete control of rootworm is responsible for the emergence of resistant populations in parts of the US corn belt. In its opinion, EFSA only discussed laboratory findings and the Gassmann et al. (2011) study, which found resistant rootworms in one state (Iowa) only. However, in the meantime several publications show that EFSA has underestimated the severity of resistance development which is, in fact, already a considerable problem in the US corn belt. Resistant rootworms were found in other states e.g. Missouri, South Dakota or Nebraska (Gray 2011, EPA 2011)

A new laboratory study even shows fitness advantages for rootworms with resistant alleles. Oswald et al. (2012) found earlier emergence and higher fecundity of resistant rootworms:

> *In fact, resistant lines emerged approximately 2–3 days earlier than control lines when reared on both MON863 and the isoline, indicating that selection for Bt resistance resulted in a general increase in the rate of larval development. In addition, resistant lines reared on Bt maize displayed higher fecundity than those reared on the isoline, which may have significant management implications."*

This study suggests there may be major consequences from the use of the Cry3Bb1 toxin that should be looked into immediately. Risk mitigation management for resistance development as proposed by EFSA relies on a strategy called "high dose/refuge". From the available data, it becomes clear that this concept is bound to fail in the case of transgenic maize lines expressing Bt toxin Cry3Bb1. So the concept should be withdrawn from the EFSA list of proposed risk mitigation

measures.

Further, there will be refuge zones covering around 20% of the maize growing areas where no measures will be taken to diminish the population of rootworms. This is very likely to cause the establishment of rootworm populations especially in those areas where the MON88017 plants are grown. Concerns that recent refuge strategies are bound to fail, even if stacked events are cultivated that express both Cry3Bb1 and Cry34/35Ab1, have been addressed in a letter written by 22 university entomologists and sent to the US EPA (Porter et al., 2012).

Under these conditions, any strategies to extinguish rootworm are bound to fail. After some years, the pest insects will have developed resistances (as expected by EFSA), and the rootworm will have been established within regions that could have been protected more efficiently by other strategies such as crop rotation. In conclusion, the overall strategy behind the introduction of MON88017 does not support sustainable agriculture in the long term and might even contribute to a new "super root worm" population with a higher fitness, causing much greater damage.

e) Herbicide tolerance

Cultivation of these herbicide resistant plants poses risks to biodiversity, plant health, soil fertility and enables the emergence of herbicide resistant weeds (Benbrook, 2009). The massive usage of glyphosate in herbicide resistant crops endangers the health of rural communities, aquatic systems as well as impacting biodiversity and soil fertility. It can cause plant diseases e.g increased infestation with fungal diseases (Johal & Huber, 2009). The negative impact on plant growth and plant health can even be transmitted to other plants cultivated in the same field in the following year (Bott et al., 2011; Bott et al., 2007).

EFSA did not include evidence that the cultivation of glyphosate tolerant plants puts populations of endangered species at risk e.g. protected butterflies. Brower et al (2011) and Pleasants & Oberhauser (2012) have shown that in the US and Mexico, a reduction in milkweed species leads to a dramatic decline in the population of Monarch butterflies. In Europe, there would be similar hazards that would need assessment when it came to large-scale cultivation. This example shows that EFSA risk assessment is deficient in regard to even the most crucial elements in environmental risk assessment.

EFSA (2011) also concludes that some negative impact on environment is to be expected: "These potential adverse environmental effects comprise (1) a reduction in farmland biodiversity, (2) changes in botanical diversity due to weed shifts, with the selection of weed communities mostly composed of tolerant species, and (3) the selection of glyphosate resistant weeds. The potential harmful effects could occur at the level of arable weeds, farmland biodiversity, food webs and the ecological functions they provide."

However, EFSA does not place sufficient emphasis on the effects on soil microbial communities. In their risk assessment of NK603 (EFSA 2009), which is also tolerant to glyphosate, for cultivation EFSA concludes, that "potential adverse environmental effects comprise (...) effects on soil microbial communities", the same conclusion is not drawn for MON88017.

Taking into account a broad range of publications showing effects on soil organisms, this EFSA opinion on MON88017 is not conclusive.

As many are aware, negative experiences with large-scale cultivation of herbicide tolerant crops in

countries such as Argentina and USA show that the cultivation of these crops cannot be regarded as sustainable. The expectation that the negative impact of large-scale cultivation can be reduced by risk mitigation measures is a matter of theoretical expectation rather than one of practical experience. Cultivation of these herbicide resistant plants poses risks to biodiversity, plant health, soil fertility and enables the emergence of herbicide resistant weeds (see also Benbrook, 2009). The massive usage of glyphosate in herbicide resistant crops endangers the health of rural communities, aquatic systems as well as impacting biodiversity and soil fertility (see also PAN AP, 2009).
The risk manager should give a clear signal that agriculture in the EU is giving sufficient weight to sustainability in agricultural production and, therefore, the cultivation of herbicide-tolerant crops such as MON88017 should not be regarded as an option.

Monitoring

The protocols used for conducting the measurements of the Bt toxins have not been fully published or evaluated by independent laboratories. As a result, independent institutions can hardly monitor the actual content of Bt concentration in the plants during cultivation or in food and feed products. No plan for surveillance as required by European regulation was made available that would allow identification of particular health or environmental impacts that might be related to the use of these genetically engineered plants in food and feed.

Monitoring of health and environmental effects has to include the risks associated with spraying glyphosate formulations and their residues in the plants. A case specific monitoring should at least be requested concerning risks for soil organism and non-target organisms such as *Coleoptera* and *Lepidoptera* species.

The usage of existing networks that are not specifically designed to monitor the impact of genetically engineered plants and the introduction of questionnaires to be filled in by farmers are not sufficient to fulfil requirements of general surveillance under practical conditions as foreseen by EU regulations.

Conclusion and recommendations

It cannot be concluded from the data for the risk assessment of MON88017 maize that this maize line is safe. There are data gaps in the environmental risk assessment and because of incomplete data, no evidence that MON88017 is safe for non-target insects. Newly published research shows that this maize has negative effects on certain leaf beetles. This recent study was not included in the EFSA opinion on maize MON88017.

Additionally, monitoring measures as proposed by EFSA are not sufficient to fulfil requirements of general surveillance under practical conditions as foreseen by EU regulations. Therefore, the risk assessment as performed by EFSA has to be rejected.

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