



TBT comment on EFSA Scientific Opinion on an application from Pioneer Hi-Bred International and Dow AgroSciences LLC (EFSA-GMO-NL-2005-23) for placing on the market of genetically modified maize 59122 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/2003

Introduction

Maize 59122 expresses the Cry34Ab1 and Cry35Ab1 proteins from *Bacillus thuringiensis*, which confer resistance to corn rootworm (*Diabrotica virgifera*). It also expresses pat gene, which makes Maize 59122 tolerant to the herbicide glufosinate. According to the applicants, Maize 59122 will not be marketed as a glufosinate resistant plant in the EU. The two toxins produced by Maize 59122 have certain synergies, but only in combination do they show sufficient toxicity to have a lethal effect on rootworms. The exact mode of action is not known in all its relevant details.

Molecular characterisation

Open reading frames were identified that can give rise to unintended gene products in the plants. The DNA construct is flanked by DNA that is known to be functional and involved in the plant metabolism. Given these findings, the impact of the additional DNA constructs on the regulation and activity of the plant genome should have been investigated in detail.

Comparative analysis (for compositional analysis and agronomic traits and GM phenotype)

Many significant differences in compositional analysis were observed in comparison with the plant's conventional counterparts. In the assessment of these findings, reference was made to historical data unrelated to the actual field trials such as the ILSI database (EFSA, 2007). Since it is not sufficiently clear under which specific conditions these additional historical data were generated, this kind of comparison inevitably contains major uncertainties.

Several statistically significant findings in agronomic parameters came to light in the 2004 European field trials (germination, plant height, ear height, final population). According to EFSA, the differences were not consistent in locations and over the years. The reason for this might be that these differences only emerge under particular environmental conditions. The differences should therefore trigger more detailed analyses.

There are no standardised protocols for these Bt toxins in order to achieve reliable results which can be reproduced by other laboratories. (see also Szekacs et al., 2011). The content of the additional proteins Cry34Ab1 produced in the plant is highly variable. This may indicate genetic

instability and result in unexpected reactions to specific environmental conditions. 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 on the Bt content in the plants (Then& Lorch, 2008). 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.

In the light of these uncertainties, further studies under controlled environmental conditions are necessary to gain a better understanding of the genome x environment interactions and the changes in composition and agronomic performances. Further, the maize has only been grown in Spain, Bulgaria and Hungary and not in other regions of the EU, thus further data representing the true bioclimatic diversity within the EU are necessary.

It must be noted that the compositional assessment and expression data rely entirely on company data. So far, no independent data on composition or agronomic parameters regarding Maize 59122 have been published.

Toxicology

The applicants performed two toxicological 90 day studies. In the first (Malley et al., 2007), contrary to OECD guidelines, only one dose level was used for the whole 90 day rat study. The study showed several differences in haematology and organ weights.

„... male rats receiving the maize 59122 diet showed statistically significant decreases in absolute reticulocyte count and red cell distribution width as well as increases in mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Females showed an increase in platelet count.“

„Organ weight determinations revealed a statistically significant increase in uterus weight in females receiving the maize 59122 diet.“ (EFSA 2007)

A more recent study (He et al., 2008), also found several haematological effects:

„Statistical differences ($p < 0.05$) were observed in certain hematology and serum chemistry response variables between rats consuming diets formulated with 59122 or 091 Control flour compared to AIN93G diet.“

The differences were attributed to the diet formula.

None of the feeding studies are based on acceptably scientific standards. For example, other genetically engineered plants in the diet (such as genetically engineered soy) are not excluded, thus relevant effects might be masked by these constituents in the diet used in the trials. Further, it should be a matter of concern that both studies were performed by the company.

Detailed in vitro studies should have been performed on the possible toxicity of the Cry toxins in vertebrates. As some publications show (Soberon et al., 2009, Mesnage et al., 2012), there are mechanisms and findings in regard to potential toxicity in mammals. Since the imported maize will at the very least contain residues from spraying, possible interactivity with the Bt toxin should have been considered as well.

No long term feeding studies, including reproductive parameters were performed and the potential impact on wildlife was not considered at all. So far, no independent data regarding the food and feed safety of Maize 59122 have been published.

Allergenicity

No empirical investigations were performed concerning allergies or other impacts on the immune system. The level of most relevant maize allergens was not determined. Adjuvant effects and impact on the immune system were not considered, despite it being known that bacterial proteins such as Bt can affect the immune system.

Nutritional assessment

According to EFSA, the studies as presented by the applicants indicate that feed produced from Maize 59122 is as nutritious and wholesome as other maize. However, all five studies that were assessed by EFSA were conducted by the applicants themselves.

In general, no conclusions on the safety of the genetically engineered plants can be drawn from nutritional studies.

Environmental risk assessment

The mode of action of Bt toxins is not fully understood and 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.

It is known that the Bt toxins (or their combination) present in Maize 59122 are indeed less specific than supposed. As EFSA (2013) states:

„The apparent activity of Cry34Ab1/Cry35Ab1 at high concentrations against the lepidopteran species (e.g., Ostrinia nubilalis and Sitotroga cerealella) was not expected based on the known spectrum of activity (Coleoptera only) of these binary proteins. The EFSA GMO Panel considers that there are indications of a potential hazard to Lepidoptera owing to cross-order activity at high Cry34Ab1/Cry35Ab1 protein concentrations.“

In this case, the specific mode of action and potential for synergistic toxicity is not known. Thus no conclusion can be reached upon the role of specific receptors in the gut or other factors that might impact toxicity. The most effective mixture of toxins is a further unknown factor. Any change in the proportions of the single toxins during the vegetation period might influence their actual toxicity. This was not taken into account in the risk assessment.

As Pardo Lopez et al. (2009) and Pigott et al. (2008) show, synthetically derived and modified Bt toxins can show much higher toxicity than native proteins. Even small changes in the structure of the proteins can cause huge changes in toxicity. 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).

Synergistic effects can become highly problematic for non-target organisms. Interactivity between the toxins or in combination with environmental toxins, bacteria, plant enzymes or pesticides can cause unexpected higher toxicity and lower selectivity (Then, 2010). None of these potential synergies with other stressors was investigated during risk assessment – which is surprising since it

is known that the toxicity of Cry34Ab1 and Cry35Abe is based on synergistic effects.

It also should be considered that the plants might not be marketed to be sprayed with the complementary herbicide (glufosinate). However, individual farmers might nevertheless apply the herbicide. Thus interactivity between the herbicide and the Bt toxins is definitely relevant for environmental risk assessment.

To assess the possible impact of maize 59122 on non-target organisms, tiered laboratory studies were conducted by the applicants. However, the studies presented by the applicants are of very poor quality. This was also remarked upon by several Member States during the consultation period. For example, the German Competent Authority criticises following points (EFSA, 2013b):

„1.) Mainly microbial-derived toxin tests and not whole-plant tests were carried out, 2.) only a single ratio of the binary toxins was tested, 3.) the representativeness of the chosen experimental toxin ratios or concentrations remained unclear, 4.) effects were sometimes not statistically analysed, 5.) only short-term studies were conducted, 6.) often no sublethal effects were tested, 7.) not all relevant developmental stages of the non-target species were taken into account, 8.) no tri-trophic experiments were done, 9.) the relevance of species not native to Europe appears questionable, 10.) the amount of toxin uptake by non-targets was never recorded, 11.) no dose-effect relationship were established, 12.) whether the toxin was degraded during experimental period and/or the proof of bioactivity of the toxin was not recorded, 13.) only a single toxin dose was applied.“

EFSA (2013) also recognized the poor quality of the applicants' laboratory studies:

„The EFSA GMO Panel notes that some of the lower-tier studies conducted by the applicant do not adhere to the general principles of good laboratory study design [...], and therefore cannot be used to support the risk assessment.

However, instead of asking the applicants for more appropriate studies, EFSA sides with the applicants' opinion that cultivation of line 59122 poses a negligible risk to human health and the environment. In Testbiotech's opinion, this cannot be deduced from the applicants' studies because of a paucity of valid data. A comprehensive assessment would have been of huge importance because of the toxicological properties of Cry34Ab1/Cry35Ab1 proteins which differ from expectations. It was also stated by Member States that independent data regarding the possible environmental effects of Cry34/35Ab1 proteins are almost completely missing, as all studies are performed by or commissioned by the applicants.

For example, in the case of two relevant non-target organisms, ladybirds (*Coccinellidae*) and leaf beetles (*Chrysomelidae*), risk assessment shows severe deficiencies:

- Although only two studies on leaf beetles (one from Germany, one from Hungary, in which a member of the GMO Panel, Josef Kiss was involved) were considered, this was deemed sufficient to conclude on the occurrence of leaf beetles in maize fields all over Europe. Due to this premature assumption, most species were not tested at all.

- As for ladybirds, the authority in the Netherlands concluded that risks could not be excluded and that a case specific monitoring should be performed. In response EFSA simply asked the applicant, Dow AgroSciences, to perform further studies. Naturally the company was aware that a (costly) case specific monitoring might be requested and therefore had significant vested interest in the outcome of these investigations. In this case EFSA should have definitely asked for independent investigations. Instead, it was happy with the company's own studies and did not request further investigations or a case specific monitoring.

Even according to EFSA, there are no reliable data on the potential accumulation of the toxin in the soil. This deficiency cannot be replaced by monitoring after commercial cultivation but must be investigated and assessed before authorisation.

Resistance in pest insects

During the last few years, rootworm resistance against genetically engineered maize lines producing Cry3Bb1 has become wide spread in the USA. Literature shows that one of the main reasons for growing resistance is the fact that current crops do not follow the high dosage requirement in regard to western corn rootworm (WCR). According to literature, this is also true for Cry34/35Ab1 (see for example Tabashnik and Gould, 2012).

Accordingly, resistance development in rootworm feeding on Maize 59122 was as fast as it was in Bt maize expressing Cry3Bb1 (Lefko et al., 2008). This aspect is also acknowledged by EFSA (EFSA2013):

„Based on the available data, the EFSA GMO Panel concludes that WCR has the ability to evolve resistance to the Cry34Ab1/Cry35Ab1 proteins, especially if maize 59122 is used repeatedly and exclusively, and the WCR infestation levels are high.“

Further, according to EFSA, it looks like no fitness costs are related to the acquired resistance against the Cry34Ab1/Cry35Ab1 proteins – this is another indication that resistance in pest insects can become a severe problem. As Oswald et al (2012) indicate, the selection of pest insects with even higher fitness, cannot be excluded.

In response EFSA has proposed (amongst others things) a requirement for systematic crop rotation as risk mitigation where the Bt plants are grown. From the perspective of the risk manager this is an interesting option: Since so far no rootworm are known in the EU that are resistant to crop rotation, the Commission could request adequate crop rotation in all regions where rootworm might become a problem in the maize fields. This would in effect render the cultivation of any Bt plants producing insecticides against the rootworm completely meaningless.

Risk mitigation

We think the proposals made by EFSA are interesting, but will not work in practice. In the long run, this maize is not sufficiently toxic (high dosage) to effectively control the rootworm. Commercial cultivation of Maize 59122 might even exacerbate the problem by allowing the emergence of

resistances in the pest insects and by enabling the selection of pest populations with an even higher fitness as seen in the case of MON88017 (Oswald et al., 2012).

Monitoring

The monitoring plan must be rejected since no case specific monitoring has been requested for most relevant non-target organisms or for possible health effects.

Conclusions and recommendations

Maize 59122 cannot be considered safe for human and animal health or the environment. There are indications that its cultivation will lead to rise in Bt resistant insects. EFSA risk assessment must be rejected because it does not fulfill necessary scientific standards and therefore carries a level of uncertainty that is too high.

Others

The application for Maize 59122 was filed by Pioneer in 2007. Member States also had an option to comment on the application in 2007. There is no option for Member States to comment on more recent scientific studies that might change their stance on the product. There seems to be a systematic flaw in the approval process that should be revised.

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