

SCIENTIFIC OPINION

Scientific Opinion on a request from the European Commission related to the safeguard clause notified by Greece on genetically modified maize MON 810 according to Article 23 of Directive 2001/18/EC¹

EFSA Panel on Genetically Modified Organisms (GMO)^{2, 3}

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ABSTRACT

Following a request of the European Commission, the European Food Safety Authority's Panel on Genetically Modified Organisms (EFSA GMO Panel) evaluated the documentation submitted by Greece in support of its request for the prohibition of the placing on the market of the genetically modified maize MON 810 according to Article 23 of Directive 2001/18/EC. The EFSA GMO Panel notes that the majority of the publications referred to by Greece were already addressed by the EFSA GMO Panel in previous scientific outputs on maize MON 810 or other related insect-resistant genetically modified maize transformation events. In the remaining evidence provided by Greece, the EFSA GMO Panel could not identify any new data subject to scientific scrutiny or scientific information that would invalidate its previous risk assessments of maize MON 810. With regard to issues related to management and monitoring of maize MON 810, the EFSA GMO Panel refers to its recent recommendations for management and monitoring measures of maize MON 810. In conclusion, the EFSA GMO Panel considers that, based on the documentation submitted by Greece, there is no specific scientific evidence, in terms of risk to human and animal health or the environment, that would support the notification of a safeguard clause under Article 23 of Directive 2001/18/EC and that would invalidate the GMO Panel's previous risk assessments of maize MON 810.

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KEY WORDS

GMO, maize (*Zea mays*), MON 810, Greece, safeguard clause, environment, Directive 2001/18/EC

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SUMMARY

In November 2011, Greece provided to the European Commission a scientific argumentation in support of its request for the prohibition of the placing on the market of the genetically modified (GM) maize MON 810 according to Article 23 of Directive 2001/18/EC.

On 22 May 2012, the European Commission requested the European Food Safety Authority's Panel on Genetically Modified Organisms (EFSA GMO Panel) to assess the supporting documentation submitted by Greece.

The EFSA GMO Panel has scrutinized the documentation provided by Greece in support of its safeguard clause on GM maize MON 810. The EFSA GMO Panel considered the relevance of concerns raised by Greece in the light of the most recent and relevant scientific data published in the scientific literature. This also includes the conclusions of the EFSA GMO Panel assessments of the PMEM results on maize MON 810 during the 2009 and 2010 growing seasons.

During its evaluation of the supporting documentation, the EFSA GMO Panel has noted that the majority of the publications referred to by Greece were already addressed in its past scientific outputs on maize MON 810 or other related insect-resistant GM maize transformation events and therefore refers to them.

In the four remaining evidence related to the environmental risk assessment provided by Greece, the EFSA GMO Panel could not identify any new data subject to scientific scrutiny or scientific information that would invalidate its previous risk assessments of maize MON 810. Against this background and in order to facilitate a thorough assessment of potential risks, the EFSA GMO Panel strongly recommends Member States who invoke safeguard clauses under Directive 2001/18/EC or emergency measures under Regulation (EC) No 1829/2003 to supply scientific data of a quality which can be subjected to detailed scientific scrutiny.

In addition, Greece did not supply scientific evidence to show that the conclusions of the previous risk assessments of the EFSA GMO Panel of maize MON 810 were not applicable to Greece.

With regard to issues related to management and monitoring of maize MON 810, the EFSA GMO Panel refers to its recent recommendations for management and monitoring measures of maize MON 810.

In conclusion, the EFSA GMO Panel finds that the scientific evidence currently available does not sustain the arguments provided by Greece and that cultivation of maize MON 810 in Greece is unlikely to have an adverse effect on human and animal health and the environment.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

The placing on the market for cultivation of maize MON 810 in the European Union is authorised since 1998 through Commission Decision 98/294/EC of 22 April 1998 (EC, 1998) and the consent granted on 3 August 1998 by the Competent Authority of France.

On 29 March 2006, Greece invoked Article 23 of Directive 2001/18/EC (EC, 2001) and Article 18 of Directive 2002/53/EC (EC, 2002) to provisionally prohibit the cultivation of maize MON 810 on its territory. Later, on 13 September 2007, Greece notified to the European Commission a ministerial decision concerning the extension of validity and amendment of the previous safeguard measure invoked in 2006 to provisionally prohibit the cultivation of maize MON 810 on its territory. In both cases, and upon request of the European Commission, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to assess the documentation provided by Greece in support of its measures requiring the prohibition of maize MON 810 cultivation. Having considered the overall information packages submitted by Greece as well as a broad range of relevant scientific literature, the EFSA GMO Panel concluded, in 2006 and 2008, that no specific scientific evidence had been provided by Greece that would justify the invocation of a safeguard clause (EFSA, 2006, 2008c).

On 15 June 2009, following the request by the applicant for the continued marketing of maize MON 810 for import, processing for food & feed uses and cultivation, the EFSA GMO Panel adopted a Scientific Opinion⁴ on the renewal of maize MON 810 under Regulation (EC) No 1829/2003 (EC, 2003, EFSA, 2009). The EFSA GMO Panel concluded that *‘maize MON 810 is as safe as its conventional counterpart with respect to potential effects on human and animal health’* and that *“maize MON 810 is unlikely to have any adverse effect on the environment in the context of its intended uses, especially if appropriate management measures are put in place in order to mitigate possible exposure of non-target (NT) Lepidoptera”*. The EFSA GMO Panel recommended that, *“especially in areas of abundance of non-target Lepidoptera populations, the adoption of the cultivation of maize MON 810 be accompanied by management measures in order to mitigate the possible exposure of these species to maize MON 810 pollen”*. Further, the EFSA GMO Panel advised that *“resistance management strategies continue to be employed and that the evolution of resistance in lepidopteran target pests continues to be monitored in order to detect potential changes in resistance levels in pest populations”*.

On 30 November 2011, the EFSA GMO Panel adopted a Statement supplementing its environmental risk assessment (ERA) and risk management recommendations on the GM insect-resistant maize transformation event Bt11 for cultivation (EFSA, 2011e). In its Statement on maize Bt11, the EFSA GMO Panel made recommendations for management measures and concluded that, *“subject to appropriate management measures, maize Bt11 cultivation is unlikely to raise additional safety concerns for the environment compared to conventional maize”*. In light of the similarities between both Cry1Ab-expressing transformation events maize Bt11 and MON 810 (e.g., identity of amino acid sequence of the core protein, similar biological activity against sensitive Lepidoptera, similar Cry1Ab protein expression level in pollen), the EFSA GMO Panel considered that the conclusions on the risk to non-target Lepidoptera from maize Bt11 apply equally to maize MON 810.

Furthermore, the EFSA GMO Panel was requested by the European Commission to assess the post-market environmental monitoring (PMEM) reports submitted by the applicant on the cultivation of maize MON 810 in 2009 and 2010. The EFSA GMO Panel therefore adopted a Scientific Opinion on the 2009 and 2010 PMEM reports on maize MON 810, on 7 September 2011 (EFSA, 2011c) and 7 March 2012 (EFSA, 2012a), respectively. The EFSA GMO Panel noted shortcomings in the methodology for case-specific monitoring (CSM) and general surveillance (GS), and therefore made recommendations for improvement of the PMEM of maize MON 810. However, the EFSA GMO Panel stressed that the shortcomings they identified in the methodology did not have any implications

⁴ This Scientific Opinion was published on the [EFSA webpage](#) on 30 June 2009.

for conclusions on safety derived from the data submitted by the applicant in its PMEM reports. Hence, the EFSA GMO Panel did not identify adverse effects on the environment, human and animal health due to maize MON 810 cultivation during the 2009 and 2010 growing seasons.

In November 2011, Greece notified to the European Commission its scientific argumentation in support of the prohibition of the use and sale of maize MON 810 as seeds for sowing, according to Article 23 of Directive 2001/18/EC. On 22 May 2012, the European Commission asked the EFSA GMO Panel to assess the scientific information submitted by Greece and to indicate whether it might lead the EFSA GMO Panel to reconsider recent outputs related to maize MON 810.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested in accordance with Article 29 of Regulation (EC) No 178/2002 to assess the scientific information submitted by Greece in its notification concerning GM maize MON 810 and to identify whether these new scientific elements might lead the EFSA GMO Panel to reconsider its related favourable opinion on GM maize MON 810 from 15 June 2009 or its statement on Bt11 (applying equally to maize MON 810) from 30 November 2011.

ASSESSMENT

1. INTRODUCTION

The EFSA GMO Panel scrutinized the documentation provided by Greece in support of its safeguard clause on maize MON 810. The EFSA GMO Panel considered the concerns expressed by Greece related to the safety assessment of food and feed derived from maize MON 810 (see section 2), the environmental risk assessment (ERA) of maize MON 810 and mainly the impacts on non-target organisms (NTOs) (see section 3) and the post-market environmental monitoring (PMEM) of maize MON 810 (see section 4).

Concerns also raised by Greece related to the co-existence of maize cropping systems and other socio-economic issues fall outside the remit of the EFSA GMO Panel.

The EFSA GMO Panel looked for evidence for GMO-specific risks taking into consideration the EFSA Guidance Documents for the risk assessment of food and feed from GM plants (EFSA, 2011f) and for the ERA of GM plants (EFSA, 2010), as well as any recent risk assessments on other Cry1Ab-expressing maize transformation events than maize MON 810 (EFSA, 2009; 2011c,e; 2012a,b).

According to the terms of reference set by the European Commission, the EFSA GMO Panel assessed whether the submitted documentation comprises new scientific information that would invalidate the conclusions of its Scientific Opinion on the continued marketing of maize MON 810 for import, processing for food & feed uses and cultivation (EFSA, 2009) and the conclusions of its recent Statement on maize Bt11 that equally apply to maize MON 810 (EFSA, 2011e).

2. CONCERNS RELATED TO THE SAFETY ASSESSMENT OF FOOD AND FEED DERIVED FROM MAIZE MON 810

2.1. Allergenicity assessment on the whole plant

Claim made by Greece

In its supporting documentation, Greece claims that “*during the examination of the application file for the approval of the MON 810 genetic modification for foodstuffs and fodder, EFSA states in its scientific opinion that no studies of the potential allergenicity using the entire plant have been carried out on the justification that there is equivalence between the genetically modified plant and its conventional counterpart* ».

Furthermore, Greece mentions that « *the increasing concern of the scientific community regarding the potential impact of GMOs on human health has led to the conducting of new studies on experimental animals. Indicatively, Finamore et al. (2008) noted changes in the intestinal and peripheral immune response of weaning and adult mice* ».

Assessment by the EFSA GMO Panel

Maize is not considered a common allergenic food. In its 2009 Scientific Opinion on the renewal of maize MON 810 (EFSA, 2009), the EFSA GMO Panel stated that, given that no biologically relevant agronomic and compositional and phenotypic changes were identified in maize MON 810 and in stacked GM maize events containing MON 810, except for the newly introduced trait (EFSA, 2005a,b,c,d,e), no increased allergenicity is anticipated for maize MON 810. The EFSA GMO Panel also referred to studies by Nakajima et al. (2007) and Batista et al. (2005) on food allergic consumers that came to the same conclusion as the applicant that maize MON 810 is as safe as conventional maize in terms of allergenic potential.

In its supporting documentation, Greece cited the publication of a study on the intestinal immune system in rats fed maize MON 810 (Finamore et al., 2008) to dispute the EFSA Scientific Opinion on

the renewal of the maize MON 810 authorisation (EFSA, 2009) regarding the allergenicity assessment on the whole plant.

Finamore et al. (2008) fed a standard pellet diet containing about 50% maize flour for either 30 or 90 days to weaning (51 days old) mice, or for 90 days (111 days) to old male Balb/c mice in order to study the intestinal and peripheral immune response (allergenicity was not studied *per se*). The maize components were either MON 810 or its conventional counterpart (control: PR33P67 and PR33P66); the GM maize being fed for either 30 or 90 days. Both maize flours were analysed for contamination with eight different mycotoxins. The level of six of them were below the maximum allowable concentration, while fumonisin B1 was above the maximum allowable concentration in the conventional counterpart (2450 µg/kg when 2000 µg/kg is allowed), and deoxynivalenol (DON) was above allowed levels in the maize MON 810 (1300 µg/kg when 700 µg/kg is allowed).

No difference was found in the food consumption of weaning and elderly mice fed maize MON 810 or control maize, and there were no differences in the mean body weights between mice that received maize MON 810 and mice that received the control maize. Spleen lymphocytes were isolated from weaning and elderly mice fed the various maize diets and their proliferative response studied. No difference in this parameter was found in mice that received these two types of diet. However, in studies on the immunophenotype of intestinal intraepithelial (IEL), spleen, and blood lymphocytes of weaning mice fed maize MON 810 and control diet, several changes were induced by the maize MON 810 diets in percentage of lymphocyte populations (T(CD3⁺), B(CD19⁺), CD4⁺, CD8⁺, TCRγδ⁺, TCRαβ⁺) at these sites after 30 days of feeding. After ninety days of feeding only the percentage of B(CD19⁺) cells were increased and only in IEL and blood. There were also some differences in the percentage of these types of blood cells in the old mice after 90 days of maize MON 810 consumption, but the number of changes was less and not always in the same direction as in the weaning mice. In addition to studying the various sub-populations of lymphocytes in IELs, spleen and blood, the investigators measured the serum level of eleven different cytokines in the weaning and old mice. Whereas only the level of MIP-1β was increased in old mice receiving the MON 810 diet, weaning mice supplied this diet for 30 days showed increased levels of IL-6, IL-13, IL-12p70 and MIP-1β. When weaning mice were fed the maize MON 810 diet for 90 days only levels of MIP-1β was increased.

In the discussion of the observed alterations in immunological parameters the investigators noted that in addition to the maize component, the diets differed in the amount of a few mycotoxins that are known to have immunomodulatory effects but at higher concentrations than those found in the maize material used in the present study. Because the investigators noted no influence on immunophenotype of intestinal intraepithelial, spleen, and blood lymphocytes of mice that were fed standard pellet fed as compared to the mice fed the conventional counterpart, they suggested that the observed alterations are likely due to the Cry1Ab protein expressed in maize MON 810. Unfortunately, the investigators did not present data on the comparison to animals receiving standard mice feed, and gave no information on the natural variability in the studied parameters in weaning and old mice. However, they underlined that the significance of these data remains to be clarified to establish whether the observed alterations reflect significant immune dysfunctions. In order to relate these immunological observations to allergenicity, Finamore et al. (2008) mention that studies were already ongoing in late 2008 evaluating the amount of different antibodies in serum of the studied mice. No report on this issue has yet been published.

The publication by Finamore et al. (2008) has been discussed, in the context of the Austrian safeguard clause on maize MON 810 and maize T25 (EFSA, 2008a), in a bilateral technical meeting amongst Austrian scientists and representatives, several experts of the EFSA GMO Panel, the EFSA GMO unit and representatives of the European Commission on 2 December 2008⁵. At that time, GMO Panel members questioned how the study approaches should be considered in the frame of routine food and feed safety testing of whole GM foods and feeds: this type of immunological study is generally not performed on whole foods and feeds.

⁵ available at <http://www.efsa.europa.eu/en/gmomsmeeeting/docs/gmo081202at-m.pdf>

As indicated by the investigators themselves, it was not possible to conclude on the study of with regard to relevance for food and feed safety (Finamore et al., 2008). Against the background of the potential confounding effects of the mycotoxins in the animal diet, missing information on natural variability in studied parameters in weaning and old mice given various types of diet, and the biological relevance of noted differences in immunological parameters, the EFSA GMO Panel awaits further scientific data. Interestingly, two recent studies report the effects of feeding maize MON 810 to pigs: Bt-maize is well tolerated by the porcine intestinal microbiota in pigs fed maize MON 810 for 31 days (Buzoianu et al., 2012), Walsh et al. (2012) did not find any indication of Th 2 type allergenic or Th 1 type inflammatory responses to MON 810 fed to pigs for 110 days.

2.2. Broiler chicken feeding study

Claim made by Greece

In its supporting documentation, Greece claims that “*in the relevant comments by Member States⁶, it is noted that experimental testing of impact on chickens was carried out, with their fodder having undergone pelleting, a process that may have led to inactivation of the protein produced by the Cry1Ab gene. Thus, it is deemed that the results cannot be adequate*”.

Assessment by the EFSA GMO Panel

The safety assessment of newly expressed proteins in GM plants is done by a comprehensive set of investigations as described in the Guidance document of the EFSA GMO Panel for risk assessment of food and feed from GM plants (EFSA, 2011f). Usually, feeding studies on experimental animals are included among those investigations, using fairly high doses of the purified protein. These studies investigate toxicological endpoints. As previously reported, studies on the Cry1Ab protein did not raise concern (EFSA, 2009). In addition, confirmation of the absence of adverse effects of dietary exposure to maize MON 810, which express the Cry1Ab protein, has been obtained in 90-day feeding studies in rats supplied diets containing this transformation event or maize MON 810 stacked with other GM events (EFSA, 2005a, 2005b, 2005c, 2005d, 2005e, 2009).

In animal feeding studies aimed at investigating the nutritional wholesomeness of a feed, the animals for food production are usually supplied the feed in a form traditionally used in animal feeding. Animal feeds are prepared by various processing steps. It is well known that different types of processing, e.g. ensiling or heat treatment, may degrade proteins (e.g., Alexander et al., 2007; Lutz et al., 2006).

In the broiler chicken feeding study provided in the application for the renewal of maize MON 810 for import, processing for food & feed uses and cultivation, it is stated that after the starter diets had been pelleted and crumbled and the grower/finisher diets had been pelleted, subsamples were sent to the applicant for identification of the maize material in the respective feed. The method used for this identification requires that for a positive signal the Cry1Ab protein is not destroyed. The YieldGard (MON 810) test diets were confirmed positive for the presence of the Cry1Ab protein, while diets containing the conventional counterpart or maize non-GM reference varieties were confirmed negative. Thus, the feed analysis confirmed that the broiler chickens received appropriate diets and that the Cry1Ab protein was still present in the maize MON 810-containing diet. As stated in its 2009 Scientific Opinion on the renewal of maize MON 810 (EFSA, 2009), the EFSA GMO Panel concludes that the broiler chicken feeding study revealed no unexpected findings, and that maize MON 810 is as wholesome as conventional maize.

As mentioned in the previous Scientific Opinion on maize MON 810, Rossi et al. (2005) came to similar conclusions in another 42-day feeding performance study on Ross male broiler chickens fed diets containing 55-60% maize MON 810 or non-GM control maize. Further confirmation on this conclusion was obtained from feeding studies with dairy cows and salmon (EFSA, 2009).

⁶ See Annex G to EFSA, 2009

The EFSA GMO Panel confirms that the validity of the broiler chicken feeding study provided in the application for the renewal of authorisation for the continued marketing of maize MON 810 under Regulation (EC) No 1829/2003, to assess the nutritional wholesomeness of maize MON 810, is not compromised by the methodology to produce the chicken feed.

2.3. Publication by Steinke et al. (2010)

Claim made by Greece

In its supporting documentation, Greece claims that *“it appears that the metabolic process of the produced Cry1Ab toxin is not clear. In a recent study by Steinke et al. (2010), the toxin was identified in the milk produced by cows that fed on MON 810 maize, even though milk production and composition did not change”* and *“from all the data presented above, it appears that there remains uncertainty concerning the safety of the MON 810 genetic modification for human health”*.

Assessment by the EFSA GMO Panel

The EFSA GMO Panel notes that the study reported by Steinke et al. (2010) neither investigated the absorption, distribution, metabolism, and excretion (metabolic process), nor whether the the Cry1Ab protein is present in the milk of cows fed diets with maize MON 810. In the referred study, the Cry1Ab protein level was measured by a specific and sensitive ELISA technique in various maize components (silage, whole-crop cobs and grains) and in the diets fed to the dairy cows.

The study of Steinke et al. (2010) reported on the performance of lactating dairy cows in a long-term feeding study with maize MON 810. Two groups, each with eighteen cows, were fed with diets containing 71% of whole-crop silage, kernels and whole-crop cobs from maize MON 810 or its conventional counterpart. There were no relevant differences in chemical composition and estimated net energy content of the diet with maize MON 810 and the diet with the conventional counterpart. The study ran over 25 months and included two consecutive lactations. Each week, dry matter intakes were calculated and diets analyzed for the presence of the Cry1Ab protein. Cows fed maize MON 810-containing diet had a daily and fairly stable Cry1Ab protein intake of 6.0 mg in the first lactation and 6.1 mg in the second lactation. In order to study the long-term effects of the maize feeding, eleven parameters (dry matter intake, milk yield, energy correct milk, milk fat concentration, milk protein concentration, milk lactose concentration, milk urea concentration, somatic cell count, body weight, body condition score and back fat thickness) were measured. The long-term study showed that there were no consistent effects of feeding dairy cows maize MON 810 or its conventional counterpart on milk composition or body conditions. The few statistically significant differences observed in the study were considered unrelated to the dietary treatment. The authors concluded that their long-term study confirms the results of previous short-term studies on the feeding of Bt-maize in dairy cow nutrition (Barrière et al., 2001; Clark and Ipharraguerre, 2001; Folmer et al., 2002).

The EFSA GMO Panel concludes that the publication by Steinke et al. (2010) does not provide new scientific evidence that would invalidate its previous risk assessments of maize MON 810 (EFSA, 2009, 2011e).

2.4. Conclusion

The EFSA GMO Panel has assessed the claims made by Greece regarding the risk assessment of food and feed from maize MON 810, and is of the opinion that the issues regarding allergenicity assessment on the whole plant, the design of the broiler chicken feeding study, and potential presence of the Cry1Ab protein in products of farm animals being fed maize MON 810 have already been considered in previous Scientific Opinions of the EFSA GMO Panel related to maize MON 810 (EFSA, 2008a, 2009). The EFSA GMO Panel is therefore of the opinion that the supporting documentation submitted by Greece does not provide new scientific evidence that would invalidate the Panel's previous risk assessments of maize MON 810.

3. CONCERNS RELATED TO THE ENVIRONMENTAL RISK ASSESSMENT OF MAIZE MON 810

3.1. Introduction

In its supporting documentation, Greece is mainly concerned with the possible adverse impact of maize MON 810 on NTOs.

EFSA notes that, in its 2009 Scientific Opinion on the renewal of the maize MON 810 authorisation (EFSA, 2009), the EFSA GMO Panel has already addressed some of the concerns expressed by Greece related to NTOs, including predators, parasitoids, pollinators, soil and aquatic NT organisms representative of relevant functional groups (see Bakonyi et al., 2006; Naranjo, 2009; Schmidt et al., 2009; Ramirez-Romero et al., 2008 referred to in EFSA, 2009). Furthermore, publications referred to by Greece in support of the current safeguard clause, such as Faria et al. (2007) and Vercesi et al. (2006), has been previously evaluated by the EFSA GMO Panel in 2008 (for further details, see EFSA, 2008a). The paper by Hilbeck et al. (2008) was also reviewed by the EFSA GMO Panel in its 2011 Scientific Opinion on maize MON 88017 (EFSA, 2011d). Finally, in its recent Scientific Opinion on the French emergency measure to ban maize MON 810 (EFSA, 2012b), the EFSA GMO Panel considered additional publications referred to by Greece (i.e., Schmidt et al., 2009; Bøhn et al., 2008; Icoz and Stotzky, 2008).

Therefore, this Scientific Opinion is mainly based on existing scientific outputs by the EFSA GMO Panel on maize MON 810 and related insect-resistant GM maize transformation events (e.g., maize Bt11). In these, the EFSA GMO Panel reviewed and assessed the majority of the publications referred to by Greece (EFSA, 2008a,b; 2009; 2011a,b,c,d,e; 2012a,b).

In this Scientific Opinion, the EFSA GMO Panel considers the remaining documentation provided by Greece in support to the current safeguard clause on maize MON 810, namely:

- Paper by Székács A., É. Lauber, E. Takács, B. Darvas (2010), entitled ‘Detection of Cry1Ab toxin in the leaves of MON 810 transgenic maize’ in *Analytical and Bioanalytical Chemistry* 396, 2203–2211 (refereed paper in an international peer-reviewed journal);
- Thesis report for the degree of Master of Philosophy by Paejaroen P. (2008) entitled ‘Impact of Cry1Ab toxin from transgenic maize (MON 810) and microbial Bt spray (DIPEL) on the ecology of a non-target parasitoid, *Cotesia marginiventris*’, University of Southampton;
- Abstract of a presentation by Békési L., E. S. Mátray, E. Zajác, L. Harka (2010) entitled ‘Effect of insect-resistant (Bt) GM corn pollen on the development of honeybee (*Apis mellifera*) larvae *in vitro*’, page 7 in the Proceedings of the COLOSS Workshop “Method standardization for larval tests”, 7.-9.6.2010, Graz, Austria, (<http://www.coloss.org/publications/ProceedingsCOLOSSLarvalRearingGraz2010.pdf>);
- Interview⁷ of Prof. Dr. H.H. Kaatz (University of Halle-Wittenberg) carried out by Christof Potthof (Gen-ethischen Netzwerk), in 2009 Testbiotech report entitled ‘Risk reloaded – Risk analysis of genetically engineered plants within the European Union’ (Then & Pothoff, 2009).

3.2. Paper by Szekacs et al. (2010) on the distribution and expression levels of Cry1Ab protein in maize MON 810 leaves

Claim made by Greece

In their 2010 paper, Szekacs et al. demonstrate that the distribution of the Cry1Ab protein in MON maize 810 leaves detected by enzyme-linked immunosorbent assay (ELISA) varies spatially within a

⁷ Interview is available at <http://www.gmwatch.org/component/content/article/11621-gm-crops-and-honey-bee-research>

plant (i.e., lengthwise along a single leaf and between lower and higher leaf levels of a plant) and also from plant to plant. Greece claims that this variability in Cry1Ab protein expression makes the assessment of the effects of maize MON 810 on NTOs difficult.

Assessment by the EFSA GMO Panel

Szekacs et al. (2010) reported some variability in the distribution and Cry1Ab expression levels of the Cry1Ab protein in maize MON 810 leaves within a plant (i.e., lengthwise along a single leaf and between lower and higher leaf levels of a plant) and also from plant to plant. The EFSA GMO Panel considers the variation in protein expression as a natural phenomenon that can be observed in GM and non-GM plants (Zhang et al., 2002; Walschus et al., 2002). Plant tissue and stage of plant development are the main parameters affecting the Cry1Ab contents of maize MON 810 e.g., as indicated by large-scale field experiments already carried out in 2002 and 2003 (Nguyen and Jehle, 2007), but no adverse effects on NTO were observed in these field trials (see Gathmann et al., 2006; Toschki et al., 2007). Later in 2009, the possible adverse effects of maize MON 810 cultivation on NTOs – including variation of Cry1Ab protein expression - were addressed in depth by the EFSA GMO Panel in its Scientific Opinion for the renewal of the placing on the market of maize MON 810 (EFSA, 2009). At that time, the EFSA GMO Panel considered a broad range of lower- and higher-tier data on NTOs (e.g., herbivores, predators, parasitoids, pollinators, soil and aquatic NT organisms) representative of relevant functional groups (for further details, see Section 6.1.4 of EFSA, 2009). The EFSA GMO Panel concluded that there was no evidence to indicate that the placing of maize MON 810 and derived products on the market is likely to cause adverse effects on NTOs in the context of its proposed uses.

In recent scientific outputs (e.g., EFSA, 2011e, 2012b), the EFSA GMO Panel supplemented its assessment of possible effects that maize MON 810 may have on NTOs (e.g., on NT Lepidoptera and coccinellids respectively) and in those GMO Panel outputs answered concerns expressed by Greece. For example, green maize plants are not an important resource of food for NTOs including indigenous Lepidoptera with the exception of a few pest species. Therefore, the main potential risk to NTOs, specifically NT Lepidoptera is expected to be the exposure to potentially harmful amounts of pollen deposited on host-plants in or near maize MON 810 fields (EFSA, 2009).

During its work on the mathematical model for the estimation of exposure of NT Lepidoptera to Bt-maize pollen deposited on host plants in or near Bt-maize fields (EFSA, 2009, 2011e; Perry et al., 2010, 2011), the EFSA GMO Panel accounted for the differences of Cry1Ab protein levels in maize MON 810 leaves and pollen. The Cry1Ab protein level in maize MON 810 pollen is lower than 0.1 µg/g fresh weight (fw), whereas in leaf tissues of maize MON 810 plants, the amount of the Cry protein ranges between 0.3 and 8.6 µg/g fw (EFSA, 2009). In this respect, the EFSA GMO Panel already took a precautionary worst-case view when assessing the effects of GM pollen on NTO Lepidoptera. The predicted outcome of the mathematical model is an average deterministic solution which is unlikely to be greatly affected by variation in expression levels within or between plants, as verified by the stochastic solution obtained by Perry et al. (2010) to address the spatial heterogeneity of pollen (see for example Hofmann, 2007, 2009). The EFSA GMO Panel took this kind of variation into account, as well as the variation due to the fact that there may well be different levels of expression in different genetic lines and hybrids. In its opinion, the EFSA GMO Panel takes variation of expression into account, both in time and space, between plants, different genetic lines, and in leaves and pollen.

Conclusion

The EFSA GMO Panel is of the opinion that this paper by Szekacs et al. (2010) does not impact on the aforementioned mathematical model or on its previous risk assessments of maize MON 810.

3.3. Thesis report by Paejaroen (2008) on the effects of maize MON 810 and Bt insecticidal spray DIPEL on a non-target parasitoid, *Cotesia marginiventris*

Claim made by Greece

In his 2008 thesis report, Paejaroen has demonstrated some direct effects of the Cry1Ab protein on the growth and development of the lepidopteran pest, *Spodoptera littoralis*, feeding on maize MON 810 as well as indirect tritrophic effects (e.g., a decrease in the number of parasitoid cocoons and adults) of the hymenopteran species *Cotesia marginiventris*, which acts parasitically on *S. littoralis*. The author attributed this decreased parasitism and non-emergence of parasitoids to the decreased quality of hosts, which subsequently cannot provide sufficient nutrients for the development of the parasitoid larvae.

Assessment by the EFSA GMO Panel

In general, invertebrate parasitoids appear to be more sensitive than predators to diets containing Cry proteins under laboratory conditions (Lövei et al., 2009), though in most cases neutral results were obtained in tritrophic experiments. The meta-analysis conducted by Naranjo (2009) confirms the higher sensitivity of parasitoids in tritrophic experiments, and Naranjo was able to identify the importance of host quality in determining such results. Parasitoids can be exposed to the Cry1Ab protein through one or more trophic levels (e.g., direct feeding on Bt-plant material or their host organisms feeding on Bt-plant tissue) and therefore host quality has an important effect (e.g., Dutton et al., 2002, 2005; Vojtech et al., 2005; Romeis et al., 2010).

Ramirez-Romero et al. (2007) observed that the exposure to Cry1Ab protein via hosts fed Bt-maize tissue sublethally affected the parasitoid *Cotesia marginiventris*, the same species studied by Paejaroen. In experiments where hosts fed maize MON 810 were compared with those fed control maize, and where the former accumulated low concentrations of Cry1Ab protein, Ramirez-Romero et al. (2007) found that these effects still occurred. Moreover, host size did not differ between treatments. The authors were able to demonstrate the importance of the genetic modification in the plant in causing unintended effects at the third trophic level, since negative results were not observed when pure protein-containing diet was used in the tritrophic experiments. Thus these results suggest an adverse effect on the parasitoid when delivered via the host feeding on plant tissue.

However, in EFSA (2009), the hazard demonstrated by Ramirez-Romero et al. (2007) was placed in context through an exposure assessment that included the meta-analyses of Marvier et al. (2007) and Wolfenbarger et al. (2008); these and other evidence demonstrated that the overall risk was low. The EFSA GMO Panel concluded that maize MON 810 will not cause reductions to natural enemies that are significantly greater from those caused by conventional farming where pesticides are used to control corn borers. The data of Paejaroen (2008) are consistent with those of Ramirez-Romero et al. (2007) but, since they were derived from laboratory experiments, they give no additional information concerning exposure. Therefore they do not affect the previous conclusion of the EFSA GMO Panel concerning risk.

Conclusion

The EFSA GMO Panel is of the opinion that this thesis report by Paejaroen (2008) does not provide new scientific elements that would invalidate its previous risk assessments of maize MON 810.

3.4. Abstract of a presentation by Békési et al., 2010 on the effect of insect-resistant (Bt) GM corn pollen on the development of honeybee (*Apis mellifera*) larvae *in vitro*

Claim made by Greece

Békési et al. (2010) reported in a short conference abstract that a lower weight was observed in bee larvae fed 20% maize MON 810 pollen compared to bee larvae fed isogenic maize pollen. After three days, larval mortality increased and only the 5-10 % of the larvae survived longer. '*In histological preparations of the midgut, the peritrophic membrane of larvae fed with Bt pollen, seemed to be discontinuous and incomplete that might explain deficient digestion and lower weight gain*' (Békési et al., 2010).

Assessment by the EFSA GMO Panel

In its 2009 Scientific Opinion on the renewal of maize MON 810 (EFSA, 2009), the EFSA GMO Panel already reviewed available scientific data on potential adverse effects on honeybees of the Cry1Ab protein or Bt-pollen of maize gathered either under laboratory or semi-field conditions (e.g., Babendreier et al., 2005; Ramirez-Romero et al., 2005, 2008; Rose et al., 2007; Duan et al., 2008; BEETLE report, 2009; Malone and Burgess, 2009; Hendriksma et al., 2011). The EFSA GMO Panel also considered the low pollen consumption of honeybee larvae compared to adults (Babendreier et al., 2004) as well as the low concentration of Cry1Ab in maize MON 810 pollen and hence concluded on a low exposure. In 2009, the EFSA GMO Panel therefore concluded that the likelihood of adverse effects on honeybees is expected to be very low even though it agreed that in field settings honeybees might face additional stresses that could theoretically affect their susceptibility to Cry proteins or generate indirect effects.

In its 2008 Scientific Opinion in response to a previous safeguard clause invoked by Greece on maize MON 810 (EFSA, 2008c), the EFSA GMO Panel concluded that the low exposure level of bees to maize pollen combined with the low toxicity of the Cry1Ab protein in maize MON 810 is unlikely to result in any adverse effects on bees.

The EFSA GMO Panel is not in a position to assess the research by Békési et al. (2010) due to the limitation of the publication format (an abstract) and lack of specific data. In addition, the EFSA GMO Panel is not aware of any literature including peer-reviewed publication(s) with new relevant and detailed scientific data that would support the observations of Békési et al. (2010). Against this background and in order to facilitate a thorough assessment of potential risks, the EFSA GMO Panel strongly recommends Member States who invoke safeguard clauses under Directive 2001/18/EC (EC, 2001) or emergency measures under Regulation (EC) No 1829/2003 (EC, 2003) to supply relevant new scientific data of a quality which can be subjected to detailed scientific scrutiny.

Conclusion

The EFSA GMO Panel is not in a position to assess the research by Békési et al. (2010) due to the limitation of the publication format (an abstract) and lack of specific data.

3.5. Interview of H.H. Kaatz (University of Halle-Wittenberg)

Claim made by Greece

Greece provided a written interview⁸ of H.H. Kaatz (University of Halle-Wittenberg) carried out by Christof Potthof (Gen-ethischen Netzwerk). This interview is part of the 2009 Testbiotech report entitled '*Risk reloaded – Risk analysis of genetically engineered plants within the European Union*' (Then & Potthoff, 2009). H.H. Kaatz cited a research project he had led on honeybees to claim: (i)

⁸ Interview is available at <http://www.gmwatch.org/component/content/article/11621-gm-crops-and-honey-bee-research>

that honeybees fed Bt-maize suffered a higher mortality rate than those on a control diet; and (ii) that there was a possibility of horizontal gene transfer from Bt-maize to bacteria in the digestive tract of honeybees. Neither claim was supported either by any data or by any related scientific publication.

Assessment by the EFSA GMO Panel

In absence of peer-reviewed publication(s) including new relevant scientific data, the EFSA GMO Panel is not in a position to assess the impact of the research by H.H. Kaatz on its previous risk assessments of maize MON 810. Against this background and in order to facilitate a thorough assessment of potential risks, the EFSA GMO Panel strongly recommends Member States who invoke safeguard clauses under Directive 2001/18/EC (EC, 2001) or emergency measures under Regulation (EC) No 1829/2003 (EC, 2003) to supply relevant new scientific data of a quality which can be subjected to detailed scientific scrutiny.

Conclusion

The EFSA GMO Panel is not in a position to assess the impact of the research by H.H. Kaatz due to the limitation of the publication format (an interview) and lack of specific data.

4. CONCERNS RELATED TO THE POST-MARKET ENVIRONMENTAL MONITORING OF MAIZE MON 810

In its supporting document, Greece found that “*the most recent monitoring for potential impact (monitoring for 2009) does not cover the necessary requirements*” (Züghart et al., 2011).

The EFSA GMO Panel notes that Greece refers to a more general policy paper representing the view of the National Environment Agencies in Austria and Switzerland and the Federal Agency for Nature Conservation in Germany on GMO monitoring (Züghart et al., 2011). This policy paper contributes to the implementation of an adequate EU-wide standard concerning the environmental monitoring of GMO and thus contains a number of general recommendations, which have been, together with other publications, considered previously by the EFSA GMO Panel (EFSA, 2011a).

In relation to issues related to management and monitoring of GM plants, the EFSA GMO Panel assesses the scientific quality of the initial PMEM plans and subsequent PMEM reports submitted by applicants, whilst the final endorsement of both the specific PMEM plan and reports is the responsibility of risk managers.

Against this background, in July 2011, the EFSA GMO Panel adopted an updated Guidance Document on the PMEM of GM plants (EFSA, 2011a) which provides applicants and risk managers with guidance on the strategy, methodology and reporting of PMEM of GM plants. Furthermore, in its Scientific Opinions on annual PMEM reports on maize MON 810 (EFSA, 2011c, 2012a), the EFSA GMO Panel concluded that no adverse effects on the environment, human and animal health due to maize MON 810 cultivation were identified during the 2009 and 2010 growing seasons. However, the EFSA GMO Panel identified weaknesses in the methodology and therefore provided detailed recommendations to the applicant for the improvement of its insect resistance management (IRM) plan, case-specific monitoring (CSM) and general surveillance (GS) of maize MON 810. The applicant was provided with specific recommendations to improve its IRM plan (e.g., non-Bt *refugia*, sampling over time in ‘hotspot areas’ with high uptake of maize MON 810 and multivoltine target pests) as well as the methodology of the GS of maize MON 810 (see Appendix 1 to EFSA, 2011c, 2012a providing a methodological guidance for the assessment of the farmer questionnaires).

In addition, in 2009, the EFSA GMO Panel already recommended (EFSA, 2009) that, especially in areas of abundance of non-target Lepidoptera populations, the adoption of the cultivation of maize MON 810 be accompanied by appropriate management measures in order to mitigate the possible exposure of these species to maize MON 810 pollen. The implications of these management measures should be considered in the PMEM plan. Further details on the framework to implement appropriate

risk mitigation measures, wherever it is necessary, are given in the recent Statement of the EFSA GMO Panel on the similar Cry1Ab-expressing maize Bt11 (for further details, see EFSA, 2011e). The EFSA GMO Panel reiterates that, through its Statement on maize Bt11, risk managers are provided with guidance to: (i) estimate the mortality of exposed non-target Lepidoptera with a range of various sensitivities to maize Bt11 (and by analogy to maize MON 810) pollen ; and (ii) choose risk mitigation measures proportionate to the level of identified risk and to the protection goals pertaining to their region.

CONCLUSIONS

The EFSA GMO Panel has scrutinized the documentation provided by Greece in support of its safeguard clause on GM maize MON 810. The EFSA GMO Panel considered the relevance of concerns raised by Greece in the light of the most recent and relevant scientific data published in the scientific literature. This also includes the conclusions of the EFSA GMO Panel assessments of the PMEM results on maize MON 810 during the 2009 and 2010 growing seasons (EFSA, 2011c, 2012a).

During its evaluation of the supporting documentation, the EFSA GMO Panel has noted that the majority of the publications referred to by Greece were already addressed in its past scientific outputs on maize MON 810 or other related insect-resistant GM maize transformation events and therefore refers to them (EFSA, 2008a, 2009, 2011d, 2012b).

In the remaining evidence provided by Greece referred to above (see sections 2, 3 and 4), the EFSA GMO Panel could not identify any new data subject to scientific scrutiny or scientific information that would invalidate its previous risk assessments of maize MON 810. Against this background and in order to facilitate a thorough assessment of potential risks, the EFSA GMO Panel strongly recommends Member States who invoke safeguard clauses under Directive 2001/18/EC (EC, 2001) or emergency measures under Regulation (EC) No 1829/2003 (EC, 2003) to supply scientific data of a quality which can be subjected to detailed scientific scrutiny.

In addition, Greece did not supply scientific evidence to show that the conclusions of the previous risk assessments of the EFSA GMO Panel of maize MON 810 were not applicable to Greece.

With regard to issues related to management and monitoring of maize MON 810, the EFSA GMO Panel refers to its recent recommendations for management and monitoring measures of maize MON 810.

In conclusion, the EFSA GMO Panel finds that the scientific evidence currently available does not sustain the arguments provided by Greece and that cultivation of maize MON 810 in Greece is unlikely to have an adverse effect on human and animal health and the environment.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission, dated 22 May 2012, to the EFSA Executive Director requesting the assessment by EFSA of the scientific elements supporting the prohibition of the placing on the market of GM maize MON 810 for cultivation purposes in Greece.
2. Acknowledgement letter, dated 9 June 2012, from the EFSA Executive Director to the European Commission.

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