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SCIENTIFIC OPINION

Request from the European Commission to review scientific studies related to the impact on the environment of the cultivation of maize Bt11 and 1507[1]

Scientific Opinion of the Panel on Genetically Modified Organisms

(Question No EFSA-Q-2008-679)

Adopted on 29 October 2008

PANEL MEMBERS*

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SUMMARY

On 19 January 2005 and 20 April 2005, the Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) issued scientific opinions on genetically modified maize Bt11 and 1507, both including the scope of cultivation.

At a meeting of the European Commission with national competent authorities on 19 June 2006, some Member States raised objections to the original opinions of the GMO Panel. Most of these objections related to potential effects of maize Bt11 and 1507 on non-target organisms and in particular lepidopteran species and to post-market environmental monitoring. Following the meeting with competent authorities and upon request of the European Commission, the GMO Panel amended its previous scientific opinions on 7 November 2006 by adopting an Annex of clarifications. In the Annex, the GMO Panel concluded that the information available for maize Bt11 and 1507 addresses objections and questions raised by Member States, and confirmed that maize Bt11 and 1507 are unlikely to have adverse effects on human and animal health or the environment in the context of their proposed uses.

[1] For citation purposes: Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission to review scientific studies related to the impact on the environment of the cultivation of maize Bt11 and 1507. *The EFSA Journal* (2008), 851, 1-27.

* This opinion is not shared by 0 members of the Panel. / (conflict of interest) 0 members of the Panel did not participate in (part of) the discussion on the subject referred to above because of possible conflicts of interest.

On 24 July 2008, the GMO Panel received a new request from the European Commission to review the previous scientific opinions of maize Bt11 and 1507 in the light of 11 scientific publications, published after the adoption of the complemented scientific opinions of the GMO Panel, as well as any other relevant study.

The GMO Panel concludes that neither the 11 scientific publications selected and provided by the European Commission, nor recent peer-reviewed papers identified as relevant by the GMO Panel, invalidate the former risk assessments of maize Bt11 and 1507 performed by the GMO Panel.

Key words: **GMOs, maize (*Zea mays*), Bt11, 1507, insect resistance, Cry1Ab, Cry1F, human health, animal health, environment, Directive 2001/18/EC**

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BACKGROUND

On 19 January 2005 and 20 April 2005, the Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) adopted 2 scientific opinions on notifications for the placing on the market under Part C of Directive 2001/18/EC, respectively, of genetically modified maize 1507 for import, feed, industrial processing and cultivation from Pioneer Hi-Bred International/Mycogen Seeds (Notification reference C/ES/01/01) (EFSA, 2005a) and of genetically modified maize Bt11, for feed, industrial processing and cultivation from Syngenta Seeds (Notification reference C/F/96/05.10) (EFSA, 2005b).

On 19 June 2006, the European Commission convened a technical meeting with Member States, EFSA and applicants to discuss the 2 pending cultivation applications for the maize events Bt11 and 1507 under Directive 2001/18/EC. The aims of the meeting were to discuss and assess the objections that several Member States had raised on both notifications and corresponding EFSA GMO Panel opinions, and to ensure that all scientific views on the subject were considered. Several Member States maintained their opinion that the 2 above referred opinions of the GMO Panel did not adequately address their concerns and therefore asked to provide more information about the potential impact of maize Bt11 and 1507 on non-target organisms and in particular lepidopteran species, as well as clarifications about the monitoring thereof. In this respect, the GMO Panel was asked by the European Commission to reconsider its previous risk assessments of maize Bt11 and 1507, including direct and indirect long-term effects.

For the reconsideration of its previous risk assessments of maize Bt11 and 1507, the GMO Panel considered data provided in the respective notifications C/F/96/05.10 and C/ES/01/01, additional information provided by the applicant, the initial risk assessment prepared by the lead Member State, specific questions and concerns raised by Member States and the most relevant scientific literature. On 7 November 2006, the GMO Panel provided the requested clarifications by complementing its initial scientific opinions with an Annex of clarifications (EFSA, 2006a). In the Annex, the GMO Panel concluded that the information available for maize Bt11 and 1507 addresses objections and questions raised by Member States, and confirmed that maize Bt11 and 1507 are unlikely to have adverse effects on human and animal health or the environment in the context of their proposed uses.

In a letter dated 24 July 2008, the European Commission requested the GMO Panel to review the previous scientific opinions on maize Bt11 and 1507 in the light of 11 scientific publications, published after the adoption of the complemented scientific opinions of the GMO Panel, as well as any other relevant study. In its request, the European Commission asks EFSA to confirm "*its risk assessment of Bt11 and 1507 maize or comment on whether they would lead EFSA to alter its conclusions or refine it*".

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

On 24 July 2008, EFSA was requested by the European Commission "*to review eleven scientific studies published after the adoption of the EFSA opinions as well as any other relevant study and to confirm its risk assessment of Bt11 and 1507 maize or comment on whether they would lead EFSA to alter its conclusions or refine it*".

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ASSESSMENT

1. Evaluation of documents delivered by the European Commission

The GMO Panel has assessed the 11 scientific publications submitted by the European Commission. The GMO Panel looked for evidence for GMO-specific risks related to maize Bt11 and 1507 taking into consideration the EFSA guidance document for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006b) as well as any related risk assessments carried out in the past. In addition, the GMO Panel considered the relevance of the concerns in the light of available scientific data and peer-reviewed publications. In this respect, the GMO Panel is continually considering any new or additional scientific publication that could have consequences for the environmental safety assessment of GMOs and in this specific case maize Bt11 and 1507.

2. Assessment by the GMO Panel

The European Commission selected and provided the following 11 scientific publications:

- Prasifka, P.L., Hellmich, R.L., Prasifka, J.R., Lewis, L.C. (2007) Effects of Cry1Ab-expressing corn anthers on the movement of monarch butterfly larvae. *Environmental Entomology*, 36: 228-233

- Hilbeck, A., Schmidt, J.E.U. (2006) Another view on Bt proteins - How specific are they and what else might they do? *Biopesticides International*, 2: 1-50

- Rosi-Marshall, E.J., Tank, J.L., Royer, T.V., Whiles, M.R., Evans-White, M., Chambers, C., Griffiths, N.A., Pokelsek, J., Stephen, M.L. (2007) Toxins in transgenic crop byproducts may affect headwater stream ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, 104: 16204-16208

- Faria, C.A., Wäckers, F.L., Prichard, J., Barrett, D.A., Turlings, T.C.J. (2007) High susceptibility of *Bt* maize to aphids enhances the performance of parasitoids of lepidopteran pests. *PLoS ONE* 2: 1-11 (e600)

- Nguyen, H.T., Jehle, J.A. (2007) Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810. *Journal of Plant Diseases and Protection*, 114: 82-87

- Douville, M., Gagne, F., Blaise, C., Andre, C. (2007) Occurrence and persistence of Bacillus thuringiensis (Bt) and transgenic Bt corn cry1Ab gene from an aquatic environment. *Ecotoxicology and Environmental Safety*, 66: 195-203

- Mulder, C., Wouterse, M., Raubuch, M., Roelofs, W., Rutgers, M. (2006) Can transgenic maize affect soil microbial communities? *PLoS Computional Biology*, 2: 1165-1172 (e128)

- Rose, R.I., Dively, G.P., Pettis, J. (2007) Effects of Bt corn pollen on honey bees: emphasis on protocol development. *Apidologie*, 38: 368-377

- Johnson, K.L., Raybould, A.F., Hudson, M.D., Poppy, G.M. (2007) How does scientific risk assessment of GM crops fit within the wider risk analysis? *Trends in Plant Science*, 12: 1-5

- Andow, D.A., Zwahlen, C. (2006) Assessing environmental risks of transgenic plants. *Ecology Letters*, 9: 196-214

- Butler, S.J., Vickery, J.A., Norris, K. (2007) Farmland biodiversity and the footprint of agriculture. *Science*, 315: 381-384

The GMO Panel critically notes that none of the 11 scientific studies report new data and results from maize 1507. Only 2 publications (Faria et al., 2007; Rose et al., 2007) provide new datasets derived from experiments carried out with maize Bt11. For 2 other publications (Hilbeck and Schmidt, 2006; Rosi-Marshall et al., 2007) it is unclear whether the performed experiments included at least one of the maize events Bt11 and 1507. The remaining 7 publications deal either exclusively with data derived from other genetically modified maize events (MON810: Douville et al., 2007; Prasifka et al., 2007 and Nguyen and Jehle, 2006; MON810 and Bt176: Mulder et al., 2006), with reviews on originally published literature to conclude on the risk assessment of transgenic plants in general (Andow and Zwahlen, 2006; Johnson et al., 2007), or to discuss environmental aspects of genetically modified herbicide-tolerant plants (Butler et al., 2007).

In this opinion, the GMO Panel indicates where information derived from other maize events (i.e., Bt176 and MON810) is used in its assessment on potential impacts of maize Bt11 and 1507. The Cry1Ab protein expressed in maize Bt11 (also in MON810 and Bt176) and the Cry1F protein expressed in maize 1507 bind specifically to the brush border membrane of target pest Lepidoptera species such as corn borer larvae. Although Cry1F and Cry1Ab proteins have different high-affinity binding sites, their binding stability and mode of action are similar. The amount of biologically active Cry protein in pollen of maize Bt11, 1507 and MON810 is relatively low resulting in similar toxicological effects on non-target lepidopteran populations exposed to pollen from these events (Mendelsohn et al., 2003), in contrast to maize Bt176 which contains higher levels of the Cry1Ab protein in pollen (Hellmich et al., 2001). For green tissues of MON810 Bt-maize plants, the amount of biologically active Cry protein is in a similar range compared to maize Bt11 and Bt176.

In the following section, each scientific publication selected and provided by the European Commission is discussed in detail and assessed by the GMO Panel.

2.1. Prasifka et al. (2007)

This publication addresses exclusively laboratory experiments performed with maize MON810 and in which the potential impact on monarch butterfly, *D. plexippus*, larvae due to exposure to Cry1Ab-containing anthers was studied.

2.1.1. Summary of the publication

Prasifka et al. (2007) detected decreased larval feeding and weight of the monarch butterfly after 4 days of exposure in the laboratory to anthers that contained a high concentration of

Bacillus thuringiensis Cry1Ab protein (Bt). To assess whether larvae exposed to Bt-anthers exhibit increased wandering, which in turn would result in less feeding and lower weight gain, the authors exposed 2-days-old monarch butterfly larvae to milkweed leaf disks with no anthers, anthers that express Bt (MON810), or other non-Bt-anthers and observed the larvae via a video-tracking system. As had been shown in previous studies, larvae exposed to Bt-anthers were shown to feed less on milkweed and to gain less weight than larvae exposed to non-Bt or no anthers, even though there was no evidence of feeding on anthers. Total distance moved, maximum displacement from release point, percentage of time spent moving or near anthers, or mean turn angle did not differ across treatments. However, the authors observed that larvae exposed to Bt-anthers spent more time off milkweed leaf disks than those exposed to no anthers and were more likely to move off the leaf than larvae exposed to non-Bt-anthers.

2.1.2. Conclusions of the authors of this publication concerning the safety of Bt-maize

Results led Prasifka et al. (2007) to suggest that larvae exposed to Bt-anthers behave differently and that ingestion may not be the only way Bt can affect non-target insects like the monarch butterfly. However, the authors pointed out that it is unclear whether the changed behavioural measures (increased time spent off leaf disks and increase frequency of larvae moving off leaf disks) would translate into changes in behaviour on intact insect host plants in the field as larvae might have the option of moving to the underside of the host milkweed leaf (which would not receive deposits of anthers).

2.1.3. GMO Panel assessment

Although decreased larval feeding and weight of monarch butterfly larvae have been reported after exposure in the laboratory to a high density of Cry1Ab-expressing anthers (MON810) as compared to larvae exposed to milkweed leaf disks with no anthers or non-Bt-anthers (Hellmich et al., 2001; Anderson et al., 2004, 2005), an examination of anthers in and near maize fields showed that toxic levels of anthers are uncommon (Anderson et al., 2004). The GMO Panel concludes that intact Bt-anthers alone or in combination with Bt-pollen are not likely to pose a significant risk to monarch butterflies. Although Anderson et al. (2004) and Prasifka et al. (2007) reported a reduction in feeding and weight gain due to behavioural changes under laboratory conditions, a point that still remains to be explained is how this change might translate to the field. Under field conditions early instar larvae, which are most susceptible to the Cry1Ab protein, are less exposed, as they mainly feed on the upper third of milkweed plants where the lowest densities of anthers occur (Pleasants et al., 2001; Anderson et al., 2004). In addition, larvae can move to the underside of leaves where they would avoid any contact with anthers (Pleasants et al., 2001; Jesse and Obrycki, 2003).

The GMO Panel concludes that, in terms of risk to human and animal health and the environment, the provided information in Prasifka et al. (2007) does not present new scientific evidence that would invalidate the previous risk assessments of maize Bt11 and 1507.

2.2. Hilbeck and Schmidt (2006)

This publication addresses potential adverse impacts on non-target organisms of Bt-maize and Bt-proteins in a general review. The authors discuss in more detail potential direct effects reported from a laboratory Bt-toxin feeding study, to document the sensitivity of green lacewing, *Chrysoperla carnea*, larvae to purified Cry protein concentrations or via Bt-maize (presumably Bt176) raised lepidopteran larvae fed to the lacewing predator. The original datasets were previously published by Hilbeck et al. (1998a,b, 1999).

2.2.1. Summary of the publication

Reviewing the use of *B. thuringiensis* and its toxins in the context of pest control, Hilbeck and Schmidt (2006) argued that several details about the mode of action of Cry toxins are still not understood well enough. Although there is considerable experience with the application and the environmental safety of Bt-based insecticides, the authors claimed that a number of research papers reporting adverse effects on non-target organisms are not sufficiently considered. These and the widespread use of genetically modified Bt-plants stimulated Hilbeck and Schmidt (2006) to review the published laboratory feeding studies on effects of Bt-toxins and genetically modified Bt-plants on non-target invertebrates. In their publication, the authors described those reports that documented adverse effects in non-target organisms in more detail, focusing on the example of green lacewing, *C. carnea*.

2.2.2. Conclusions of the authors of this publication concerning the safety of Bt-maize

Discussing their findings, Hilbeck and Schmidt (2006) argued firstly that the evidence for adverse effects in non-target organisms is compelling enough that it would merit more biosafety research. They further concluded from their in-depth analysis that the published reports studying effects of Bt-toxins from Bt-pesticides and genetically modified Bt-plants on green lacewing larvae provide complementary and not contradictory data. And, finally, Hilbeck and Schmidt (2006) found that key experiments, explaining the mode of action not only in this particular affected non-target species, but also in most other affected non-target species are still missing. Considering the steadily increasing global production area of Bt-crops, for Hilbeck and Schmidt (2006) it seemed prudent to thoroughly understand how Bt-toxins might affect non-target organisms. In particular, they concluded that Bt-toxins do not have the same mode of action in predatory lacewing larvae like in herbivorous caterpillars.

2.2.3. GMO Panel assessment

The GMO Panel has reviewed in detail a wide range of publications concerning the safety of Bt-maize, including the original data presented in Hilbeck et al. (1998a,b 1999) (EFSA, 2005a,b). According to Rodrigo-Simón et al. (2006), the Cry1Ab protein does not show specific binding *in vitro* to brush border membrane vesicles from the midgut of *C. carnea* larvae, which is a prerequisite for toxicity. When *C. carnea* larvae are fed lepidopteran larvae reared on Cry1Ab-expressing maize, the laboratory studies by Hilbeck et al. (1998a,b, 1999) indicate significantly prolonged larval development and increased mortality. However, the key experiments on what caused the significantly higher mortality in Bt-exposed lacewings larvae in these studies are still missing to date. In addition, from the protein binding studies noted above, it can also be concluded that these effects are likely to be a consequence of the lepidopteran prey apparently being of lower nutritional quality (Romeis et al., 2006). This is supported by data showing that *C. carnea* larvae are unaffected when feeding on non-susceptible *Tetranychus urticae* containing large amounts of biologically active Cry1Ab protein (Dutton et al., 2002). *C. carnea* larvae in the field are known to feed mainly on aphids, whereas lepidopteran larvae are not considered an important prey, especially after their first moult. Because aphids do not accumulate the Cry1Ab protein, the risk that this trophic chain poses for *C. carnea* larvae can be regarded as negligible. Even though chronic effects cannot be completely ruled out, the GMO Panel emphasizes that the continuous exposure of *C. carnea* to diets exclusively based on lepidopteran larvae is unlikely under field conditions (Canard, 2001; Dutton et al., 2003). In addition, Li et al. (2008) demonstrated that adults of *C. carnea* are not affected by Bt-maize pollen and are not sensitive to the Cry1Ab and Cry3Bb1 proteins at concentrations exceeding those observed in pollen of Bt-maize.

No negative effects on *C. carnea* have been documented in the field; sampling from Cry1Ab-expressing maize fields has not shown a decline in their abundance (Bourguet et al., 2002; Eckert et al., 2006).

The GMO Panel concludes that, in terms of risk to human and animal health and the environment, the provided information in Hilbeck and Schmidt (2006) does not present new scientific evidence that would invalidate the previous risk assessments of maize Bt11 and 1507. The GMO Panel reiterates that no new scientific data were supplied confirming adverse effects due to the cultivation of maize Bt11 and 1507 neither on the environment, nor on human and animal health.

2.3. Rosi-Marshall et al. (2007)

This publication addresses the potential impact on non-target aquatic organisms of Bt-maize in general, without providing information on the specific Bt-maize event used in the study.

2.3.1. Summary of the publication

Rosi-Marshall et al. (2007) reported that Bt-maize is widely planted in the Midwestern United States, often adjacent to headwater streams. The authors have shown that maize byproducts, such as pollen and detritus, enter headwater streams and are subject to storage and transport to downstream water bodies. Laboratory feeding trials revealed that consumption of Bt-maize byproducts reduced growth and increased mortality of non-target stream insects such as Trichopterans. The authors pointed out the importance of stream insects as prey for aquatic and riparian predators, and suggested that the widespread planting of Bt-maize might have unexpected ecosystem-scale consequences.

2.3.2. Conclusions of the authors of this publication concerning the safety of Bt-maize

Rosi-Marshall et al. (2007) concluded that Bt-maize byproducts may have negative effects on the biota of streams in agricultural areas. Based on these findings, they suggested that the assessment of potential non-target effects from genetically modified crops should be expanded to include relevant aquatic organisms, such as stream insects. Moreover, since headwater streams in the Midwestern United States are already impaired by nutrient enrichment and extensive habitat degradation, the authors argued that Bt-crop byproducts could represent an additional stressor to these systems, which in turn could have implications for stream restoration and riparian management in agricultural landscapes.

2.3.3. GMO Panel assessment

The GMO Panel has assessed the study of Rosi-Marshall et al. (2007) at its 37th plenary meeting held on 22-23 November 2007 (EFSA, 2007a), and like other scientists (ACRE, 2007; Beachy, 2008; Parrott, 2008), it argued that the study shows some weaknesses and that their results do not support their speculative conclusions, even though the study has its strengths in quantifying the exposure of headwater streams to maize biomass (Bt or non-Bt) in general.

Rosi-Marshall et al. (2007) measured degradation rates in aquatic systems and found no difference between Bt and non-Bt-maize plant material, but since no concentrations of the Cry1Ab protein were measured in leaves and pollen, no dose-response relationship with Bt-protein can be made. It is thus unclear whether degradation of the Bt-protein is equal to degradation of plant material and thus what levels of the Cry1Ab protein were present in the samples they examined, if any.

The GMO Panel is of the opinion that important background information on levels of Bt-maize exposure and plant material used is missing (e.g., identity of the Bt-maize event(s) used, potential use of isogenic controls, data on the amount of maize material fed to test organisms). Therefore, the GMO Panel argues that the conclusions made by Rosi-Marshall et al. (2007) are not fully supported by the data presented in the paper. From the study performed, it can only be concluded that a potential hazard for Trichopterans has been identified under laboratory conditions when exposed to high doses of Cry toxins. However, due to the low level of exposure to Trichopterans in aquatic ecosystems, the GMO Panel considers that it is unlikely that Bt-toxins in GM crop byproducts would cause toxic effects on aquatic insects.

Based on the present publication, the GMO Panel concludes that, in terms of risk to human and animal health and the environment, the provided information in Rosi-Marshall et al. (2007) does not present new scientific evidence that would invalidate the previous risk assessments of maize Bt11 and 1507.

2.4. Faria et al. (2007)

This publication addresses experiments performed with maize Bt11, MON810 and Bt176 under confined conditions, and assesses potential tritrophic effects of Cry1Ab-expressing maize on parasitoids via their targeted host organisms.

2.4.1. Summary of the publication

Faria et al. (2007) showed a positive effect of Bt-maize on the performance of the corn leaf aphid *Rhopalosiphum maidis*, which in turn enhanced the performance of parasitic wasps that feed on aphid honeydew. Within 5 out of 6 pairs of maize lines that were evaluated, genetically modified maize lines were shown to be significantly more susceptible to aphids than their near-isogenic equivalents, with the remaining pair being equally susceptible.

Phloem element content and analyses derived from the selected maize lines revealed marginally, but significantly higher amino acid levels in Bt-maize, which according to the authors might partially explain the observed increased aphid performance. Larger colony densities of aphids on Bt-plants resulted in an increased production of honeydew that can be used as food by beneficial insects. The authors observed that *Cotesia marginiventris*, a parasitoid of lepidopteran pests, lived longer and parasitized more pest caterpillars in the presence of aphid-infested Bt-maize than in the presence of aphid-infested isogenic maize.

2.4.2. Conclusions of the authors of this publication concerning the safety of Bt-maize

Depending upon aphid pest thresholds, Faria et al. (2007) pointed out that the observed increased susceptibility of Bt-maize to aphids may be either a welcome or an undesirable side effect. Based on the observations made, the authors concluded that as a long as aphid numbers do not reach pest status, the increase of aphid susceptibility to Bt-maize may pose an advantage in maintaining beneficial insect fauna in Bt-maize.

2.4.3. GMO Panel assessment

The GMO Panel agrees with the conclusions of Faria et al. (2007). Even though aphid performance fell within the normal variation observed among conventional maize varieties, different studies reported that aphids perform better on Bt-maize than on near isogenic counterparts (e.g., Bourguet et al., 2002; Dutton et al., 2002; Lumbierres et al., 2004; Pons et al., 2005; Eizaguirre et al., 2006). With the larger colony densities of aphids on Bt-maize, more honeydew was produced, in turn increasing parasitoid longevity and rate of parasitism.

The GMO Panel concludes that, in terms of risk to human and animal health and the environment, the provided information in Faria et al. (2007) does not present new scientific evidence that would invalidate the previous risk assessments of maize Bt11 and 1507.

2.5. Nguyen and Jehle (2007)

This publication addresses the measurement of the Cry1Ab protein content in maize MON810 in field experiments performed in Germany.

2.5.1. Summary of the publication

Nguyen and Jehle (2007) determined the tissue-specific expression and seasonal abundance of the Cry1Ab protein in genetically modified maize plants (MON810, variety ‘Novelis’) from 2 field trials located near Bonn and Halle, Germany. A total of 1085 samples were analysed by using Double Antiserum-Enzyme Linked Immunosorbent Assay (DAS-ELISA). The authors quantified Cry1Ab contents of various plant tissues (root, stem, upper leaf, lower leaf, anther, pollen and kernel) at 4 different plant growth stages (BBCH19, BBCH30, BBCH61 and BBCH83) collected in 2001, 2002 and 2003. MON810 showed the highest Cry1Ab contents in leaves (5.5 – 6.4 µg g^{-1} fresh weight [fw]) at BBCH83, whereas the lowest Cry1Ab contents were detected in the pollen (1 – 97 ng g^{-1} fw). The Cry1Ab content of residual root stocks collected in the field 9 months after harvest was 15 – 17 ng g^{-1} fw. This demonstrated that the Cry1Ab concentration in residual root stocks was reduced to about one-hundredth of the fresh roots. The monitoring of the Cry1Ab expression showed that Cry1Ab contents varied strongly between different plant individuals.

2.5.2. Conclusions of the authors of this publication concerning the safety of Bt-maize

Nguyen and Jehle (2007) concluded that their analysis was the first large-scale expression monitoring of the Cry1Ab protein under European field conditions and provided a comprehensive dataset of the temporal distribution of the Cry1Ab protein in maize MON810. Cry1Ab expression was shown to be the lowest in pollen, very low in stalks, low in roots, whilst the highest expression levels were observed in leaves. Although their study confirms previously reported tendencies of Cry1Ab contents in MON810 (Mendelsohn et al., 2003), the authors observed a considerable variation in expression levels of *cry1Ab*. The observed variation exceeds variation levels reported previously and may be due to the large number of analysed samples and different growing years. The authors suggested a certain plant to plant variation in the Cry1Ab expression. Cry1Ab levels also varied in different plant tissues of MON810 at different growth stages. The overall small differences but similar patterns of Cry1Ab levels at the 2 field sites clearly indicated that plant tissue and plant development are the main parameters affecting Cry1Ab contents of maize MON810.

2.5.3. GMO Panel assessment

The GMO Panel evaluated the reported variations in the Cry1Ab protein content of maize MON810 (Nguyen and Jehle, 2007) and concluded that these protein concentrations do not give rise to any effect that was not assessed previously in the GMO Panel opinion concerning maize MON810. These variations in protein concentration can be explained by the larger assortment of tissues sampled at different physiological and environmental conditions. In addition, the data were generated within a larger set of biosafety research in Germany, and within the same locations, biotic effects were monitored. No adverse effects were observed on a wide range of non-target organisms during the 3-year experimental phase (Gathmann et al., 2006; Baumgarte and Tebbe, 2005; Eckert et al., 2006; Toschki et al., 2007).

The GMO Panel concludes that, in terms of risk to human and animal health and the environment, the provided information in Nguyen and Jehle (2007) does not present new scientific evidence that would invalidate the previous risk assessments of maize Bt11 and 1507.

2.6. Douville et al. (2007)

This publication addresses datasets derived exclusively from maize MON810, and focuses on the occurrence and persistence of the *cry1Ab* gene in aquatic environments near a field plot where Bt-maize was grown.

2.6.1. Summary of the publication

The aims of the Douville et al. (2007) publication were to examine the occurrence and persistence of the *cry1Ab* gene from conventional Bt-based pesticides (Bt *kurstaki*) and Bt-maize (MON810) in aquatic environments near fields where Bt-maize was cultivated. The *cry1Ab* gene was shown to persist for more than 21 and 40 days in surface water and sediment, respectively. In sediments, the *cry1Ab* gene from Bt-maize was still detected after 40 days in clay and sand-rich sediments. Field surveys revealed that the *cry1Ab* gene from maize MON810 and from naturally occurring Bt was more abundant in the sediment than in the surface water. The authors detected the *cry1Ab* transgene as far away as 82 km downstream from the MON810 maize cultivation plot, suggesting that there were multiple sources of this gene and/or that it undergoes transport by the stream. Sediment-associated *cry1Ab* gene from Bt-maize was shown to decrease with distance from the Bt-maize field. The authors observed that sediment concentrations of the *cry1Ab* gene were significantly correlated with those of the *cry1Ab* gene in surface water.

2.6.2. Conclusions of the authors of this publication concerning the safety of Bt-maize

The data indicate that DNA from Bt-maize and conventional Bt *kurstaki* were persistent to some extent in aquatic environments and were detected in rivers draining farming areas. The authors suggested from their findings that environmental concentrations were low, but that the null hypothesis (i.e., that the *cry1Ab* transgene was not present in aquatic environments) cannot be accepted at the present time and thus that further research and monitoring efforts are required.

2.6.3. GMO Panel assessment

The GMO Panel considers that DNA presence/absence alone is not a reliable indicator that enables to draw conclusions on the toxicity to non-target organisms. A more reliable indicator of toxicity to non-target organisms would be the presence and concentrations of the Cry1Ab protein in surface water and sediment. In a previous study of the same group of researchers, it was reported that the presence of the Cry1Ab protein in body waters was either absent or just above the detection limit (Douville et al., 2005), suggesting that Cry1Ab protein concentrations would remain far below any toxic level.

The GMO Panel concludes that, in terms of risk to human and animal health and the environment, the provided information in Douville et al. (2007) does not present new scientific evidence that would invalidate the previous risk assessments of maize Bt11 and 1507. The GMO Panel also took into account the publication by Douville et al. (2009), which is a preliminary study on the occurrence of *cry1Ab* genes from Bt and Bt-maize in mussels without any indications of environmental impact.

2.7. Mulder et al. (2006)

This publication addresses exclusively pot experiments performed with maize MON810 and Bt176, and assesses the potential impact of Cry1Ab-expressing maize on soil microbial communities.

2.7.1. Summary of the publication

The aim of the Mulder et al. (2006) laboratory experiment was to determine if temporal variations of belowground activity reflect the influence of the Cry1Ab protein from genetically modified maize on soil bacteria and, hence, on a regulatory change of the microbial community (ability to metabolize sources belonging to different chemical guilds) and/or a change in numerical abundance of their cells. Effects of the addition of crop residues on respiration and catabolic activities of the bacterial community were examined in microcosm experiments. Four maize cultivars of 2 different isolines (each one including the conventional crop and its Bt-cultivars MON810 or Bt176) and 1 control of bulk soil were included in the experimental design. Growth models suggested a dichotomy between soils amended with either conventional or genetically modified maize residues. According to Mulder et al. (2006), the Cry1Ab protein appeared to influence the composition of the microbial community. The highly enhanced soil respiration observed during the first 72 hours after the addition of Bt-maize residues was assigned by the authors to the presence of genetically modified crop residues. This result was confirmed by agar plate counting, as the averages of the colony-forming units of soils in conventional treatments were about one-third of those treated with genetically modified plant material. Furthermore, the addition of Bt-maize appeared to induce increased microbial consumption of carbohydrates in BIOLOG EcoPlates. Three weeks after the addition of maize residues to the soils, the authors did no longer detect differences between the consumption rate of specific chemical guilds by bacteria in soils amended with genetically modified maize and bacteria in soils amended with conventional maize.

2.7.2. Conclusions of the authors of this publication concerning the safety of Bt-maize

Mulder et al. (2006) concluded that their data support the existence of short Bt-induced ecological shifts in the microbial communities of croplands' soils.

2.7.3. GMO Panel assessment

Mulder et al. (2006) reported short-term effects of Bt-maize (MON810 and Bt176) which induced ecological shifts in the microbial communities of croplands' soils in laboratory testing. However, the GMO Panel notes that there were differences in agronomic and compositional characteristics between the tested Bt-maize and the near isogenic comparator, which may have caused the shift in microbial communities, so that no conclusions on the impact of the genetic modification can be made. Microbial activity could have been mainly affected by, for instance, sugar content (Biavati and Sorlini, 2007) rather than the level of the Cry1Ab protein. Percentage differences in sugar content were relatively higher than those observed in levels of the Cry1Ab protein. The highly enhanced soil respiration observed during the first 72 hours after the addition of Bt-maize residues can be interpreted as being related to the presence of other macronutrient crop residues. However, 3 weeks after the addition of maize residues to the soils, no differences between the consumption rate of specific chemical guilds by bacteria in soils amended with genetically modified maize and bacteria in soils amended with conventional maize were detectable in the Mulder et al. (2006) experiments.

Due to the close interaction between crops and microbe-mediated soil processes, soil organisms in the rhizosphere are likely to be exposed to the Cry1Ab protein released from Bt-maize as root exudates. Some studies demonstrated consistent significant differences between soils with Bt and non-Bt-maize in relation to soil microorganisms. Root exudates of Bt-maize (Bt176) were shown to reduce presymbiotic hyphal growth of the arbuscular mycorrhizal fungus, *Glomus mosseae*, as compared with those of another Bt-maize (Bt11) and control maize (Turrini et al., 2004). Castaldini et al. (2005) also reported consistent differences in rhizosphere heterothrophic bacteria and mycorrhizal colonization (including *G. mosseae*) between Bt-maize (Bt176) and its conventional counterpart. According to the authors, the genetic modification in maize Bt176 might have led to changes in plant physiology and composition of root exudates, which in turn may have affected symbiotic and rhizosphere microorganisms. In this respect, Widmer (2007) suggested that effects observed on symbiotic microorganisms living in close association with crops will only be disadvantageous for the crop itself, without representing a concern for the ecosystem. In addition, a number of other studies (reviewed by Widmer, 2007 and Icoz and Stotzky, 2008), performed under laboratory, glasshouse and field conditions and covering a large array of classical and more recent analytical tools, revealed only some minor changes in soil microbial community structure with Bt-maize compared to non-Bt-maize (Blackwood and Buyer, 2004; Brusetti et al., 2004; Mulder et al., 2005; Griffiths et al., 2006) or generally show no adverse effects of the Cry1Ab protein released by Bt-maize in root exudates or from biomass incorporated into soil on microorganisms or microorganism-mediated processes (Saxena and Stotzky, 2001a; Flores et al., 2005; StMUGV, 2006; Hönemann et al., 2008; Icoz et al., 2008). In studies were effects on microbial communities have been reported, these effects were in general considered spatially and temporally limited, and small compared with those induced by differences in geography, temperature, seasonality, plant variety or soil type (Fang et al., 2005, 2007; Griffiths et al., 2005, 2006; Lilley et al., 2006; Icoz and Stotzky, 2008). Factors such as plant growth stage and field heterogeneities much largely affected soil microbial community structure, as compared with Bt-maize (MON810) (Baumgarte and Tebbe, 2005; Griffiths et al., 2007b).

Studies in which the decomposition of Bt-maize was compared with that of non-Bt-isogenic lines mostly show that Cry1Ab-expressing maize does not cause differences in decomposition rate or mass of carbon remaining over time (e.g., Cortet et al., 2006; Tarkalson et al., 2008). Litter-bag experiments with Bt-maize (Bt11) reported by Zwahlen et al. (2007) did not reveal major changes in the decomposition rate of Bt-maize residues. Similarly, but for MON810 instead of Bt11, various studies found no evidence for any effect related to the genetic modification when examining the decomposition rate of Bt-maize (Griffiths et al., 2007a,b; Hönemann et al., 2008; Lehman et al., 2008; Tarkalson et al., 2008). These recent findings confirm that previously reported decreases in decomposition rate (e.g., Saxena and Stotzky, 2001b; Flores et al., 2005; Fang et al., 2007; Raubuch et al., 2007) do not result from an effect on soil microorganisms by the Cry1Ab protein, but more likely from increased lignin contents in specific maize varieties. Altered lignin content in specific maize varieties has been shown not to be a generic effect of the cry1Ab gene insertion (Griffiths et al., 2007b). Moreover, other factors such as climatic conditions affected decomposition rates and mineralisation (e.g., Cortet et al., 2006).

Based on the findings of studies discussed above, the GMO Panel is of the opinion that potential adverse effects on soil microorganisms due to maize MON810 and Bt176 will, if at all, be transient, minor and local in field settings.

The GMO Panel concludes that, in terms of risk to human and animal health and the environment, the provided information in Mulder et al. (2006) does not present new scientific evidence that would invalidate the previous risk assessments of maize Bt11 and 1507.

2.8. Rose et al. (2007)

This publication addresses exclusively laboratory and field experiments performed with maize Bt11, in which potential effects of Bt-maize pollen on honeybees were studied, as well as relevant protocol development aspects.

2.8.1. Summary of the publication

Laboratory feeding studies performed by Rose et al. (2007) showed no effects on the weight and survival of honeybees feeding on Cry1Ab-expressing sweet maize Bt11 pollen for 35 days. Colonies foraging in sweet maize plots and fed Bt-pollen cakes for 28 days were not affected by the Cry1Ab protein, as no adverse effects on bee weight, foraging activity, and colony performance were observed. Brood development was not shown to be affected by exposure to Bt-pollen but was reduced significantly by the positive insecticide control. The authors reported that the number of foragers returning with pollen loads, pollen load weight, and forager weight were the most consistent endpoints as indicators of foraging activity. They also discussed methods to ensure exposure to pollen, duration of exposure, positive controls, and appropriate endpoints to consider in planning laboratory and field studies to evaluate the non-target effects of transgenic pollen.

2.8.2. Conclusions of the authors of this publication concerning the safety of Bt-maize

The study of Rose et al. (2007) has identified several experimental design elements, sensitive endpoints, and factors to consider in planning short term field studies with open, functional hives to evaluate non-target effects of transgenic pollen. Even though there is no evidence so far of any lethal or sublethal effects of Bt-proteins, the authors concluded that insecticidal products expressed by other transgenes may need more extended field testing on a case-by-case basis to assess the longer term consequences of sublethal changes in colonies and subtle modifications in honeybee behaviour.

2.8.3. GMO Panel assessment

The GMO Panel agrees with the conclusion of the Rose et al. (2007) study in which no adverse effects on honeybee weight, foraging activity, and colony performance were observed. Similarly, in a flight cage study maintained in controlled conditions, no significant differences were reported in honeybee mortality, syrup consumption and olfactory learning performance when honeybee colonies were exposed to different syrups containing Cry1Ab protoxin (Ramirez-Romero et al., 2005). In this respect, Ramirez-Romero et al. (2008) recently concluded that negative effects of the Cry1Ab protein on foraging behaviour and olfactory learning performance of honeybees are unlikely in natural conditions. Feeding behaviour and olfactory learning performance were disturbed only when honeybees were exposed to extremely high concentrations of the Cry1Ab protein (5000ppb), which do not occur under normal apicultural or field conditions (Ramirez-Romero et al., 2008).

Based on a meta-analysis of 25 independent laboratory studies assessing direct effects on honeybee survival of Cry proteins from currently commercialised Bt-crops, Duan et al. (2008) concluded that the assessed Cry proteins do not negatively affect the survival of either honeybee larvae or adults in laboratory settings. However, as argued by Duan et al. (2008), the GMO Panel agrees that in field settings honeybees might face additional stresses, which could theoretically affect their susceptibility to Cry proteins or generate indirect effects.

The GMO Panel concludes that, in terms of risk to human and animal health and the environment, the provided information in Rose et al. (2007) does not present new scientific evidence that would invalidate the previous risk assessments of maize Bt11 and 1507.

2.9. Johnson et al. (2007)

This publication addresses general aspects about risk assessment and risk management of genetically modified plants and the interplay thereof in the context of risk analysis. No specific information is provided on any Bt-maize event.

2.9.1. Summary of the publication

According to Johnson et al. (2007), the debate concerning genetically modified crops illustrates confusion between the role of scientists and that of the wider society in regulatory decision-making. The authors identified 2 fundamental misunderstandings, which, if rectified, would allow progress with confidence. First, scientific risk assessment needs to test well-defined hypotheses, not simply collect data. Second, risk assessments need to be placed in the wider context of risk analysis to enable the wider 'non-scientific' questions to be considered in regulatory decision-making. The authors claimed that such an integration and understanding are urgently required because challenges to regulation will escalate as scientific progress advances.

2.9.2. Conclusions of the authors of this publication concerning the safety of Bt-maize

Johnson et al. (2007) concluded that the debate about genetically modified crops has exemplified how both scientists and members of society can misunderstand their roles as well as those of scientific risk assessment and risk analysis. According to the authors, the result of these misconceptions has been the persistent outrage that has enveloped the debate about genetically modified crops in Europe over the past decade. From this there has been a larger breakdown in society's trust of both scientific applications and the regulatory process safeguarding people and the environment against potential risks. The authors expect that the breakdown in trust could have much more far-reaching consequences for science and the acceptance of other emerging scientific innovation such as future advances in medicine (e.g., stem cell therapy), technological solutions to climate change and nanotechnology. They argued that sustainable development in its widest sense can only be achieved if our innovations are developed in the context of communication, accountability and trust.

2.9.3. GMO Panel assessment

The publication of Johnson et al. (2007) addressed risk assessment and risk management in general terms, and does not provide new or additional information about exposure or impact of Cry proteins to non-target organisms. The GMO Panel therefore concludes that, in terms of risk to human and animal health and the environment, the information provided in Johnson et al. (2007) does not present new scientific evidence that would invalidate the previous risk assessments of maize Bt11 and 1507.

2.10. Andow and Zwahlen (2006)

This publication is a review paper on general aspects of risk assessment of genetically modified plants and does not provide new original datasets on Bt-maize.

2.10.1. Summary of the publication

Andow and Zwahlen (2006) reiterated that by the end of the 1980s, a broad consensus had been developed that genetically modified plants might pose environmental risks that require an assessment and that this assessment must be done on a case-by-case basis, taking into account the transgene, recipient organism, intended environment of release, and the frequency and scale of the intended introduction. The authors explain that since 1990 there have been gradual but substantial changes in the environmental risk assessment process. In their review, Andow and Zwahlen (2006) focused on changes in the assessment of risks associated with non-target species and biodiversity, vertical gene flow, and the evolution of resistance in targeted pests. The authors reported that non-target risk assessments are now focusing on risks of genetically modified plants to the intended local environment of release. They also observed that measurements of vertical gene flow indicate that it occurs at higher rates than believed in the early 1990s, mathematical theory is beginning to clarify expectations of risks associated with vertical gene flow, and that management methods are being developed to reduce vertical gene flow and possibly mitigate its effects. Andow and Zwahlen (2006) also discussed that potential resistance development in targeted insect pests is now managed using a high-dose/refuge or a refuge-only strategy, and the present research focuses on monitoring for resistance and encouraging compliance to requirements. Andow and Zwahlen (2006) synthesized previous models for tiering risk assessment and proposed a general model for tiering.

2.10.2. Conclusions of the authors of this publication concerning the safety of Bt-maize

Andow and Zwahlen (2006) concluded that their review has focused on the assessment of environmental risks of genetically modified plants, but that potential environmental benefits also require rigorous scientific evaluation. Risk assessment methodologies were anticipated to continue evolving in the future, and additional research would be essential to ensure that this evolution is based on sound scientific information. In the authors' opinion, risk assessment is always an imperfect science, and it should be developed so that there are many ways to manage this uncertainty. Despite its imperfections, however, it provides the main way for scientific methodologies and data to influence decisions to regulate commercialisation of genetically modified crops, and should be supported and improved by environmental scientists. Scientific understanding of factors affecting environmental risk is still developing, and it is unlikely that controversies over environmental risks of genetically engineered organisms will be resolved in the near future. The authors therefore concluded that here is both the need and time for environmental scientists to have significant influence on the development of risk assessment methodologies for genetically modified plants.

2.10.3. GMO Panel assessment

The publication of Andow and Zwahlen (2006) addresses risk assessment in general terms, and discusses generic risk assessment issues associated with exposure and impacts of Cry proteins on target and non-target organisms. It should be noted that in order to further develop and improve scientific approaches on risk assessment of GMOs, there is a commitment by EFSA and its GMO Panel to continuously monitor progress in science and risk assessment methods. As part of this process EFSA organised the Colloquium on Environmental Risk Assessment of Genetically Modified Plants - Challenges and Approaches (EFSA, 2007b) and invited participation from a wide range of scientists and experts from EU and outside. Participants discussed and agreed on the current case-by-case approach to perform environmental risk assessment (ERA) of target and non-target organisms as outlined in the EFSA GMO Panel's Guidance Document (EFSA, 2006b), and agreed that EFSA should provide more detailed guidance information on the testing of non-target organisms.

In line with its previous scientific opinions, the GMO Panel maintains its requirement that the potential development of resistance in target pests continues to be monitored for detecting potential changes in resistance levels in pest populations. Applicants are generally requested to monitor resistance development in target pests under case-specific monitoring as part of their insect resistance management requirements and/or consider it under general surveillance through farmer questionnaires (EFSA, 2005a,b; Schmidt et al., 2008).

The GMO Panel concludes that, in terms of risk to human and animal health and the environment, the provided information in Andow and Zwahlen (2006) does not present new scientific evidence that would invalidate the previous risk assessments of maize Bt11 and 1507.

2.11. Butler et al. (2007)

This publication does not provide any specific information on Cry-expressing maize or its environmental impact, but rather assesses the impact on farmland biodiversity and ecosystem services of both novel farming practices associated to the use of genetically modified herbicide-tolerant (GMHT) crops and UK agri-environment schemes.

2.11.1. Summary of the publication

Butler et al. (2007) reported that sustainable development requires the reconciliation of demands for biodiversity conservation and increased agricultural production. Assessing the impact of novel farming practices on biodiversity and ecosystem services is deemed fundamental to this process according to the authors. Using farmland birds as a model system, the authors presented a generic risk assessment framework that accurately predicts each species' current conservation status and population growth rate associated with past changes in agriculture. Butler et al. (2007) demonstrated its value by assessing the potential impact on biodiversity of 2 controversial land uses, GMHT crops and UK agri-environment schemes. The authors argued that the proposed framework can be used to guide policy and land management decisions and to assess progress toward sustainability targets.

2.11.2. Conclusions of the authors of this publication concerning the safety of Bt-maize

According to Butler et al. (2007), the proposed framework provided a robust basis for assessing risk, and its application to GMHT crops and agro-environmental management has important implications for policy- and decision-makers. Butler et al. (2007) believed that their framework can also contribute greatly to the economic evaluation of proposed agricultural changes that alter the functioning of ecosystem services through their impact on biodiversity.

2.11.3. GMO Panel assessment

The publication of Butler et al. (2007) addresses risk assessment and environmental management in general terms, and assesses the environmental impact of novel herbicide regimes associated to the cultivation of GMHT crops. The GMO Panel therefore concludes that, in terms of risk to human and animal health and the environment, the provided information in Butler et al. (2007) does not present new scientific evidence that would invalidate the previous risk assessments of maize Bt11 and 1507. Moreover, since mainly novel herbicide management regimes used in maize cropping systems are determining the environmental impact of GMHT crops, the GMO Panel encourages that both applicants and appropriate competent authorities in Member States establish and implement herbicide management systems for GMHT crops that do no more environmental harm than conventional systems and which are consistent with the environmental protection goals and biodiversity action plans in each Member State (EFSA, 2008).

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel has assessed the 11 scientific publications selected and provided by the European Commission, and concludes that these publications do not provide new information that would change previous environmental risk assessments – including potential long-term effects – conducted on maize Bt11 and 1507 (EFSA, 2005a,b, 2006a). Having also considered other recent scientific publications, the GMO Panel reaffirms its previous conclusions on the environmental safety of maize Bt11 and 1507, expressed on 19 January 2005, 20 April 2005 and 7 November 2006.

DOCUMENTATION PROVIDED TO EFSA

1. Letter, dated 24 July 2008, including a list of scientific publications, from Jos Delbeke, Acting Director-General Environment EC, to Catherine Geslain-Lanéelle, Executive Director EFSA (ref ENV/B3/CB/AA D(2008) ARES (2008)16755) – Request to review recent scientific studies relating to the impact on the environment of the cultivation of two genetically modified maize plants: 1507 and Bt11.
2. Letter, dated 8 August 2008, from Catherine Geslain-Lanéelle, Executive Director EFSA, to Jos Delbeke, Acting Director-General Environment EC, requesting for a comprehensive data package.
3. E-mail from DG ENV providing the EFSA GMO Panel with some of the publications listed in the list.
4. Letter, dated 25 September 2008, from Catherine Geslain-Lanéelle, Executive Director EFSA, to Jos Delbeke, Acting Director-General Environment EC, acknowledging the mandate.

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