Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safeguard clause invoked by Greece according to Article 23 of Directive 2001/18/EC and to Article 18 of Directive 2002/53/EC[1] (Question No EFSA-Q-2006-048)

Opinion adopted on 7 November 2006

SUMMARY

On 29 March 2006, Greece invoked Article 23 of Directive 2001/18/EC and Article 18 of Directive 2002/53/EC (safeguard clause) to provisionally prohibit the cultivation of the authorised genetically modified maize MON810 on its territory. The European Commission received from Greece a written submission, composed of a scientific report, listing detailed reasons for supporting measures taken by Greece, and of 71 publications and statements.

As a consequence, the European Commission requested in a letter dated 4 May, 2006 a scientific opinion as to whether the scientific report and publications submitted by the Greek authorities show that there is an imminent danger for human health and the environment due to the cultivation of the maize varieties with the genetic modification MON810 expressing CRY1Ab protein.

Following investigation of the evidences presented in the Greek submission, EFSA's Scientific Panel on Genetically Modified Organisms (GMO Panel) concludes that, in terms of risk to human health and the environment, no new scientific evidence was presented that would invalidate the risk assessment of genetically modified maize MON810 established under Directive 90/220/EEC (repealed by Directive 2001/18/EC from 17 October 2002). The GMO Panel concluded that MON810 maize is unlikely to have adverse effects on human and animal health or on the environment due to the cultivation of the maize varieties with the genetic modification MON810 in Greece.

Key words: GMOs, maize (*Zea mays*), MON810, Greece, safeguard clause, *Ostrinia nubilalis* European Corn Borer (ECB), human health, environment, Directive 2001/18/EC, Directive 2002/53/EC.

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BACKGROUND

On 22 April 1998 the 15 EU Member States voted in favour of the Commission Decision N° 98/294/EC (EC, 1998) allowing the competent authority of the lead Member State, France, to give consent for the placing on the European market of the genetically modified maize (*Zea mays* L.) MON810, pursuant to Council Directive 90/220/EEC (EC, 1990). Before the decision, the European Commission had sought the opinion of the relevant Scientific Committees on this notification, as required by the legislation when competent authorities of Member States raised objections to the MON810 notification (reference C/F/95/12-02) that had been forwarded to the European Commission with a favourable opinion by the lead competent authority (France). The Scientific Committee on Plants on 10 February 1998 delivered an opinion, which concluded that there is no reason to believe that the placing on the market of MON810 maize would have any adverse effects on human or animal health and the environment (SCP, 1998). MON810 maize was authorised in the European Union for all intended uses, with the exception of food, by Commission Decision 98/294/EC on 22 April 1998 (EC, 1998) and final consent was granted by the French competent authority on 3 August 1998. Food use of maize MON 810 derivatives was notified according to Article 5 of Regulation (EC) 258/97 (EC, 1997) on 6 February 1998 (EC, 2004).

On 29 March 2006, the European Commission received a request from Greece related to a national ban of the marketing in Greece of maize hybrids with the genetic modification MON810 inscribed in the common catalogue of varieties. Greece provided a written submission, composed of a scientific report, listing detailed reasons for supporting measures taken by Greece, and of 71 publications and statements. On 4 May 2006 the European Food Safety Authority (EFSA) received a request from the European Commission to provide a scientific opinion on the Greek submission in the context of the safeguard clause invoked under Article 23 of Directive 2001/18/EC (EC,

2001) and under Article 18 of Directive 2002/53/EC (EC, 2002). The mandate for the request was adopted at the plenary meeting of the GMO Panel on 4-5 July 2006. EFSA was asked *'whether the scientific report and the scientific publications submitted by Greece show that there is an imminent danger for human health and the environment due to the cultivation of the maize varieties with the genetic modification MON810*'. An informal meeting with Greek representatives took place to further clarify some issues raised by Greece.

Greece is not the only country that invoked a safeguard clause on MON810 maize, either under Article 16 of Directive 90/220/EEC (EC, 1990) or under Article 23 of Directive 2001/18/EC (replacing Directive 90/220/EEC from 17 October 2002). On 1 June 1999 Austria invoked Article 16 of Directive 90/220/EEC. On 24 September 1999 the Scientific Committee on Plants delivered an opinion indicating that the justification and information submitted by the Austrian authorities did not impact on the original assessment of the genetically modified (GM) maize in terms of risks to human or animal health and the environment (SCP, 1999). In January 2003 Austria provided the European Commission with additional information which had been submitted to the EFSA's Scientific Panel on Genetically Modified Organisms (GMO Panel) for an opinion. On 8 July 2004 the GMO Panel concluded that there was no new scientific evidence, in terms of risk to human health and the environment, that would invalidate the risk assessments of genetically modified maize MON810 established under Directive 90/220/EEC or Directive 2001/18/EC (EC, 2001) and that would justify a prohibition of these genetically modified crops authorised under Directive 90/220/EEC or Directive 2001/18/EC in Austria (EFSA, 2004). On 21 January 2005, Hungary invoked Article 23 of Directive 2001/18/EC to provisionally prohibit the production, use and distribution of seeds derived from the authorised MON810 maize, along with the importation into its territory. The prohibition does not apply to food and feed uses of MON810 maize. On 8 June 2005, following an analysis of the evidence presented in the Hungarian submission, the GMO Panel concluded that, with regard to risk to human health and the environment, there is no new scientific evidence which would invalidate the risk assessment of MON810 maize established under Directive 90/220/EEC (EFSA, 2005d).

In 2005, the Member States (Austria, France, Germany, Greece and Luxemburg) which invoked safeguard clauses on one or more GMOs (Bt176, T25 and MON810 maize, and Ms1xRf1 or Topas 19/2 oilseed rape) under Article 16 of Directive 90/220/EEC were asked by the European Commission to lift their bans or to re-confirm the measures invoked under article 23 of Directive 2001/18/EC replacing Directive 90/220/EEC from 17 October 2002. In that context, on 10 November 2005, EFSA received a request from the European Commission to reconsider the previous scientific opinions assessing the safety of these GMOs in the light of any new information subsequent to these assessments. The GMO Panel was of the opinion that, with respect to the specific questions raised by the European Commission and on the basis of current scientific knowledge, there was no reason to believe that the continued placing on the market of Bt176, T25 and MON810 maize, and Ms1xRf1 and Topas 19/2 oilseed rape was likely to have adverse effects on human and animal health or on the environment, under the conditions of their respective consents (EFSA, 2006a).

TERMS OF REFERENCE

EFSA was requested, under Article 29(1) and in accordance with Articles 22(2) and 22(5)(c) of Regulation (EC) No 178/2002, to provide a scientific opinion as to whether the scientific report and the scientific publications submitted by the Greek authorities

show that there is an imminent danger for human health and the environment due to the cultivation of the maize varieties with the genetic modification MON810. EFSA should confine itself to the above terms of reference and did not need to consider elements, such as the fact that the decision of MON810 was given in 1998 and should be renewed, discussions in the Council, reference to the Cartagena Protocol, reference to the Greek Constitution, to co-existence issues contained in the introductory note. EFSA should analyse the scientific report documenting the hazard together with the bibliography.

ASSESSMENT

1. Introduction

Consents for the deliberate release of GMOs into the environment, including the placing on the market of GMOs, were formerly granted under the previous Directive 90/220/EEC, which was repealed by Directive 2001/18/EC on 17 October 2002. Of these authorized products, seeds from MON810 maize have been authorised for the placing on the market in the EU, including for cultivation purposes.

Article 23 of Directive 2001/18/EC related to the safeguard clause states that:

- Where a Member State, as a result of new or additional information made available since the date of the consent and affecting the environmental risk assessment or reassessment of existing information on the basis of new or additional scientific knowledge, has detailed grounds for considering that a GMO as or in a product which has been properly notified and has received written consent under this Directive constitutes a risk to human health or the environment, that Member State may provisionally restrict or prohibit the use and/or sale of that GMO as or in a product on its territory. The Member State shall ensure that in the event of a severe risk, emergency measures, such as suspension or termination of the placing on the market, shall be applied, including information to the public. The Member State shall immediately inform the Commission and the other Member States of actions taken under this Article and give reasons for its decision, supplying its review of the environmental risk assessment, indicating whether and how the conditions of the consent should be amended or the consent should be terminated, and, where appropriate, the new or additional information on which its decision is based.

- A decision shall be taken on the matter within 60 days in accordance with the procedure laid down in Article 30(2). For the purpose of calculating the 60 day period, any period of time during which the Commission is awaiting further information which it may have requested from the notifier or is seeking the opinion of the Scientific Committee(s) which has/have been consulted shall not be taken into account. The period of time during which the Commission is awaiting the opinion of the Scientific Committee(s) consulted shall not exceed 60 days. Likewise, the period of time the Council takes to act in accordance with the procedure laid down in Article 30(2) shall not be taken into account.

Article 18 of Directive 2002/53/EC related to the safeguard clause states that:

> If it is established that the cultivation of a variety included in the common catalogue of varieties could in any Member State be harmful from the point of view of plant health to the cultivation of other varieties or species, or present a risk for the environment or for human health, that Member State may upon application, be authorised in accordance with the procedure referred to in Article 23(2) or in Article 23(3) in the case of a genetically modified variety to prohibit the marketing of the seed or propagating material of that variety in all or part of its territory. Where there is imminent danger of the spread of harmful organisms or imminent danger for human health or for the environment, that prohibition may be imposed by the Member State concerned as soon as its application has been lodged until such time as a final decision has been taken. That decision shall be taken within a period of three months in accordance with the procedure laid down in Article 23(2) or in Article 23(3) in the case of a genetically modified variety.

The GM maize line MON810 has therefore been evaluated at the national and EU level prior to market approval and thereafter in the context of previous safeguard clauses. MON810 maize was assessed by the Scientific Committee on Plants (SCP, 1998; 1999). The Scientific Committee on Plants concluded that there is no evidence indicating that the seeds of insect resistant maize MON810 when grown, imported and processed are likely to cause adverse effects on human or animal health and the environment.

2. Evaluation of documents delivered by Greece in relation to current scientific knowledge

The GMO Panel has examined the Greek submission and supporting references of the Greek Ministry of Rural Development and Food in its document '*Scientific data concerning the decision to prohibit in Greece the marketing of maize hybrids with the genetic modification MON810 inscribed in the common catalogue of varieties of the European Union*'. Greece presented 71 references and statements in support of their invocation.

The GMO Panel looked for evidence for GMO-specific risks taking into consideration the EFSA guidance document for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006b).

Two main aspects were considered:

- whether new scientific evidence had been presented by Greece which would change the initial risk assessment conducted on MON810 maize which currently has marketing consent in the EU;
- whether there was scientific evidence supplied which would indicate that the cultivation conditions, the environment or the ecology of Greece merited a separate environmental risk assessment, including potential long-term effects, from that applied to other regions of EU.

Risk assessment and approval of GMOs according to Directive 2001/18/EC is carried out on a case-by-case basis. The Directive provides the possibility for Member States to raise objections to the marketing of specific GMOs. If necessary, the risk assessment

may include features specific to certain geographical regions or sub-regions. Furthermore, Article 23 of the Directive provides safeguards in the case where new or additional information would affect the risk assessment of an authorised GMO. The provisions foreseen by Greece seek to provisionally prohibit MON810 seeds from cultivation.

The Greek submission specifies five subjects of concern:

A. Environmental impacts of MON810 maize, particularly in relation to biodiversity, ecosystem stability and potential adverse effects on non-target fauna, taking into account the specific climatic and agricultural conditions in Greece;

B. Genome scrambling in MON810 maize ;

C. Impact of MON810 maize on the agricultural environment due to resistance development in target insects ;

D. Impact of MON810 maize on the large-scale beekeeping industry in Greece;

E. Impact of MON810 maize on human health.

The GMO Panel evaluated the information and documents provided by the Greek authorities which were subsequently clarified in an informal meeting with Greek representatives. The assessment follows the structure of the Greek submission.

A. Environmental impacts of MON810 maize, particularly in relation to biodiversity, ecosystem stability and potential adverse effects on non-target fauna, taking into account the specific climatic and agricultural conditions in Greece

The GMO Panel noted that Greece referred to an extensive set of information mainly related to effects of GMOs observed outside the territory of Greece. The GMO Panel comments on the Greek presentation of scientific literature and statements as follows:

i) Potential effects of MON810 maize on pest Lepidoptera

MON810 maize varieties expressing CRY1Ab protein are not only protected against *Ostrinia nubilalis,* the European Corn Borer (ECB), but are also used to control other lepidopteran pests species such as *Sesamia nonagrioides* (Eizaguirre *et al*., 2006 ; González-Cabrera *et al.,* 2006; Novillo *et al*., 2003).

The GMO Panel considers that low infestation levels of *Ostrinia nubilalis* and other lepidopteran pests in maize in Greece do not alter the environmental risks associated with MON810 cultivation. MON810 cultivation only gives an economic advantage when pest infestation is above certain levels and so MON810 maize is only likely to be grown in areas where lepidopteran pests will cause significant damage. For example, in the low *Ostrinia nubilalis* infestation areas of Spain, only a small proportion of farmers have chosen to grow CRY1Ab-expressing maize (Departament d'Agricultura, Ramaderia i Pesca, 2006).

ii) Potential effects of MON810 maize on green lacewings

According to Rodrigo-Simon *et al*. (2006), CRY1Ab protein does not show specific binding *in vitro* to brush border membrane vesicles from the midgut of *Chrysoperla carnea* larvae, which is a prerequisite for toxicity. When *Chrysoperla carnea* larvae are

fed lepidopteran larvae reared on CRY1Ab-expressing maize, laboratory studies indicate significantly prolonged larval development and increased mortality (Hilbeck *et al*., 1998a,b; 1999). Obrycki *et al*. (2001), cited by Greece, relates to the original research of Hilbeck *et al*. (1998a, b; 1999). However, from the protein binding studies noted above, it can 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 *Chrysoperla carnea* larvae are unaffected when feeding on non-susceptible *Tetranychus urticae* containing large amounts of biologically active CRY1Ab protein (Dutton *et al*., 2002). *Chrysoperla 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 molt. Because aphids are not harmed by CRY1Ab protein, the risk that this crop poses for *Chrysoperla carnea* larvae can be regarded as negligible. No negative effects on these predators have been documented in the field; sampling from CRY1Ab-expressing maize fields has not shown a decline in their abundance (Bourguet *et al*., 2002).

iii) Potential unanticipated adverse effects of MON810 maize on parasitoids

Greece cited Hafez *et al*. (1997), who studied the effects of a conventional Bt spray (Dipel). This is of limited relevance to MON810 maize because this Bt formulation contains a number of different CRY proteins so that any observed effect cannot be directed to the truncated CRY1Ab protein. The effects of Bt plants on hymenopteran parasitoids developing in herbivores reared on transgenic plants have been investigated in several studies (see Romeis *et al*., 2006). Effects on mortality, development, weight or longevity were observed in all cases where CRY1Ab-susceptible lepidopteran herbivores were used as hosts. This information is not surprising, given that host-parasitoid relationships are usually tight and parasitoids are very sensitive to changes in host quality. Parasitoids developing in Bt-fed larvae of a resistant strain of *Plutella xylostella* (diamondback moth) were not affected. This confirms that host quality was most likely the cause of effect in the studies cited by Greece. The publication of Prutz *et al*. (2004) was a laboratory 'tier 2' study confirming the tight relationship between parasitoids and their hosts. In higher tier studies, Bourguet *et al*. (2002) and Siegfried *et al*. (2001) found that populations of specific natural enemies of *Ostrinia nubilalis* are less abundant in CRY1Ab-expressing maize fields than in non-Bt maize fields. This is not thought to be due to the direct effects of the CRY1Ab protein consumed while predating or parasitizing *Ostrinia nubilalis* but to decreased availability of specific prey.

iv) Potential effects of MON810 maize on Coleoptera

Greece cited a study of Wold *et al*. (2001) who observed a significant effect in one year of their two-year study. The authors' statistical analyses of their inconsistent numerical data suggested that there was insufficient evidence to conclude that CRY1Ab protein had any adverse effect on beneficial insects in the field. Unlike the case of Bt-cotton, to date, only trace amounts of the CRY proteins have been detected in phloem-feeders (aphids) on different Bt maize events. Thus, aphidofagous predators, such as several lady beetle species, are unlikely to be significantly exposed to the CRY1Ab protein via their preys. Laboratory studies indicate that direct feeding on Bt plant material poses a negligible risk for these predators (Romeis *et al*., 2006). Thus, predators preferentially feeding on aphids, such as lady beetles, are unlikely to be at risk. The diversity of carabid assemblage on MON810 maize plots did not differ from the one on isogenic plots (Szekeres *et al*., 2006).

v) Potential long-term exposure to CRY1Ab protein from MON810 maize in the field

The GMO Panel has recommended that monitoring for resistance is a requirement for all Bt crops cultivated in the EU. Six year studies of exposure to CRY1Ab maize in Spain

(Eizaguirre *et al*., 2006) have shown no resistance development in *Ostrinia nubilalis*. In addition they showed no adverse effects on non-target species compared with non-GM plant material. Also CRY1Ab-expressing maize had no adverse effects on predator species studied using criteria such as life-table parameters (e.g. longevity or fecundity) or abundance (e.g. Eckert *et al.*, 2006; Romeis *et al*., 2006).

vi) Potential adverse effects of MON810 maize on Collembola

The review paper of Losey *et al*. (2004) refers to a website of the U.S. Environmental Protection Agency (Website EPA) for a study that might have shown a significantly higher mortality and reduced reproduction rate in the soil-dwelling collembolan, *Folsomia candida* exposed to the Bt176 maize event. The EPA website was accessed by the GMO Panel. There was no information available that would relate to the above mentioned study on Bt176 maize. In other studies MON810 maize has shown no negative effects on the collembollan *Protaphorura armata* (Heckmann *et al*., 2006). In addition, no adverse effect of CRY1Ab-expressing maize on Collembola was observed in an intensive field study with Bt176 maize (Candolfi *et al*., 2004). The GMO Panel concludes that no adverse effect of CRY1Ab-expressing maize on Collembola has been reported.

vii) Potential adverse effects of MON810 maize on non-target Lepidoptera

It is well documented that a range of lepidopteran species may be affected by CRY1Ab proteins (Glare and O'Callaghan, 2000) and some of these species may be present in maize fields (Schmitz *et al*., 2003; for a review see Evans, 2002). However, the exposure of any populations of Lepidoptera to the protein is restricted to those consuming the CRY1Ab plant or its products. In the vicinity of the MON810 maize field, larvae may be most exposed to the protein when MON810 maize pollen is deposited on plants on which they are feeding. Losey *et al*. (1999) reported harm to monarch butterfly (*Danaus plexippus)* from Bt176 maize pollen in a tier 1 (toxicity) laboratory study. Subsequently, a series of ecologically based studies have been carried out on higher tiers to evaluate rigorously the impact of pollen from such crops and to complete the risk assessment based on quantitative and exposure data. The results of this investigation demonstrated that the commercial large-scale cultivation of tested Bt maize hybrids (including MON810) did not pose a significant risk to the *Danaus plexippus* populations (Gatehouse *et al*., 2002). Dively *et al.* (2004) concluded in their overall risk assessment that it is unlikely that MON810 maize will significantly affect *Danaus plexippus* populations in North America. Zangerl *et al*. (2001) reported effects of exposure of monarch butterflies and Black Swallowtail caterpillars to Bt176 maize pollen under field conditions. The effect is not surprising given the higher level of Cry1Ab expression in pollen of the Bt176 compared to MON810 maize. A recent study in Germany has demonstrated that MON810 maize poses negligible risk to non-target Lepidoptera (Gathmann *et al*., 2006). Maize, an introduced species into Europe, is not a significant food source for endemic Lepidoptera in Europe and impacts due to pollen dispersal are likely to be transient and minor as demonstrated by studies on monarch butterflies in the USA (Dively *et al*., 2004) or on other butterflies in Germany (Gathmann *et al*., 2006). Greece did not show any quantitative data on silkworm exposure to maize pollen, e.g. the maize pollen distribution on mulberry trees near maize fields (similar to Fan *et al*., 2003). Traditionally silkworms are fed with mulberry leaves taken from the trees and transported into special cocoon houses to avoid silkworm losses by birds (and insecticides) in the field. This measure would decrease the direct exposure of silkworms to maize pollen. In this respect, Yao *et al.* (2006) conducted a series of laboratory bioassays to evaluate the effect of the pollen from a GM rice line with a fused *cry1Ab/cry1Ac* gene on silkworms. No significant adverse effects were observed on the survival, growth and development of silkworm young larvae, even after the neonates had been exposed to Bt pollen at the highest density of 3,395.0 grains/cm^2 for 48 h.

The tested pollen density is far higher than the highest pollen density on mulberry leaves found under field conditions, 1,635.9 grains/cm². Yao *et al.* (2006) concluded that Bt rice pollen pose little effect on silkworm rearing in natural settings. Taking into account the available literature data, the GMO Panel is of the opinion that the risk of MON810 pollen to silkworm and other non-target lepidopteran species is negligible due to its low CRY1Ab content and the low levels of exposure of wild species to maize pollen.

viii) Potential effects of MON810 maize residues on soil function and soil organisms (specifically earthworms)

CRY proteins are rapidly decomposed in soil (Glare and O´Callaghan, 2000; Hopkins & Gregorich 2003, 2005; Baumgarte & Tebbe, 2005). Saxena and Stotzky (2001a) reported that CRY1Ab protein had no apparent effect on earthworms and nematodes in a 45-day study. Zwahlen *et al.* (2003) reported a 200-day study investigating the impact of Bt11 maize on immature and adult *Lumbricus terrestris* in a single worst-case laboratory study and in a single small scale field test. At the end of the laboratory test the earthworms showed a significant weight loss of 18% (compared with their initial weight) when fed CRY1Ab-expressing maize litter whereas a weight gain of 4% occurred with non-GM control maize. No difference was found in the higher tier small scale field test. Due to the experimental design, the authors stated that they were unable to exclude the possibility that the weight loss of earthworms fed with CRY1Ab-expressing maize in the laboratory test was due to other factors. A study by Vercesi *et al*. (2006) indicates that CRY1Ab-expressing maize apparently poses minimal risks to earthworms as far as growth and reproduction is concerned. Vercesi *et al*. (2006) also recognized a small negative effect on cocoon hatching success, but this was at relatively high concentrations of finely ground CRY1Ab-expressing maize material and it can be questioned whether this effect would have any ecological significance under field conditions. The published results from laboratory and field trials showed that on short to medium time scales (up to 3 years) and under field conditions, the effects of CRY1Ab-expressing maize on soil functions and biodiversity (Blackwood and Buyer, 2004; Motavalli *et al*., 2004; Evans, 2002) does not exceed "natural" variability. No conclusive evidence has yet been presented that currently approved CRY1Ab-expressing GM crops are causing significant direct effects on the soil environment. The effects of CRY1Ab-expressing maize in these experiments were small, if they existed at all. In addition, the available data do not indicate a chain of events that might result in long-term effects. Therefore, it seems likely that in commercial cropping conditions, where crop rotations are used, the consequences of effects on soil functions and soil organisms are negligible. There have been no reports of soil function problems in countries where CRY1Ab-expressing crops have been cultivated continuously for several years. The GMO Panel is thus of the opinion that the risk of MON810 maize to soil function and soil organisms is negligible.

ix) Potential higher lignin content in MON810 maize

Saxena and Stotzky (2001b) found higher lignin contents in three maize events (Bt11, Bt176, and MON810 maize) genetically modified to express the *Bacillus thuringiensis* CRY1Ab protein. The statement of Saxena and Stotzky (2001b) was commented upon by the GMO Panel in their opinions on 1507 and Bt11 maize (EFSA, 2005a, 2005b). Poerschmann *et al.* (2005) confirmed the occurrence of pleiotropic effects with regard to lignin biosynthesis in stems of Bt maize as described by Saxena and Stotzky (2001b), although to a lesser extent. Another study suggests that the extent of lignification of Bt GM maize (several lines derived from MON810 and Bt11 maize) does not differ from the non-GM controls (Jung and Sheaffer, 2004). Recently, the decomposition of different plant species expressing Bt proteins was analysed in laboratory experiments and results were discussed in relation to lignin contents and potential environmental consequences

(Flores *et al*., 2005). Generally, Bt plants showed less decomposition than non-Bt plants. However, this effect was not clearly related to lignification or reduced microbial activity in soil. The authors concluded that lower decomposition rates may be beneficial as organic matter derived from plants would persist for a longer period of time improving soil structure and reducing erosion. Flores *et al*. (2005) also discussed potential effects on target and non-target insects due to the longer persistence of Bt proteins in soil. In relation to soil organic content, it has been shown that even distinct increases in decomposition resistant compounds such as lignin result in only modest increases in organic carbon in the topsoil. Changes in soil management have a much more pronounced effect (Sessitsch *et al*., 2004). Considering the available information on potential effects of Bt plants on the soil environment and in particular on soil non-target organisms, the GMO Panel concluded that adverse effects due to slightly altered lignin contents are unlikely.

x) Potential impact of MON810 maize on biodiversity

There are considerable data on the long-term ecological and biodiversity effects of CRY1Ab-expressing maize (e.g. Dutton *et al*., 2003 a,b ; Rauschen *et al*., 2004 ; Lövei and Arpaia, 2005 ; O'Callaghan *et al*., 2005 ; Romeis *et al*., 2006 ; Eckert *et al*., 2006 ; Eizaguirre *et al*., 2006 ; Gathmann *et al*., 2006). Dolezel *et al.* (2006) concluded that there is no evidence of environmental harm from CRY1Ab-expressing maize and that only long-term monitoring studies of commercialised crops could provide data sufficient to indicate ecological impact. The GMO Panel agrees with Mendelsohn *et al*. (2003) that MON810 maize poses no specific significant risk to the environment or to human health compared with other maize types.

xi) Potential food-chain effects of MON810 maize

The GMO Panel agrees that tri-trophic effects e.g. on non-target organisms (like maize lepidopteran pest) are an important issue to consider during the environmental risk assessment (ERA). However, the overall data for effects of MON810 maize on biodiversity indicate that the environmental risks to non-target species are negligible. Genetic modified maize did not have a negative impact on non-target species in the field after six years of CRY1Ab-expressing maize cultivation (Bt176 and MON810 maize) in Spain (Eizaguirre *et al*., 2006). More aphids and leafhoppers but similar numbers of cutworms and wireworms were counted in Bt *versus* non-Bt fields. Eizaguirre *et al*. (2006) observed no difference in the numbers of the most relevant predators in fields containing GM or non-GM maize. No adverse effects of MON810 maize on non-target insects including butterflies were observed during a three-year field study in Germany (Rauschen *et al*., 2004; Gathmann *et al*., 2006; Eckert *et al*., 2006). In these two studies, potential long-term effects were addressed due to the continuous growth of MON810 maize without any crop rotation. The GMO Panel considers that MON810 maize will have effects similar to those of comparable non-GM maize cultivars on the environment. In addition, reports and reviews of studies of the effects of the CRY1Ab protein on biodiversity, including the abundance of non-target and biocontrol species, indicate that significant adverse environmental effects due to CRY1Ab-expressing maize cultivation are unlikely (Amman, 2005; Clark *et al*., 2005; Dolezel *et al*., 2006; Eizaguirre *et al*., 2006; Rodrigo-Simon *et al*., 2006; Romeis *et al*., 2006).

xii) MON810 maize and the Greek environment

The Greek submission referred to local particulars such as wind, irrigation and cultivation management which could increase environmental risks associated with MON810 cultivation. The link between maize irrigation, resulting in an increase in local humidity, and an increased incidence of *Ostrinia nubilalis* is an issue relevant to maize cultivation in other regions as well e.g. in Spain, where incidence of *Ostrinia nubilalis* is

related to other geographical and climatic factors independent of irrigation. The GMO Panel is not aware of any implication of long-lasting dry and hot periods accompanied by strong wind for the biosafety of the MON810 maize in comparison to non-GM maize. Again such conditions also occur in Spain and there have been no reports of related problems with the growing CRY1Ab-expressing crops. The specific Greek concern of CRY1Ab protein exposure to honeybees is addressed in more detail in section D below.

Conclusions: The GMO Panel included in its review of the risk assessment of MON810 maize consideration of the potential long-term effects and effects due to the co-cultivation and continuous cultivation of maize and cotton. The GMO Panel took into account the environmental parameters and particular circumstances described by Greece. The GMO Panel concludes that the Greek submission provided no new scientific data or information in support of their particular concerns or the more general issues discussed above.

B. Genome scrambling in MON810 maize

The Greek submission questioned the molecular characterization of MON810 maize. In particular, Greece stresses that unintended effects due to the so-called '*genome scrambling*', might have occurred during the transformation process of MON810 maize event. The GMO panel recognizes that the process of introducing genes by genetic transformation technologies such as *Agrobacterium* or particle bombardment-mediated gene transfer can result in DNA re-arrangements at the site of insertion and, for example that non-target sequences e.g. from the vector backbone may also be inserted. The GMO Panel is also aware of the potential for genetic and compositional changes caused by somaclonal variation in tissue/cell culture rather than by foreign gene insertion *per se*. The phenomena described above could indeed give rise to unintended effects, which is why the GMO Panel maintains a comprehensive and holistic approach to risk assessment, which does not rely on any single approach. The approaches are well described in the EFSA Guidance document (EFSA, 2006b).

With regard to molecular characterization, applicants are required to provide information on the transgene locus. This includes the DNA sequence of the inserts and flanking regions. This allows the identification of unintended sequences, if any, and potential re-arrangements of the transgene locus. Bioinformatic studies are then used to assess the possibility that open reading frames are interrupted and to indicate the possibility that potential fusion proteins may be produced which influence allergenicity and toxicity potential of the product.

The molecular analysis is complemented by further analyses e.g of agronomic performance and chemical composition of the GMO to provide an indication of possible unintended effects. These analyses would also reveal changes caused by somaclonal variation. For each species examined the GMO Panel requires appropriate non-GM controls to be included in the comparative analyses and also takes into account the extent of natural variation e.g. in chemical composition as this provides an important benchmark with respect to history of safe use. The GMO Panel uses approaches, which are in line with those recommended by OECD, WHO etc. After having analysed all the data, no specific risk was identified by the GMO Panel due to the possibility of genome scrambling.

As the *cry1Ab* gene in MON810 maize is driven by the CaMV 35S promoter, Greece has concern that the CaMV 35S promoter may be transferred, possibly integrated, and might influence the expression of other bacterial genes, and genes in viruses and

mammalian tissue (Ho *et al*., 2000; Myhre *et al*., 2006). The GMO Panel dealt with issues related to the use of the 35S CaMV promoter in a previous opinion (EFSA, 2003). The CaMV 35S promoter is derived from the common cauliflower mosaic virus (CaMV) and is a promoter frequently used in the genetic modification of (crop) plants. It has been suggested (Ho *et al*., 1999) that the CaMV 35S promoter could result in an inadvertent activation of plant genes or endogenous viruses, promote horizontal gene transfer, or might even recombine with mammalian viruses with unexpected consequences. Arguments for the safety of the promoter in GM crops are provided by Hull *et al*. (2000). Furthermore, in 2002 the UK Advisory Committee on Releases of GM crops into the Environment considered the CaMV 35S promoter issue (ACRE, 2002) and concluded that no new data or direct experimental evidence had been presented to support the hypothesis that the promoter is inherently unsafe. Moreover, humans and animals have been eating plant material containing the 35S promoter via natural CaMV infection and no adverse effects have been reported. Recent findings by Myhre *et al*. (2006) are in line with previous studies showing that the CaMV 35S promoter can be active *in vitro* in mammalian cells. However there is no evidence that the transfer and integration of active promoter fragments occurs *in vivo*. Therefore the GMO Panel is of the opinion that the conclusions of the ACRE study regarding the safety of the CaMV 35S promoter are still valid.

Greece is concerned about accepting that safety studies of CRY proteins are performed with proteins produced in bacteria, as the nucleotide sequence of the corresponding genes integrated into GM crops may not be precisely the same as the nucleotide sequence of the bacterial gene. However, an analysis of the suitability of bacterially produced proteins has already been carried out by the Competent Authorities of France and the Scientific Committee on Plants in their original assessment (SCP, 1998). The altered sequence of the *cry1A*b gene in MON810 maize has been assessed by the GMO Panel in previous opinion (EFSA, 2005b).

Greece expressed concern over the detection methods. Hernández *et al*. (2003) describes a specific real-time quantitative PCR detection system for identifying the MON810 maize event. Subsequently, other event specific detection methods have been proposed by, for example, Germini *et al*. (2004, 2005), Huang and Pan (2004), Bordoni *et al*. (2005), Onishi *et al*. (2005), Yoshimura *et al*. (2005), and Hernández *et al*. (2005). Issues related to specific molecular detection methodologies as well as on their validation are not within the scope of the GMO Panel.

Conclusions: The GMO Panel concludes that the Greek submission provided no new scientific data or information in support of their particular concerns on molecular characterization.

C. Impact of MON810 maize on the agricultural environment due to resistance development in target insects

In the Greek submission concerns were expressed that the development of CRY1Ab protein resistance in target pest species is only focused on *Ostrinia nubilalis,* the European Corn Borer (ECB). Greece cited a paper of Munkvold & Hellmich (1999) which describes the situation in the USA, where *Sesamia nonagrioides* is not found. However, in Greece and Europe the main target pest is *Sesamia nonagrioides*. Both lepidopteran species are the subject of monitoring and resistance management studies in Europe, for example in the EU 6th-Framework research project ProBenBt (http://ec.europa.eu/research/quality-of-life/cell-factory/volume2/projects/qlk3-2002-01969_en.html). According to the results generated in this project, gene flow between

insect populations will in all likelihood be high enough to restrict the development of resistant populations when applying the high-dose/refuge resistance management strategy. Lepidopteran pest studies demonstrated similar susceptibility of *Ostrinia nubilalis* populations to CRY1Ab protein. Gene flow between e.g. lepidopteran populations is expected to be sufficiently high to be maintained by adequate resistance management strategies. For future resistance monitoring only few *Ostrinia nubilalis* populations per country/geographically similar region may be necessary as representative populations for susceptibility screening/monitoring. No major alleles that make pest resistant to CRY1Ab-expressing maize have been detected so far in EU populations of *Ostrinia nubilalis* and *Sesamia nonagrioides*. This implies that recessive resistance alleles are rare in European populations and the frequency of resistance alleles are below 1 x 10^{-3}. Up to now, resistant *Ostrinia nubilalis* or *Sesamia nonagrioides* have not been found in fields in the US or in Europe (Evans, 2002; Tabashnik *et al*., 2005; Bourguet *et al*., 2002; Farinós *et al*., 2004) or in Spain (Eizaguirre *et al*., 2006). Although laboratory tests have shown that *Ostrinia nubilalis* populations are capable of developing some degree of tolerance to the CRY1Ab protein, laboratory selection and F2 screening to generate highly resistant *Ostrinia nubilalis* strains have failed so far (Bourguet, 2002). However, another lepidopteran pest (*Plutella xylostella*) has developed resistance to Bt proteins (Tabashnik *et al*., 2005). The GMO Panel concludes that large scale cultivation of MON810 maize over several years will increase the selection pressure on *Ostrinia nubilalis*, which might result in the development of resistance. This could have several consequences including the use of alternative phytosanitary measures to control the pest e.g. the use of insecticides other than Bt proteins. The GMO Panel agrees that the likelihood of resistant *Ostrinia nubilalis* occurring is low since, under field conditions and several years of cultivation, no resistance has been reported. However, cultivation of CRY1Ab-expressing maize in Europe is currently on a small scale and limited to a few geographic regions. Thus it is difficult to predict future responses of *Ostrinia nubilalis* populations in Europe. Therefore, the GMO Panel has recommended that monitoring for resistance is a requirement for all Bt crops cultivated in the EU.

The development of resistance is complex and is influenced by a variety of interacting factors, including the extent of selection pressure by Bt plants, the mode of inheritance, and migratory behaviour of the adult butterflies (Tabashnik *et al*., 2005; Kranthi *et al*., 2006). The most promising resistance management strategy entails the use of plants with a high dose of protein in combination with the maintenance of refuge crops that produce Bt-susceptible insects within the pest population. The three most important pre-requisites for a successful functioning of refuge strategy are "recessive resistant allele," "high dose" expression of CRY proteins in Bt plants, and "rare resistant allele" in field populations (Kranthi *et al*., 2006). Although the inheritance of resistance to Cry1Ac Bt cotton in Indian populations of *Helicoverpa armigera* was defined as a semi-dominant trait (Kranthi *et al*., 2006), the studies indicated that resistance to CRY1Ac in *Helicoverpa armigera* strains from China was inherited as an incomplete recessive trait, suggesting that the heterozygous genotype was still susceptible to CRY1Ac protein (Liang *et al*., 2000). Also, it was reported that the main varieties of Bt cotton being used in China could not be considered high dose for *Helicoverpa armigera* because some larvae could survive on Bt cotton in the late season (Wu *et al*., 2003). However, according to Wu *et al*. (2006), the susceptibility to CRY1Ac of the field populations sampled in China was not different from the baseline in 1997, and no movement toward resistance among *Helicoverpa armigera* populations was apparent. The CRY1Ac example demonstrates the need for a case-specific monitoring of *Helicoverpa armigera*, if significant CRY1Ab containing conventional Bt spays are used in both maize and cotton cultivation. Where CRY1Ab containing conventional Bt spays are also used in both maize and cotton cultivation in Greece (for *Helicoverpa armigera*, control), the

management of CRY1Ab resistance in target pests should also consider Bt protein exposure through this route.

Conclusions: The GMO Panel is of the opinion that the risk to the agricultural environment due to resistance development in target insects and the specific conditions prevailing in Greece mentioned in the Greek statement is currently low. The GMO Panel concludes that the evidence provided by Greece to support its concern can be adequately addressed by implementing case-specific monitoring as is being conducted in Spain.

D. Impact of MON810 maize on the large-scale beekeeping industry in Greece

Greece expressed concerns over adverse effects of MON810 maize pollen to bee health due to the observation that bees may visit the male flowers for pollen collection and transport the collected pollen to their hives for feeding. Potential adverse effects of specific Bt-proteins or of Bt-maize pollen on honey bees have been investigated several times either under laboratory or under 'semi-field' conditions. Experimental approaches aiming at simulating responses of honey bee field colonies exposed to Bt are very complex and characterized by shortcomings caused by methodological and biological difficulties e.g. due to the short life cycle of bees (max.: 2 month) or ecological interactions at a population level. Malone (2004) reviewed previous publications which included effects of CRY1Ba (not CRY1Ab) on lepidopteran/coleopteran pests (Naimov *et al*., 2001) and the foraging/longevity of honey bees (Malone *et al*., 2001). Malone *et al*. (2001) provided young tagged bees in enclosed cages, with a specific diet (CRY1Ba-free pollen or pollen enriched with 625 µg/g dw CRY1Ba protein) for 7 days. The bees were transferred back to their hives for investigations under 'normal' hive conditions. Even after the short period of separation from their 'home-colonies', the bees had lost their typical hive-odours. Consequently, from colony to colony a variable number of bees were rejected and died soon after return. Due to these colony-effects the numbers of surviving bees differed substantially, so that differences between colonies were greater than differences between treatments. Under these conditions Malone *et al*. (2001) found the mean longevity of CRY1Ba fed bees to be 1,3 days shorter than that of control bees, resulting in a life span reduction of 6,8 %. Malone *et al.* (2001) could not find any impact of the CRY1Ba diet on flying behaviour. Since the Bt-effect found was not unequivocally attributable to CRY1Ba, Malone *et al.* (2001) concluded "*that transgenic Bt-plants will be similarly harmless to bees, rather than a mixture of proteins, spores and vegetative stages, as is the case with Bt bio-pesticides*." This conclusion is of special importance since realistic expression levels of CRY1Ab protein in MON810 pollen are at < 90 ng/g dw. Cry-protein levels added to maize pollen in the bee feeding study of Malone *et al*. (2001) were ca. 10.000 times higher than the levels of CRY1Ab protein found in pollen of MON810 maize. In the review, Malone (2004) concludes: "*Evidence available so far show that none of the GM plants currently commercially available have significant impacts on honey bee health*". The GMO Panel agrees with this statement for MON810 maize and does not see the need for further risk assessment concerning the direct exposure of bees to MON810 maize.

The GMO Panel considered other available literature as well. Babendreier *et al.* (2005) investigated the suitability of hypo-pharyngeal gland development of worker bees as an indicator of potential disturbances in honey bee colony development due to pollen expressed CRY1Ab proteins or protease inhibitors. The hypo-pharyngeal gland is used to prepare food for the young bees. The authors fed young bees for 10 days with CRY1Ab maize pollen or with purified CRY1Ab protein solubilized in sugar solutions. The authors found no significant differences either in diameter or in weight development of hypo-

pharyngeal glands of control bees and bees fed with CRY1Ab pollen or CRY1Ab-containing sugar solutions. By contrast protease inhibitors caused significant differences which indicated the sensitivity of the method. Ramirez-Romero *et al*. (2005) used a semi-field approach for investigating effects of CRY1Ab protein on bee colonies in controlled flight rooms. They applied 1000 ng/g CRY1Ab, which is more than 10 times the concentration in MON810 pollen. Other treatments included applications of the pyrethroid derivative deltamethrin and the chloro-nicotinyl systemic insecticide, imidacloprid. Bees ingested the insecticides from artificial syrups offered as feed enriched with the substances of interest. Ramirez-Romero (2005) measured mortality, syrup consumption, foraging activity, and learning performances as indicators of bee health. The effects of the insecticides of interest were tested on one single colony with no independent control and no replication. Pesticide effects were derived from various feeding treatments offered consecutively: (1) no insecticide (2) insecticide (3) no insecticide; each treatment was offered for 2 - 4 days. In the treatment with CRY1Ab-enriched feed no significant differences in bee mortality were found at different treatment stages. However, foraging activity of bees fed with CRY1Ab declined continuously through the treatment stages without any recovery between treatments. An interpretation of these results is difficult since no control data are available. It is not clear, for example, if the foraging activity decrease was due to a season effect since the CRY1Ab-experiment was carried out in winter time and investigations on deltamethrin and on imidacloprid in the summer. The authors themselves could not exclude a seasonal effect on activity. By contrast foraging activity decreased significantly in summer time during the periods of feeding with the synthetic insecticides but rapidly recovered when bees were transferred back to insecticide-free syrup. These types of responses can be interpreted as typical of the repellent or anti-feedant effect in insects exposed to certain pesticides of low toxicity. The GMO Panel does not share the view by Ramirez-Romero *et al.* (2005) that the above results were mainly CRY1Ab dependent. Negative effects on bees are likely not directly associated with exposure to the CRY1Ab protein because of the design of the experiment and lack of simultaneous controls or replication.

As the pollen shed in a given maize field usually takes place for approximately 10 days each season, the GMO Panel concludes that potential bee exposure to MON810 pollen will be limited. Thus the proportion of maize pollen as a total of all pollen collected and fed to larvae during a summer will be low in most cases. Also considering the low concentration of CRY1Ab protein in MON810 pollen, it is likely that larvae will be exposed to very low concentrations of the protein. The literature cited in the submission does not alter this conclusion and therefore the GMO Panel considers that the low exposure level combined with the selective activity of CRY1Ab is unlikely to result in any adverse effects on bees.

The dispersal of GM maize pollen to other maize crops as mentioned in the documents submitted by Greece is not a biosafety issue within the mandate of EFSA, but a coexistence issue, which should be considered when Greece is developing its coexistence regimes. However, the GMO Panel points out that bees make very little contribution to pollination in maize, as bees rarely visit the female flowers.

Conclusions: The GMO Panel concludes that the Greek submission provided no new scientific data or information in support of an adverse effect of MON810 maize on the large-scale beekeeping industry in Greece.

E. Impact of MON810 maize on human health

The section titled '*Impact on human health*' of the Greek submission contained fifteen scientific references to support its claim of an adverse effect on human health. However, the GMO Panel has reviewed all of them and came to the following conclusions. Some of these references do not appear to contain scientific information pertinent to the human health issues. For example, one reference is an editorial analysis discussing the sometimes quite marked differences in attitude to safety testing of GMOs between the main body of scientists and official risk assessors on the one hand, and individual scientists and non-governmental interest groups on the other (Butler *et al*., 1999). Other references are texts from the internet, which have not gone through a traditional reviewing process, and only mention MON810 maize in passing. The GMO Panel does not find specific comments related to other GM crops, and those never approved in the EU, appropriate for the case in question. However, three publications are dealing specifically with MON810 maize. Two of these have already been discussed in previous sections of this opinion. Saxena and Stotzky (2001a) claimed increased lignin content in MON810 maize but mainly deal with environmental aspects. Hernandez *et al*. (2003) described a detection method for this GM maize (discussed in sections A and B, respectively). The third is addressed below.

Although not mentioned explicitly by the Greek Authority, there seems to be a concern that transgenic DNA from GM food, including MON810 maize, is transferred to cells of the consumer and give rise to adverse effects. Greece draws attention to the transfer of MON810 DNA fragments from feed to animal tissues (blood, liver, spleen and kidney) (Mazza *et al*., 2005). Various studies have been undertaken to determine whether fragments of transgenic and naturally occurring DNA in feed could be detected in animal tissues and food products such as meat, milk and eggs. These studies, which recently have been reviewed by the Council for Agricultural Science and Technology (CAST, 2006), indicate that fragments of both natural and transgenic DNA can be found in animal tissues and fluids. The detection of DNA in meat, milk and eggs is likely to be a function of its abundance and the analytical sensitivity. These observations confirm that transgenic DNA does not behave in a different way to DNA occurring in conventional food and feed products. However, the presence of such gene fragments has never shown any adverse effects on animals. Incorporation of functional plant gene fragments from consumed plant material into mammalian cells *in vivo* has never been observed and is considered extremely improbable (Hohlweg and Doerfler, 2001).

The GMO Panel has assessed several applications on hybrids containing MON810 (EFSA, 2005 a,b,c,d). In this context, the GMO Panel has assessed the molecular characterisation of the MON810 event together with data on the levels of the CRY1Ab protein, and chemical composition of the crop, as well as assessed potential toxicity and allergenicity of the respective hybrids. In the opinion of the GMO Panel the assessed hybrids containing MON810 are considered as safe as conventional maize.

Conclusions: The GMO Panel therefore affirms its conclusions that, on the basis of current scientific knowledge, MON810 maize is unlikely to have adverse effects on human and animal health or on the environment in the context of its intended uses.

CONCLUSIONS

The GMO Panel has investigated in depth the claims and documents provided by Greece. In these documents, the GMO Panel did not identify any new data subject to

scientific scrutiny or scientific information that would change the risk assessment conducted on MON810 maize which currently has marketing consent in the EU. In addition, the Greek submission did not supply scientific evidence that the environment or ecology of Greece was different from other regions of the EU sufficient to merit separate risk assessments from those conducted for other regions in the EU. The GMO Panel considered the available data for MON 810 maize on molecular characterisation, food and feed safety together with available data on environmental impact. The GMO Panel also reviewed new literature on CRY1Ab-expressing maize. The GMO Panel concluded that MON810 maize is unlikely to have adverse effects on human and animal health or on the environment in the context of its proposed uses. The GMO Panel therefore re-affirms its previous conclusions on the safety of MON810 maize.

The GMO Panel, having considered the scientific information submitted by Greece, is of the opinion that

- there is no new data that would invalidate the initial risk assessment conducted on MON810 maize established under Directive 90/220/EEC or Directive 2001/18/EC,

- there is no specific scientific evidence, in terms of risk to human health and the environment, that would justify a prohibition of cultivation of the MON810 maize authorised under Directive 90/220/EEC or Directive 2001/18/EC in Greece.

In conclusion, the GMO Panel finds that the scientific evidence currently available does not sustain the arguments provided by Greece and that there is no imminent danger for human health and the environment due to the cultivation of the maize varieties with the genetic modification MON810.

DOCUMENTATION PROVIDED TO EFSA

1. Letter to EFSA, dated 4 May 2006 with ref. DA/bt 510293 from Mrs. Paola Testori Coggi from Health & Consumer Protection Directorate-General SANCO requesting a consultation of the Scientific Panel on Genetically Modified Organisms with supporting documents:

   a written submission, made of a scientific report, listing detailed reasons for supporting measures taken by Greece, and of 71 publications and statements.

2. Letter dated 29 March 2006 of Mr Kondolaki of the Permanent Representation of Greece to the European Commission, DG SANCO comprising in particular an introductory note, a scientific report and a dossier with 71 publications, opinions.

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