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SCIENTIFIC OPINION

Applications (EFSA-GMO-RX-MON810) for renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto[1]

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Scientific Opinion of the Panel on Genetically Modified Organisms

(Question No EFSA-Q-2007-150, EFSA-Q-2007-153, EFSA-Q-2007-164)

Adopted on 15 June 2009

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SUMMARY

This document provides a scientific opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on 3 applications

[1] For citation purposes: Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-RX-MON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto. *The EFSA Journal* (2009) 1149, 1-85.

[*] (minority opinion) 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.

submitted under Regulation (EC) No 1829/2003 for renewal of the authorisation of (1) existing food and food ingredients produced from genetically modified (GM) maize MON810 (Unique Identifier MON-ØØ81Ø-6); (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, developed by Monsanto to provide resistance to lepidopteran target pests.

The scopes of the 3 renewal applications cover the continued marketing of:

- existing food and food ingredients produced from maize MON810 (Reference EFSA-GMO-RX-MON810$_{[8\text{-}1a]}$) that have been placed on the market in accordance with Article 5 of Regulation (EC) No 258/97;

- feed consisting of and/or containing maize MON810 that were authorised under Directive 90/220/EEC (Commission Decision 98/294/EC), including the use of seed for cultivation (Reference EFSA-GMO-RX-MON810$_{[20\text{-}1a]}$);

- food additives produced from maize MON810 that were authorised under Directive 89/107/EEC, and feed produced from maize MON810, i.e., feed additives lawfully placed on the market under Directive 70/524/EEC and feed materials (Reference EFSA-GMO-RX-MON810$_{[8\text{-}1b/20\text{-}1b]}$).

After the date of entry into force of the Regulation (EC) No 1829/2003, the products mentioned above were notified to the European Commission according to Articles 8 or 20 of this Regulation and subsequently included in the Community Register of GM food and feed.

Maize MON810 expresses a Cry1Ab insecticidal protein, derived from *Bacillus thuringiensis* subsp. *kurstaki*, which confers protection against lepidopteran target pests such as the European corn borer (*Ostrinia nubilalis*) and species belonging to the genus *Sesamia*.

In delivering its scientific opinion, the EFSA GMO Panel considered the 3 renewal applications (EFSA-GMO-RX-MON810$_{[8.1.a]}$, EFSA-GMO-RX-MON810$_{[20.1.a]}$ and EFSA-GMO-RX-MON810$_{[8.1.b/20.1.b]}$); additional information supplied by the applicant; the scientific comments submitted by Member States; the report of the Spanish Competent Authority and its Biosafety Commission; and relevant information published in the scientific literature.

The EFSA GMO Panel assessed maize MON810 with reference to the intended uses and appropriate principles described in the guidance document of the EFSA GMO Panel for the risk assessment of GM plants and derived food and feed. The scientific assessment included molecular characterisation of the inserted DNA and expression of target proteins. A comparative analysis of agronomic traits and composition was undertaken, and the safety of the new protein and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plan were undertaken.

Maize MON810 was generated by particle acceleration technology. Maize MON810 expresses a *cry1Ab* coding sequence that encodes an insecticidally active Cry1Ab protein. The molecular characterisation data established that a single insert is integrated in the maize

genomic DNA. Appropriate analyses of the integration site including sequence determination of the inserted DNA and flanking regions and bioinformatic analysis have been performed. Updated bioinformatic analysis of junction regions demonstrated the absence of any potential new open reading frames coding for proteins known to be toxic for humans and other mammals and/or allergens. The expression of the genes introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The EFSA GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of maize MON810 does not raise any safety concern, and that sufficient evidence for the stability of the genetic modification was provided.

Analyses carried out on materials from maize MON810, including stacked GM maize events where maize MON810 was one of the parental lines, and their comparators indicate that maize MON810 is compositionally, phenotypically and agronomically equivalent to the non-GM counterparts and conventional maize, except for the newly expressed trait.

The Cry1Ab protein shows no homology with proteins known to be toxic for humans and other mammals and/or allergens. In addition, this protein is rapidly degraded under simulated gastric conditions. Furthermore, the Cry1Ab protein has been extensively assessed in previous opinions of the EFSA GMO Panel. No concerns for humans and animals were identified regarding the safety of the Cry1Ab protein.

In a 90-day feeding study in rats, no indications of adverse effects were observed. In addition, a 42-day broiler feeding study provided evidence of nutritional equivalence of maize MON810 kernels to kernels of conventional maize. The toxicological and nutritional data on maize MON810 and appropriate non-GM maize control published during the last 10 years confirm that these maize varieties have comparable influence on the test systems. Therefore, the EFSA GMO Panel is of the opinion that maize MON810 is as safe as its non-GM counterparts and that the overall allergenicity of the whole plant is not changed through the genetic modification.

The Spanish Competent Authority and its Biosafety Commission provided to EFSA its report on the environmental risk assessment in line with Articles 6.3(e) and 18.3(e) of Regulation (EC) No 1829/2003. The Spanish Competent Authority and its Biosafety Commission conclude that "*according to the current state of scientific knowledge and after examining the existing information and the data provided by the Monsanto Company, the Spanish Commission on Biosafety could give a favourable opinion to the renewal of commercialisation in the EU of maize MON810 if the proposals and conditions defined in this environmental risk assessment report are implemented*".

Since maize MON810 has no altered survival, multiplication or dissemination characteristics, the EFSA GMO Panel agrees with the assessment that the likelihood of unintended environmental effects due to the establishment and spread of maize MON810 will be no different from that of conventional maize varieties.

On the basis of the data provided by the applicant and obtained from a literature survey and a modelling exercise on the effect of the cultivation of maize MON810 on non-target lepidopteran species in representative maize cultivation regions in the European Union (EU), the EFSA GMO Panel concludes that the likelihood of adverse effects on non-target

organisms or on ecological functions is very low, especially if appropriate mitigation measures are adopted. In agreement with the environmental risk assessment by the applicant and the assessment conducted by the Spanish Competent Authority and its Biosafety Commission, the EFSA GMO Panel identifies the possible evolution of resistance in target species, as a potential risk linked to the cultivation of maize MON810.

In conclusion, the EFSA GMO Panel considers that the information available for maize MON810 addresses the scientific comments raised by Member States and that maize MON810 is as safe as its conventional counterpart with respect to potential effects on human and animal health. The EFSA GMO Panel also concludes that maize MON810 is unlikely to have any adverse effect on the environment in the context of its intended uses, especially if appropriate management measures are put in place in order to mitigate possible exposure of non-target Lepidoptera. Moreover, the EFSA GMO Panel advises that pest resistance management strategies continue to be employed.

Key words: **GMO, maize (*Zea mays*), MON810, insect resistant, Cry1Ab, food safety, feed safety, human and animal health, environment, Regulation (EC) No 1829/2003, Directive 2001/18/EC, Directive 90/220/EEC, renewal, existing products**

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BACKGROUND

On 29 June 2007, the European Food Safety Authority (EFSA) received from the European Commission 3 applications for renewal of authorisation of (1) existing food and food ingredients produced from genetically modified (GM) maize MON810 (Unique Identifier MON-ØØ81Ø-6); (2) feed consisting of and/or containing maize MON810, and maize MON810 for feed use (including cultivation); and of (3) food and feed additives, and feed materials produced from maize MON810, submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on GM food and feed.

The scopes of the 3 renewal applications cover the continued marketing of:

- existing food and food ingredients produced from maize MON810 (Reference EFSA-GMO-RX-MON810$_{[8\text{-}1a]}$) that have been placed on the market in accordance with Article 5 of Regulation (EC) No 258/97;

- feed consisting of and/or containing maize MON810 that were authorised under Directive 90/220/EEC (Commission Decision 98/294/EC), including the use of seed for cultivation (Reference EFSA-GMO-RX-MON810$_{[20\text{-}1a]}$);

- food additives produced from maize MON810 that were authorised under Directive 89/107/EEC, and feed produced from maize MON810, i.e., feed additives lawfully placed on the market under Directive 70/524/EEC and feed materials (Reference EFSA-GMO-RX-MON810$_{[8\text{-}1b/20\text{-}1b]}$).

After the date of entry into force of the Regulation (EC) No 1829/2003, the products mentioned above were notified to the European Commission according to Articles 8 or 20 of this Regulation and subsequently included in the Community Register of GM food and feed.

In agreement with the European Commission, it has been decided that the Panel of Genetically Modified Organisms (GMO Panel) of EFSA would assess these 3 renewal applications together.

Maize MON810 was the subject of an earlier safety assessment (SCP, 1998) and has been authorised (EC, 1998) under Directive 90/220/EEC. More recently, maize MON810 has been evaluated by the EFSA GMO Panel as a component of stacked GM maize events under Directive 2001/18/EC and Regulation (EC) 1829/2003 (EFSA, 2005a,b,c,d,e).

After receiving the 3 renewal applications and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed Member States as well as the European Commission and made the summary of these applications publicly available on the EFSA website. EFSA initiated a formal review of the 3 renewal applications to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 16 and 22 December 2008, EFSA received additional information requested under completeness check (requested on 20 December 2007) and on 29 January 2008 EFSA declared the applications as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

On 20 December 2007, following a call for expression of interest among Competent Authorities under Directive 2001/18/EC and in accordance with Articles 6.3(c) and 18.3(c) of Regulation

(EC) No 1829/2003, EFSA requested the Spanish Competent Authority to assess the initial environmental risk assessment of application EFSA-GMO-RX-MON810$_{[20\text{-}1a]}$ for the continued marketing of maize MON810 for cultivation. This call was initiated by EFSA on 23 October 2007 and the Spanish Competent Authority gave its conformity on 14 December 2007.

EFSA made the valid applications available to Member States and the European Commission and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member State bodies had 3 months after the date of receipt of the valid applications (until 29 April 2008) within which to make their opinion known.

The Spanish Competent Authority and its Biosafety Commission asked the applicant for additional data on maize MON810 on 13 May 2008 and 22 July 2008 that have been provided by the applicant on 9 June 2008 and 20 August 2008, respectively.

The EFSA GMO Panel asked the applicant for additional data on maize MON810 on (1) 24 April 2008, 22 July 2008, 12 November 2008 and 11 February 2009 for application EFSA-GMO-RX-MON810$_{[8\text{-}1a]}$; (2) 24 April 2008, 12 November 2008 and 11 February 2009 for application EFSA-GMO-RX-MON810$_{[20\text{-}1a]}$; and on (3) 24 April 2008, 22 July 2008, 12 November 2008 and 11 February 2009 for application EFSA-GMO-RX-MON810$_{[8\text{-}1b/20\text{-}1b]}$. The applicant provided the requested information on (1) 15 May 2008, 30 September 2008, 5 December 2008 and 27 February 2009 for application EFSA-GMO-RX-MON810$_{[8\text{-}1a]}$; (2) 15 May 2008, 2 December 2008, 5 December 2008 and 27 February 2009 for application EFSA-GMO-RX-MON810$_{[20\text{-}1a]}$; and on (3) 15 May 2008, 30 September 2008, 5 December 2008, 17 February 2009 and 27 February 2009 for application EFSA-GMO-RX-MON810$_{[8\text{-}1b/20\text{-}1b]}$. After receipt and assessment of the full data package, the EFSA GMO Panel finalised its risk assessment of maize MON810.

The EFSA GMO Panel carried out a scientific assessment of the intended uses of maize MON810 in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into consideration the information provided by the applicant in the 3 renewal applications; the scientific comments of Member States; the additional information provided by the applicant; the environmental risk assessment report from the Spanish Competent Authority and its Biosafety Commission; and relevant information published in the scientific literature.

In giving its opinion on maize MON810 to the European Commission, Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of 6 months from the receipt of the valid applications. As additional information was requested by the Spanish Competent Authority and its Biosafety Commission and by the EFSA GMO Panel, the time-limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, the EFSA opinion shall include an assessment report stating the reasons for its opinion and the information on which its opinion is based. This document is to be seen as the report requested under Articles 6(6) and 18(6) of that

Regulation and thus will be part of the overall opinion in accordance with Articles 6(5) and 18(5).

To present and clarify some of the comments raised by some Member States on 6 May 2009, a multilateral technical meeting between some Member States, several experts of the EFSA GMO Panel and EFSA staff was held on 26 May 2009 (EFSA, 2009b). Representatives of the European Commission attended the technical meeting as observers.

TERMS OF REFERENCE

The EFSA GMO Panel was requested to issue a scientific opinion for the renewal of authorisation of (1) existing food and food ingredients produced from maize MON810 that have been placed on the market in accordance with Article 5 of Regulation (EC) No 258/97; (2) feed consisting of and/or containing maize MON810 that were authorised under Directive 90/220/EEC, including the use of seed for cultivation; and of (3) existing food additives that were authorised under Directive 89/107/EEC, and feed produced from maize MON810, i.e., feed additives lawfully placed on the market under Directive 70/524/EEC and feed materials. After the date of entry into force of the Regulation (EC) No 1829/2003, these products were notified to the European Commission according to Articles 8 or 20 of this Regulation and subsequently included in the Community Register of GM food and feed.

Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environments and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)e of Regulation (EC) No 1829/2003.

The EFSA GMO Panel was not requested to give an opinion on information required under Annex II of the Cartagena Protocol, nor on the proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to GMO risk management.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Groups on Molecular Characterisation, Food/Feed and Environment, the Spanish Competent Authority and its Biosafety Commission, as well as the *ad hoc* expert: Rosie Hails, the hearing expert: Félix Ortego, and the following members of its staff: Anna Christodoulidou, Yann Devos, Ana Gomes and Karine Lheureux for the preparation of this opinion.

ASSESSMENT

1. Introduction

Maize MON810 was developed to provide protection against certain lepidopteran target pests (such as the European corn borer (*Ostrinia nubilalis*) and species belonging to the genus *Sesamia*) by the introduction of a part of a *Bacillus thuringiensis* gene encoding the insecticidal Cry1Ab protein. This Bt-protein binds to specific receptors on the epithelial surface of the midgut of lepidopteran species. As a consequence, pores are formed in the membranes of the midgut cells of the insect larvae that subsequently causes cells to burst and enables midgut bacteria to enter the body cavity, which leads to septicemia and death of the larvae (Crickmore, 2005; Broderick et al., 2006, 2009; Jimenez-Juarez et al., 2007; Soberón et al., 2007, 2009; Bravo and Soberón, 2008; Lemaux, 2009).

Maize MON810 is assessed with reference to its intended uses and the appropriate principles described in the guidance document of the EFSA GMO Panel for the risk assessment of GM plants and derived food and feed (EFSA, 2006a). In delivering its scientific opinion, the EFSA GMO Panel considered the information provided by the applicant in the 3 renewal applications (EFSA-GMO-RX-MON810$_{[8.1.a]}$, EFSA-GMO-RX-MON810$_{[20.1.a]}$ and EFSA-GMO-RX-MON810$_{[8.1.b/20.1.b]}$) including (1) an update on peer-reviewed scientific data on maize MON810; (2) a report on areas and quantity of production, importation, use in Europe of maize MON810 and information on known and estimated human and animal exposure; (3) updated molecular characterisation, including sequence data for the flanking regions; (4) updated information on allergenicity and toxicology; (5) updated information on environmental issues; and (6) post-market (environmental) monitoring plan, as well as the additional information submitted by the applicant in reply to questions from the EFSA GMO Panel.

The assessment presented here is also based on the scientific comments submitted by Member States (Annex G); the report of the Spanish Competent Authority and its Biosafety Commission (Annex H); and relevant information published in the scientific literature.

In its assessment of maize MON810, the EFSA GMO Panel considered, when appropriate, information available from other GM maize events expressing Cry1Ab proteins, in particular maize Bt176 and Bt11. Due to the use of a different promoter, pollen from maize Bt176 contains approximately 40 times higher concentrations of the Cry protein than pollen of maize Bt11 and MON810 (Mendelsohn et al., 2003). The promoters used in maize Bt11 and MON810 are almost inactive in pollen resulting in Cry1Ab protein levels lower than 0.1 µg/g fresh weight (fw) (Hellmich et al., 2001; Dutton et al., 2003; Nguyen and Jehle, 2007). In leaf tissues of MON810 maize plants, the amount of the Cry protein ranges between 0.3 and 8.6 µg/g fw (Nguyen and Jehle, 2007). This range is similar and approximately 3 times higher than that observed in maize Bt11 and Bt176, respectively (Dutton et al., 2003; Mendelsohn et al., 2003). The EFSA GMO Panel indicates in its opinion where information derived from maize Bt176 and Bt11 is used in its assessment on potential impacts of maize MON810.

2. Issues raised by Member States

Issues raised by Member States are addressed in Annex G of the EFSA overall opinion.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs

Maize MON810 was generated by particle acceleration technology using plasmids PV-ZMBK07 and PV-ZMGT10. Plasmid PV-ZMBK07 contained the CaMV35S promoter with duplicated enhancer region (e35S); an intron from the maize *Hsp70* (heat-shock protein) gene; the *cry1Ab* gene encoding the nature identical Cry1Ab protein; *nos 3'* - a 3' non-translated region of the nopaline synthase gene (transcriptional termination; polyadenylation); a *lac* operon fragment (a partial *Escherichia coli lacI* coding sequence, the promoter *lac* and a partial coding sequence for β-D-galactosidase or *lacZ* protein from pUC119); *ori-pUC* (replication origin for pUC plasmids, originally derived from plasmid ColE1); and the *npt*II gene as a selectable marker.

Plasmid PV-ZMGT10 contained the e35S promoter; the *Hsp70* intron; transit peptides *CPT1* and *CPT2* (from *Arabidopsis thaliana*); the CP4 *epsps* gene (from *Agrobacterium* sp.) which allows for selection on glyphosate; and the *gox* gene (from *Ochrobactrum anthropi* sp.) which encodes a glyphosate metabolising enzyme, the *nos 3'* terminator, the *lacZ* region, *ori-pUC* and the *npt*II gene.

3.1.2. Transgenic construct in the genetically modified plant

In a previous molecular characterisation of maize MON810, it has been reported that MON810 contains a single insertion event which consists of elements derived from plasmid PV-ZMBK07, including the enhanced 35S promoter, the maize *Hsp70* intron, and a *cry1Ab* coding sequence sufficient to encode an insecticidally active Cry1Ab protein. Additional experiments confirmed that the MON810 insert contains a portion of the 3' end of the e35S promoter as well as a portion of the 5' end of the *cry1Ab* coding sequence. Data indicated that no other portion of plasmid PV-ZMBK07 DNA and no portion of plasmid PV-ZMGT10 were present in maize MON810. This included the absence of *npt*II. In a recent study, the insert in maize MON810 was re-characterised and Southern analysis used to confirm the presence of a single copy insert, the integrity of the inserted elements from plasmid PV-ZMBK07, the absence of plasmid backbone sequences, and the absence of elements from PV-ZMGT10. Probes were derived from sequences spanning the *cry1Ab* expression unit in PV-ZMBK07, the plasmid backbone sequence that encompasses both PV-ZMBK07 and PV-ZMGT10 backbone, and elements from plasmid PV-ZMGT10. The data confirm that MON810 contains part of the e35S promoter, the *Hsp70* intron, and part of the *cry1Ab* coding sequence, but does not contain the *nos* transcriptional termination sequence.

The organisation of the elements within the insert in maize MON810 was confirmed by PCR. The insert was sequenced to further confirm the organisation of the elements within the insert. Sequence data indicate that the e35S promoter that regulates expression for the *cry1Ab* gene has been modified into a shorter promoter version e35SMON810 (307 bp at the 3' end of the 620 bp promoter), that the *Hsp*70 is intact and that 2448 bp of the *cry1Ab* coding sequence (corresponding to the 5' end of the 3470 bp gene) encompassing the insecticidally active tryptic core is present. A portion from the 3' end of the *cry1Ab* gene as well the *nos* terminator have been deleted as the result of the integration process.

PCR analysis was performed on genomic DNA extracted from maize MON810 and conventional maize to demonstrate that the DNA sequences flanking the 5' and 3' ends of the insert in MON810 are native to the maize genome. The 5' and 3' DNA sequences flanking the insert in maize MON810 were previously reported (EFSA, 2005d,e). Bioinformatic analyses were performed to assess the potential for allergenicity, toxicity, or bioactivity of putative polypeptides encoded by the 5' and 3' inserted DNA-maize genomic DNA junctions. No biologically relevant structural similarities to allergens, toxins, or pharmacologically active proteins were observed for any of the putative polypeptides and no short (8 identical amino acid) polypeptide matches were shared between any of the putative polypeptides and proteins in the allergen database. These data demonstrate the lack of both structurally and immunologically relevant similarities to allergens for all putative polypeptides analysed. These data also demonstrate the lack of structurally relevant similarities towards toxins or other pharmacologically active proteins for all putative polypeptides analysed.

Additional information provided in 2007 confirmed the DNA sequences of the 5' and 3' DNA flanking regions originally provided, but supplied additional sequence information. This revealed an additional 400 bp of maize DNA at the 3' flank and an additional 1000 bp of maize DNA at the 5' flank. Updated analysis of open reading frames (ORFs) indicated no new potential chimeric proteins showing homologies with potential toxins or allergens, confirming the original bioinformatic assessment. *In silico* analysis did reveal that the 3' genomic region corresponded to a gene putatively coding for the HECT-ubiquitin ligase protein. As further discussed in sections 4.1.2 and 4.1.3, there is phenotypic and compositional equivalence between maize MON810 and its conventional counterparts giving no evidence of any safety implications resulting from the interruption of this gene sequence.

A recent publication by Rosati et al. (2008) confirmed that the 3' genomic region corresponded to a gene putatively coding for the HECT E3 ubiquitin ligase. In addition, using RT-PCR they showed that this 3' region produced cDNA variants of different length. *In silico* translation of these transcripts identified 2 and 18 putative additional aminoacids in different variants, all derived from the adjacent host genomic sequences, added to the truncated Cry1Ab protein. These putative recombinant proteins did not show homology with any known protein and do not raise any new safety concerns.

3.1.3. Information on the expression of the insert

In 1994, field trials were conducted at 6 locations distributed throughout the major United States (US) maize growing region representing a variety of environmental conditions. Tissues of MON810 plants were analysed for the 3 proteins, Cry1Ab, CP4 EPSPS, and GOX using ELISA. The CP4 EPSPS and GOX proteins were not detected in any of the plant tissues of maize MON810. This was expected since the molecular analysis of maize MON810 established that the CP4 *epsps* and *gox* genes were not present in the nuclear genomic DNA. The level of Cry1Ab protein ranged from 7.93-10.34 μg/g fw in young leaf tissue; 3.65-4.65 μg/g fw in whole plant tissue; and 0.19-0.39 μg/g fw in harvested grain. The foliar expression of Cry1Ab protein remained high during the vegetative growth stages of the maize plant as measured in overseason leaf samples.

In 1995, 5 field trials were conducted within the major maize growing regions of France and Italy. The level of Cry1Ab protein ranged from 7.59-9.39 μg/g fw in young leaf tissue; 4.21-9.23 μg/g fw in forage tissue; and 0.42-0.69 μg/g fw in harvested grain. The 1995 analysis

confirmed that CP4 EPSPS and GOX proteins were not present in plant tissues of maize MON810. With regard to Cry1Ab, the protein levels were similar for plants grown in the US and European field trials over 2 consecutive generations. The levels of Cry1Ab detected do not raise any safety concerns.

The expression levels of Cry1Ab in maize MON810 and stacked GM maize events containing MON810 (MON863xMON810xNK03; MON863xMON810 and NK603xMON810) have also been reported and reviewed by the EFSA GMO Panel who concluded that the levels of expression of Cry1Ab protein do not raise safety concerns (EFSA, 2005a,b,c,d,e).

3.1.4. Inheritance and stability of inserted DNA

The integrity of the insert originally described in 1995 and 2001 was reaffirmed by the recent study of 2007, indicating stability of the insert. Stability of the insert over 3 generations was established by Southern analyses. ELISA analysis of Cry1Ab protein expression over 7 generations of backcrosses to one recurrent parent and 6 generations of crosses to a second, unrelated inbred line, confirmed trait stability and established a Mendelian inheritance pattern. This was confirmed by feeding assays with European corn borer.

The applicant also has seed quality and stewardship processes in place that typically include single nucleotide polymorphism, protein expression and phenotypic grow-out tests. A formal performance claims process is also in place. Any reported incidence of non-performance, for any seed product by a farmer, can be reported. Appropriate actions are undertaken to investigate the reasons for non-performance. Overall, less than 1% of the registered biotechnology products complaints are related to product performance and to date, none of these complaints have revealed trait stability issues.

3.2. Conclusion

Appropriate analysis of the integration site including flanking sequences and bioinformatic analysis have been performed to analyse the construct integrated in the GM plant. Updated bioinformatic analyses revealed that 1 ORF shared sequence similarity to a putative HECT-ubiquitin ligase protein. The EFSA GMO Panel found no safety implications resulting from the interruption of this gene sequence. The expression of the genes introduced by the genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The EFSA GMO panel considers this to be an adequate analysis and the molecular characterisation does not indicate any safety concerns.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

Having considered the information provided in the initial application evaluated by the Scientific Committee on Plants (SCP, 1998), new information supplied by the applicant, and the Member States' comments to the renewal applications, the EFSA GMO Panel requested from the applicant a comprehensive referenced review and discussion of the new scientific data relevant for food/feed safety of maize MON810 and published by independent sources since the original authorisation of this maize was given by the European Commission (EC, 1998).

4.1.1. Choice of comparator and production of material for the compositional assessment

The original field trials with maize MON810 were performed in the US in 1994 (6 sites) and in France in 1995 (4 sites). As these field trials were not replicated, only the combined data were statistical analysed. The non-GM maize control material was maize MON818 in all 1994 field trials and maize MON820 in the 1995 field trials. Both control materials were similar in pedigree to the tested maize MON810. Only grain material was analysed from the field trials in 1994, whereas both grain material and forage was analysed from the field trials performed in 1995. The set of compounds analysed in grain material was proximates, 18 amino acids, 9 fatty acids, carbohydrates (5 compounds or fractions), vitamins (3 tocopherols), minerals (calcium and phosphorous), and anti-nutrients (phytic acid). Forage was analysed for proximates, and neutral and acidic fibre. Leaf, forage and grain were also analysed for the expression of the Cry1Ab protein. In total 44 compounds were analysed.

To support the original compositional data, the applicant provided compositional data on forage and grain material collected from field trials with 3 different stacked GM maize events where maize MON810 was one of the parental GM maize lines. The studies were on MON810xMON863 grown at 4 replicated sites in Argentina in 1999, MON810xNK603 grown at 3 replicated sites in France in 2000, and MON810xMON863xNK603 (expresses Cry1Ab, Cry3Bb1, and CP4 EPSPS) grown at 4 replicated sites in Argentina during the season 2002-2003. The triple-stacked GM maize MON810xMON863xNK603 was produced from single maize events by first crossing maize MON863 with NK603, producing the double-stacked GM maize MON863xNK603, and then, after inbreeding, crossing this double-stacked GM maize with maize MON810. The field trials in Argentina 1999 compared the double-stacked GM maize MON810xMON863 with maize MON810, maize MON863 and a hybrid between non-GM maize varieties (MON846) having a comparable genetic background to that of the parental GM maize events. In the French field trials in 2000, the double-stacked GM maize MON810xNK603 was compared to maize MON810, maize NK603 and a hybrid between non-GM maize (name not given) with comparable genetic background to that of the parental GM maize events. Finally, in the field trials in Argentina 2002-2003, the triple-stacked GM maize MON810xMON863xNK603 was compared to a non-GM maize control having a comparable genetic background (DKC46-26). Although these studies on stacked GM maize events did not statistically compare levels of key compounds in maize MON810 and in the maize hybrid created by crossing the appropriate non-GM maize controls, the raw data were available for the EFSA GMO Panel to analyse and draw conclusions from. In total, there were data on 9 compositional parameters from forage and 54 from grain.

4.1.2. Compositional analysis

Grain materials from field trials in the US in 1994 were analysed for proximates (moisture, total protein, total fat, calories, carbohydrate, crude fibre and ash) and 44 specific maize constituents (amino acids, fatty acids, starch, sugars, calcium, phosphorous, tocopherols and phytic acid). For 11 of the studied compounds, 8 amino acids, crude fibre, calcium and β-tocopherol, levels were significantly higher in maize MON810 than in the control maize (MON818). However, for 8 of these compounds, concentrations in maize MON810 and its control line were within the ranges reported for maize in the literature. The level of histidine and cystine were higher than reported in the literature in both studied materials (maize MON810 and its control). On the other hand, the calcium levels in both materials were below the levels reported in the literature for maize. However, notably the levels for all 3 compounds

did not deviate from those reported by the applicant to occur in another commercial maize variety with a similar genetic background.

Thirty-six compounds were analysed in the grains collected from the French field trials in 1995 (proximates, amino acids and fatty acids), but from this field trial also proximates in forage were analysed. In this material, statistical differences in constituent levels between maize MON810 and its control (MON820) were observed for 5 compounds (increased grain moisture and palmitic acid content, and reduced levels of methionine and tryptophan, as well as increased crude protein in forage) which in no case confirmed findings from the 1994 trial.

Several independent investigators have reported on the lignin levels in maize varieties expressing the Cry1Ab protein. Some claim that lignin levels are higher in maize MON810 than in an appropriate non-GM maize control (Saxena and Stotzky, 2001b; Flores et al., 2005; Poerschmann et al., 2005), whereas other investigators claim that it is unchanged or reduced (Folmer et al., 2002; Jung and Sheaffer, 2004; Mungai et al., 2005; Anonymous, 2006). Several investigators have pointed out the importance of the chemical analytical technique for lignin analysis and have indicated that inconsistent methodology might have contributed to the variable results published for this maize constituent (Hatfield et al., 1999; Jung and Sheaffer, 2004). Notably, the most recent literature identifies no compositional difference in lignin content between various GM maize events containing MON810 (Cry1Ab), MON863 (Cry3Bb1), and DKC60-14 (stacked Cry1Ab and Cry3Bb1), and their appropriate non-GM maize controls (Lehman et al., 2008). Furthermore, Tarkalson et al. (2008) found no difference in decomposition of lignin over time in maize genetically modified to express Cry1Ab and non-GM maize control.

The applicant notes that grain of maize MON810 frequently exhibits lower mycotoxin levels than control maize grains. The EFSA GMO Panel reviewed a substantial amount of published data (Munkvold et al., 1999; Dowd, 2000, 2001; Magg et al., 2002, 2003; Schaafsma et al., 2002; Clements et al., 2003; Hammond et al., 2004, 2006; de la Campa et al., 2005; Papst et al., 2005; Rossi et al., 2005; Williams et al., 2005) regarding mycotoxin levels in maize MON810 and its non-GM maize control, and concludes that the Cry1Ab expressing maize MON810 may contain lower levels of fumonisins, and possibly also aflatoxin, than control maize not expressing this protein.

The Scientific Committee on Plants summarised the compositional information on maize MON810 when giving its opinion on the MON810 notification C/F/95/12/02 (SCP, 1998). Based on the analysis of both forage and kernels of MON810 and a non-transgenic maize control grown in field trials over 2 seasons, this Committee concluded that no significant compositional changes could be detected in maize MON810 as compared to the control. During its evaluation of several applications to place stacked GM maize events on the market, where maize MON810 was one of the parental lines used in conventional crosses with other GM maize varieties, the EFSA GMO Panel concurred with the opinion of the Scientific Committee on Plants on the chemical composition of this transformation event (EFSA, 2005a,b,c,d,e). In the present assessment, the EFSA GMO Panel considered the comprehensive compositional data supplied by the applicant and published by independent investigators after the original authorisation to market maize MON810 was given (e.g., Autran et al., 2003; Jung and Sheaffer, 2004; Bakke-McKellep et al., 2008; Venneria et al., 2008), and concludes that maize MON810 is compositionally equivalent to the non-GM maize

counterparts MON820 and MON818 and to conventional maize varieties except for the presence of the Cry1Ab protein.

4.1.3. Agronomic traits and GM phenotype

The EFSA GMO Panel has already assessed the agronomic and phenotypic characteristics of maize MON810 in relation to an appropriate non-GM maize control having a comparable genetic background in connection with giving its opinions on several stacked GM maize events (EFSA, 2005a,b,c,d,e). The information available in the renewal applications gives no reason to change the opinion that maize MON810 is agronomically and phenotypically equivalent to currently grown non-GM maize varieties, with exception of the insect resistance conferred by the Cry1Ab protein.

4.2. Conclusion

Analyses carried out on materials from maize MON810, including stacked GM maize events where maize MON810 was one of the parental lines, and their comparators indicate that maize MON810 is compositionally, phenotypically and agronomically equivalent to the non-GM counterparts and conventional maize varieties, except for expressing the Cry1Ab protein.

5. Food/Feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Product description and intended uses

The scopes of the 3 renewal applications are for (1) existing food and food ingredients produced from maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and for (3) food and feed additives, and feed materials produced from maize MON810. Maize MON810 was developed to express a Cry1Ab protein from *Bacillus thuringiensis* subsp. *kurstaki*, rendering the maize protected against certain lepidopteran target pests such as the European corn border (*Ostrinia nubilalis*) and species belonging to the genus *Sesamia*.

Maize MON810 is intended to be processed like any conventional maize. The applicant has provided information on the use of maize and derived products. The primary use of maize is for animal feed, but it is also processed into valuable food products, such as starch, syrups and oils.

5.1.2. Effect of processing

Based on compositional data of raw agricultural commodities (grain and forage) of maize MON810, stacked GM maize events containing MON810 (EFSA, 2005a,b,c,d,e) and the corresponding non-GM maize varieties with a genetic background similar to these GM maize events, the EFSA GMO Panel is of the opinion that there are no reasons to assume that the characteristics of the processed products derived from maize MON810 would be different from that of processed non-GM maize products.

There are a few independent studies on the fate of the Cry1Ab protein during processing of maize MON810. Dien et al. (2002) reported on the Cry1Ab protein in dry ground and wet-milled MON810 during ethanol production. After wet-milling the Cry1Ab protein was found in the germ, gluten, and fibre fractions, whereas after dry milling no protein could be detected after liquefaction due to denaturation of the protein at the high temperatures during this phase.

5.1.3. Toxicology

5.1.3.1. Toxicological assessment of expressed novel protein in maize MON810

Given the low expression level of Cry1Ab protein in maize MON810 and the very difficult task of isolating a sufficient quantity of purified protein from the maize plant for safety testing, Cry1Ab was produced in a recombinant *Escherichia coli* strain. As the Cry1Ab protein produced by MON810 is converted to the trypsin-resistant core protein by digestive proteases, the trypsin-resistant core protein (HD-1t), obtained through trypsinolysis of the *Escherichia coli*-produced Cry1Ab protein, was used for safety assessment. The identity of the *Escherichia coli*-expressed trypsin-resistant core protein to the trypsin-resistant core protein present in maize MON810 was confirmed by amino acid sequencing, amino acid composition, immunoreactivity (ELISA and Western blot), molecular weight (SDS-PAGE and Coomassie blue staining) and insect bioassay.

Extensive *in vivo* experience, backed by *in vitro* studies, have led to the conclusion that both the Cry1Ab protein expressed in *Bacillus thuringiensis* and the Cry1Ab protein expressed in plants are highly selective and do not target mammalian organisms (Wolfersberger, 1992; Wieczorek et al., 1999; Griffitts and Aroian, 2005; Shimada et al., 2006a,b; Stumpff et al., 2007; Bondzio et al., 2008). Therefore, the EFSA GMO Panel accepts the use of the trypsin-resistant core of Cry1Ab protein derived from *Escherichia coli* for the safety testing of the trypsin-resistant core of the Cry1Ab protein present in maize MON810.

Furthermore, the Cry1Ab protein has been extensively assessed in previous opinions of the EFSA GMO Panel and found to be safe (EFSA 2005a,b,c,d,e).

(a) Acute toxicity testing

The applicant provided a single dose acute oral toxicity study in mice using the *Escherichia coli* produced protein. No signs of systemic toxicity were observed up to the highest Cry1Ab protein dose of 4000 mg/kg body weight.

(b) Repeated dose toxicity testing

Potential toxicity after repeated dosage of the Cry1Ab protein purified from *Bacillus thuringiensis* var. *kurustaki* (strain HD-1), was recently studied by Onose et al. (2008) in F344 rats with or without chemically induced gastrointestinal impairment. The protein was administered by gavage during the second and fourth week of feeding in the 28-days study. No significant changes indicative of toxicity of the Cry1Ab protein from *Bacillus thuringiensis* were noted on any of the parameters tested.

(c) Degradation in simulated digestive fluids

The digestibility of the Cry1Ab protein produced in *Escherichia coli* was tested *in vitro* with simulated gastric fluid containing pepsin. The degradation occurred rapidly (within 2 minutes) as demonstrated by Western analysis and Cry1Ab activity measurement of Cry1Ab activity in an insect bioassay. The Cry1Ab protein was not degraded in simulated intestinal fluid containing a trypsin-like protease within 19.5 hours (only time measured), also demonstrated by Western analysis.

The digestibility of Cry1Ab derived from *Bacillus thuringiensis* subsp. *kurstaki* (strain HD-1) has been studied in simulated gastric fluid and simulated intestinal fluid also by Okunuki et al. (2002). In simulated gastric fluid, the purified protein (85% pure) was degraded to undetectable levels within 60 seconds. Degradation of a heat-treated protein was quicker. The same protein extracted from maize MON810 was degraded within 120 seconds. Simulated intestinal fluid degraded Cry1Ab from the bacteria within 240 minutes, whereas 20% of the protein extracted from maize was degraded during the same time. Preheating of the proteins resulted in a dramatically increased degradability. No degradation fragments were identified.

The *in vitro* digestion experiments demonstrate that the Cry1Ab protein is rapidly degraded at simulated gastric conditions.

(d) Degradation in the gastrointestinal tract

Several publications report on the degradability of the Cry1Ab protein, and transfer of the protein to mammalian tissue in animal feeding studies (or a feed-material in which the protein is expressed). There are even more studies on the fate of the transgenic DNA. Jennings et al. (2003) were unable to detect fragments of the *cry1Ab* gene and the Cry1Ab protein in the breast muscle of broiler chicken that had been fed a diet with 50-60% maize MON810 for 42 days. Rossi et al. (2005), in studies on male broiler chickens, were able to detect fragments of the *cry1Ab* gene only in the contents of both the crop and gizzard of chickens fed maize MON810, but not in any bird tissue. Although Nemeth et al. (2004) were able to detect DNA fragments of the high copy number maize endogenous chloroplast encoded gene rubisco in 5%, 15% and 53%, respectively, of the muscle samples from beef steers, broiler chickens, and swine fed maize MON810, *cry1Ab* DNA fragment could not be found. Of the milk samples taken from dairy cows 86% were positive for a DNA fragment of the rubisco gene, but contained no transgenic fragments. Using a sensitive analytical technique to quantify the Cry1Ab protein in blood of cows fed for 1 or 2 months on a diet containing 70% of its dry matter as maize MON810, Paul et al. (2008) were unable to detect the Cry1Ab protein in any of the plasma samples taken before or after end of feeding. Similarly, neither the intact *cry1Ab* gene nor its minimal functional unit was detected in blood, spleen, liver, kidney and muscle tissues of piglets fed for 35 days a diet containing 50% maize MON810 or conventional non-GM maize of comparable genetic background (Mazza et al., 2005). A small fragment of the *cry1Ab* transgene was, together with endogenous maize genes, detected in blood, liver, spleen and kidney of animals fed the test diet. However, no integration of the transgenic DNA in the host genome has been detected. Thus, transgenic DNA does not seem to behave differently from non-transgenic DNA with respect to transfer to animal tissue.

(e) Bioinformatics studies

Searches for amino acid sequence homology of the Cry1Ab protein expressed in maize MON810 with amino acid sequences of known toxic or allergenic proteins (databases AD8,

TOXIN6 using the FASTA algorithm and ALLPEPTIDES) did not identify any relevant sequence homology with proteins known to be toxic for humans and other mammals and/or allergenic.

5.1.3.2. Toxicological assessment of new constituents other than proteins

Since no new constituents other than the above mentioned Cry1Ab protein are expressed in maize MON810 and because there is no indication of alteration in levels of endogenous compounds, a toxicological assessment for new constituents is not applicable.

5.1.3.3. Toxicological assessment of the whole GM food/feed

The applicant provided a 90-day feeding study in Sprague-Dawley rats with grains of maize MON810 as a component of the diet. This study is available in the scientific literature (Hammond et al., 2006). Groups of 20 male and 20 female rats were fed diets containing 11% or 33% maize MON810 grain, the corresponding levels of the non-GM maize grain with a comparable genetic background, or 33% maize grain from commercial non-GM maize reference varieties. In total, 6 different commercial maize varieties were also tested in the study. When the dietary dose was 11% maize MON810 grain, a supplementation of the diet with 22% of the commercial non-GM maize used by the feed-formulating company was required to bring all diets up to 33% maize grain.

No clinically relevant reactions were noted when observing the animals. The detailed examination of the animals revealed no biologically relevant differences between treatment groups regarding body weight gain, food consumption, clinical pathology parameters (haematology, blood chemistry, urinalysis), organ weights, and gross and microscopic appearance of tissues. The only statistically significant differences observed in the haematology determinations were a slightly reduced mean corpuscular haemoglobin concentration and an increased number of platelets in female rats fed the lower dose of maize MON810. In the absence of effect at the higher dose level, and no effect in the males these effects were considered to be spurious as they were also within the literature reference and historical control ranges. In relation to serum chemistry, male rats given the diet with 33% maize MON810 showed a reduced albumin/globulin ratio, without any change in serum levels of albumin and globulin. The difference in albumin/globulin ratio in male rats given the high dose of maize MON810 was attributed to slightly lower albumin and slightly higher globulin levels.

Confirmation of the absence of adverse effects of dietary exposure to maize MON810 has been obtained in 90-day feeding studies in rats supplied diets containing maize with stacked GM maize events, in which one of the parents was MON810 (EFSA 2005a,b,c).

Sagstad et al. (2007) fed post-smolt Atlantic salmon (*Salmo salar*) for 82 days diets containing 15% or 30% maize MON810, its near-isogenic non-GM maize variety, or a reference maize variety not genetically related to the other 2 maize varieties. They then evaluated what was termed stress- and immune-response biomarkers at the transcriptional and protein levels. Small changes in stress protein activity (SOD, CAT), which were not correlated with gene expression, were noted in the liver and distal intestine. In addition, the study identified a change in white blood cell population of fish fed high levels of the MON810 containing diet as compared to the near-isogenic non-GM maize variety. However, no

difference was observed to the reference maize variety. Unfortunately, the authors do not give data on blood cell count for the two doses separately. Although fish performance in the study was good, the investigators interpreted their findings as a potential immune response.

5.1.4. Allergenicity

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation, or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (CAC, 2003; EFSA, 2006a).

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

The *cry1Ab* gene originates from *Bacillus thuringiensis* subsp. *kurstaki*, a soil microorganism that is not known to be allergenic. The Cry1Ab protein was subjected to bioinformatics analysis. The results of amino acid sequence homology searches for identical sequences of at least 8 contiguous amino acids of the Cry1Ab protein expressed by maize MON810 with a sliding window of a similar size of known allergenic proteins, identified no parts of the Cry1Ab protein to be identical to short stretches of known allergenic proteins. An additional search for overall similarity between the Cry1Ab protein and known allergens indicated no sequence identity above 35%. Furthermore, the Cry1Ab protein is not stable in acidic environments and is rapidly degraded under simulated gastric conditions. Based on these results, the EFSA GMO Panel considers that the newly expressed Cry1Ab protein is not likely to be allergenic.

Furthermore, the Cry1Ab protein has been extensively assessed in previous opinions of the EFSA GMO Panel and found not to be allergenic (EFSA 2005a,b,c,d,e).

5.1.4.2. Assessment of allergenicity of the whole GM plant

Allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, for example, through qualitative or quantitative modifications of the expression of the endogenous proteins. However, given that no biologically relevant agronomic and compositional changes were identified in maize MON810 and in stacked GM maize events containing MON810 (EFSA, 2005a,b,c,d,e) (with the exception of the introduced trait), no increased allergenicity is anticipated for maize MON810. Moreover, maize is not considered a common allergenic food.

There are also independent reports indicating no, or very low risk, for allergenicity of maize MON810. Nakajima et al. (2007) monitored the occurrence of IgE antibodies specific to the Cry1Ab protein expressed in maize MON810 in food allergic patients of the Japanese population. IgE levels were within background levels in sera of all 44 patients studied. When sera from maize allergic patients were tested against extracts of non-GM maize and maize MON810, respectively, similar staining patterns were found for both types of maize. Thus, no significant level of IgE antibodies specific to the Cry1Ab protein could be found in the studied food-allergic patients.

Batista et al. (2005) performed skin prick tests with extracts of maize MON810 on children with food and inhalant allergy and individuals with asthma-rhinitis. None of the individuals undergoing tests reacted differently to the MON810 and the non-transgenic maize samples studied. Similarly, when IgE of sera from food allergic patients were blotted to the transgenic Cry1Ab protein expressed in maize MON810, none of the tested samples contained detectable levels of IgE antibodies against the tested protein.

Thus, several investigators have come to the same conclusion as the applicant that maize MON810 is as safe as conventional maize in terms of allergenic potential.

5.1.5. Nutritional assessment of GM food/feed

The applicant provided a 42-day broiler feeding study to evaluate the nutritional performance of maize MON810. The study used a randomised complete block design and in addition to a group of broilers fed diets with maize MON810, also included broiler chickens fed diets with grains of the non-GM maize DK551 with a comparable genetic background to maize MON810, maize MON810xGA21 (expressing the Cry1Ab and an EPSPS protein), DK493AF (a suitable control maize for the double-stacked GM maize MON810xGA21), and 4 commercial maize varieties (DK539, DK521, DK537, or BX8 6). Each treatment consisted of 100 broilers kept in 10 pens with 5 birds of each sex. The starter diet contained approximately 50% maize grain and the grower/finisher diet approximately 60% maize grain. The result of the study has been published (Taylor et al., 2003a).

The mortality of young chickens was low and randomly distributed across treatments, and was not attributed to the feeding with maize MON810. Mortality during the later part of the study, mainly caused by sudden death and ascites, was comparatively high (6%), particularly in males, but was randomly distributed across treatments. The death rate ranged from 2% in maize MON810 to 11% for a commercial maize variety (DK 537).

None of the diets with GM maize material influenced performance (live weight at day 1 and 42, feed intake and feed conversion, adjusted feed conversion), carcass yield (absolute and relative chill weight, fat pad weight, thigh weight, and wing weight), breast meat (moisture, protein and fat), and thigh meat parameters (moisture, protein and fat). The only parameters statistically significantly influenced were related to carcass yield and included breast meat weight, which was increased in MON810 as compared to its control (24.12% of chill weight as compared to 23.42%), and drum weight, which was reduced as compared to its control (13.94% of chill weight as compared to 14.02%). In relation to broilers fed diets with the 4 commercial maize varieties, a difference for these parameters was found only in one case. The recorded values were also similar to historical and literature data. The EFSA GMO Panel concludes that the broiler feeding study revealed no unexpected findings, and that maize MON810 is as wholesome as conventional maize.

Rossi et al. (2005) came to similar conclusions from another 42-day feeding performance study on Ross male broiler chickens fed diets containing 55-60% maize. They found maize MON810 to have no other influence on live weight, average daily weight gain, feed intake, and feed conversion than the non-GM maize control.

In addition to these broiler chicken feeding studies with maize MON810, two 42-day feeding studies with stacked GM maize events positively assessed by EFSA (EFSA, 2005a,b,d,e)

where MON810 was one of the parental varieties (MON810xNK603, MON810xMON863) were used to assess nutritional efficiency as compared to the corresponding non-GM maize. These stacked GM maize events showed a nutritional wholesomeness comparable to the corresponding non-GM maize controls (Taylor et al., 2003b,c).

Independent research teams have also performed feeding studies with maize MON810 on other food-producing farm animals. Feed intake, milk production, milk composition, and ruminal digestibility was studied by Donkin et al. (2003) in lactating dairy cows fed *ad libitum* diets with 42-60% maize silage and 20-34% maize grain of either MON810 or its non-GM maize isogenic control in a switchback design (3 periods of 21-days). There was a similar dry matter intake, 4% fat-corrected milk production and milk composition in cows given maize MON810 and the control maize. There was also no difference in ruminal degradability between these 2 types of maize.

Sung et al. (2006) compared the influence of maize MON810 and non-GM maize control on *in vitro* rumen fermentation and digestibility, and concluded that maize MON810 has no adverse effect on these parameters compared to the isogenic maize control.

Investigators have studied the nutritional efficiency and wholesomeness of maize MON810 for Atlantic salmon by supplying a feed containing up to 12.1% maize from hatching to eight month of age (when they had an average weight of 101-116 g). Sanden et al. (2005) investigated long-term effects of feeding plant products by studying growth, somatic indices, histological parameters and cell proliferation in fish that had had received the starch component of maize MON810 or non-GM maize instead of the normal source of starch in the fish diet. The exposure to maize MON810 had comparable effects on intestinal indices, and histology of the pyloric caeca, mid and distal intestine to the non-GM maize control. Cell proliferation in the distal intestine was reduced both in fish fed maize MON810 and its non-GM control. In a follow-up publication (Sanden et al., 2006), the authors reported that the diets supported good growth and did not result in diet-related mortality. Besides minor differences on hepatosomatic index and thermal growth coefficient at a few sampling times, body composition, relative organ weights, plasma nutrient concentrations and enzyme activities did not vary among treatments at any sampling. The investigators concluded that the inclusion of maize MON810 at a level of about 12% of the salmonid diets poses little, or no, health risks to first feeding Atlantic salmon parr, and promote normal growth. In the same experiment, Bakke-McKellep et al. (2008) studied a large number of histological, digestive, metabolic, and immunological parameters. Salmon parr fed maize MON810 did not respond differently to the diet than fish fed non-GM maize of a comparable genetic background.

5.1.6. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that maize MON810 is any less safe than its non-GM counterparts. In addition, no biologically relevant agronomic and compositional changes were identified in maize MON810. Therefore, in line with its guidance document (EFSA, 2006a), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

5.2. Conclusion

The Cry1Ab protein shows no homology with known toxic proteins and/or allergens. In addition, this protein is rapidly degraded under simulated gastric conditions. No concerns were identified regarding the safety of the Cry1Ab protein. Furthermore, the Cry1Ab protein has been extensively assessed in previous opinions of the EFSA GMO Panel and found to be safe.

In a 90-day feeding study in rats, no indications of adverse effects were observed. In addition, a 42-day broiler feeding study provided evidence of nutritional equivalence of MON810 maize kernels to kernels of conventional maize. The toxicological and nutritional data on maize MON810 and appropriate non-GM maize control published during the last ten years confirm that these maize varieties have comparable influence on the test systems. The EFSA GMO Panel is of the opinion that maize MON810 is as safe as its non-GM counterparts and that the overall allergenicity of the whole plant is not changed through the genetic modification.

6. Environmental risk assessment and monitoring

Considering the intended uses of maize MON810 including cultivation, the environmental risk assessment is concerned with potential direct and indirect effects of the cultivation and the spread of the GM plant into non-cultivated environments, as well as indirect exposure through manure and faeces from the gastrointestinal tracts, mainly of animals fed maize MON810.

The environmental risk assessment was evaluated by the Spanish Competent Authority and its Biosafety Commission. The report on the environmental risk assessment of the Spanish Competent Authority and its Biosafety Commission is provided in Annex H.

In its report, the Spanish Competent Authority and its Biosafety Commission considered the following issues on the environmental risk assessment submitted by the applicant: (1) persistence and invasiveness, selective advantage or disadvantage; (2) potential for gene transfer; (3) genetic and phenotypic stability; (4) interactions between the GM plant and target organisms; (5) potential interaction of the GM plant with non-target organisms; (6) potential impacts of the specific cultivation, management and harvesting techniques; and (7) effects on biogeochemical processes. The Spanish Competent Authority and its Biosafety Commission consider that most issues have been addressed in a satisfactory way by the applicant, except the potential interaction of the GM plant with non-target Lepidoptera species representative of EU maize cultivation regions.

6.1. Evaluation of relevant scientific data

6.1.1. Unintended effects on plant fitness due to the genetic modification

Maize is highly domesticated and not generally able to survive in the environment without appropriate cultivation practices. The survival of maize is limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens, herbivory and cold climate conditions. Maize plants are only winter hardy in European regions with mild winters, and in those situations maize kernels remaining in the field after harvest can germinate, grow, flower, and locally cross-pollinate neighbouring maize plants (Gruber et

al., 2008; Palaudelmàs et al., 2009). Despite cultivation for centuries, maize plants do not occur outside cultivated or disturbed land in Europe.

Studies conducted by the applicant, published literature on the cultivation of numerous varieties of maize MON810 and monitoring observations in some EU countries (see e.g., Delos et al., 2006, 2007) indicate that this maize behaves like non-GM maize and is unlikely to establish volunteers or survive over subsequent seasons or outside cultivation, or to establish feral populations under European environmental conditions. The insect protection against Lepidoptera is not regarded as providing a significant selective advantage to maize plants in Europe, except under high infestation conditions in cultivated fields.

The EFSA GMO Panel concludes that the likelihood of unintended environmental effects due to the establishment and survival of maize MON810 will be no different to that of conventional maize varieties.

6.1.2. Gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via the dispersal of pollen and seed.

6.1.2.1. Plant to bacteria gene transfer

Based on current scientific knowledge (EFSA, 2004, 2007b, 2009a; Keese, 2008), horizontal gene transfer from GM plants to microorganisms under natural conditions is considered extremely unlikely. Since transgenic DNA is a component of many food and feed products derived from maize MON810, microorganisms in the digestive tract of humans and animals (domesticated animals and other animals feeding on fresh and decaying GM plant material) may be exposed to transgenic DNA. Moreover, exposure of microorganisms to transgenic DNA takes place in the environment during the natural decay of plant material remaining in agricultural areas after harvest.

However, the *cry1Ab* gene is under the control of an eukaryotic promoter (see section 3.1.1) with limited if any activity in prokaryotic organisms. Genes under control of prokaryotic regulatory elements conferring the same traits as expressed in the GM plants are widespread in bacteria in natural environments. In addition, the CP4 *epsps* and *gox* genes coding for glyphosate tolerance which were used as selection markers were not inserted in the genome of maize MON810 (see section 3.1.3).

Taking into account the origin and nature of the *cry1Ab* gene and the lack of selective pressure in the intestinal tract and/or the environment, the likelihood that horizontal gene transfer would result in increased fitness of microorganisms or other selective advantages is very limited. For this reason, the EFSA GMO Panel concludes that is very unlikely that the *cry1Ab* gene from maize MON810 would become transferred and established in the genome of microorganisms in the environment or in the human and animal digestive tract. In the unlikely event that such a horizontal gene transfer would take place, no adverse effects on human and animal health or the environment are expected as no new traits would be introduced into or expressed in microbial communities.

6.1.2.2. Plant to plant gene transfer

Maize is a cross-pollinated plant, relying on wind for the dispersal of its pollen. While maize pollen can be collected by honeybees and other insects, these pollinating insects play a minor role in the cross-pollination of maize plants (Eastham and Sweet, 2002; Malone and Burgess, 2009).

Compared to other wind-pollinated species, pollen grains of maize are relatively large (an average diameter of 90 μm) and heavy (0.25 μg) (Raynor et al., 1972; Di-Giovanni et al., 1995). Due to their characteristics, maize pollen grains settle to the ground rapidly (Aylor et al., 2003) and have usually a short flight range (Jarosz et al., 2005). Although vertical wind movements or gusts during pollen shedding can lift pollen up high in the atmosphere and distribute it over significant distances, concentrations of viable pollen considerably decrease with height (Aylor et al., 2006) and distance (Jarosz et al., 2005) from the source. Hence, low levels of cross-pollination can occur over longer distances under suitable climatic conditions (Bannert and Stamp, 2007; Delage et al., 2007), but most cross-pollination events occur within 50 m of the pollen source (reviewed by Eastham and Sweet, 2002; Devos et al., 2005, 2009b; van de Wiel and Lotz, 2006; Hüsken et al., 2007; Sanvido et al., 2008).

The EFSA GMO Panel does not consider pollen dispersal and consequent cross-pollination as environmental hazards in themselves, and is primarily concerned with assessing the environmental consequences of transgene flow on ecosystems by considering the spread and fitness of hybrids and backcross progeny as well as exposure to non-target organisms (see section 6.1.4).

Theoretically, seeds originating from the cross-pollination of certain cross-compatible wild/weedy relatives can mediate the potential spread and establishment of hybrids and backcross progeny (Wilkinson et al., 2003; Morales and Traveset, 2008; Devos et al., 2009a). However, in the EU, there are no cross-compatible wild/weedy relatives with which maize can hybridise and form backcross progeny (Eastham and Sweet, 2002). The only recipients of cross-pollinated transgenes from maize are other cultivated maize varieties and types (Devos et al., 2005, 2009b; van de Wiel and Lotz, 2006; Hüsken et al., 2007; Sanvido et al., 2008; Bitocchi et al., 2009). Thus cross-pollination in maize is not considered an environmental risk, but is an agricultural management and coexistence issue and is not within the remit of the EFSA GMO Panel.

Even though accidental seed dispersal of maize MON810 in Europe is occurring during its cultivation in many countries, the seed-mediated establishment of maize MON810 and its survival outside of cultivation has not been reported in spite of extensive cultivation and accidental seed dispersal. Since maize plants have lost their ability to release seeds from the cob, most seed dispersal is due to harvesting and post-harvest activities of farmers. However, the survival of maize is limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens, herbivory and cold climate conditions.

6.1.3. Interactions between the GM plant and target organisms

Maize MON810 expresses the *cry1Ab* gene, which provides resistance to lepidopteran target pests, and more particularly stem boring species. Because resistance to chemical insecticides is known to evolve in insect pests (Whalon et al., 2008), the potential evolution of insect resistance to Cry proteins constitutively expressed in Bt-crops is considered as a relevant

concern by the scientific community (e.g., BEETLE report, 2009). Field-evolved resistance to Bt-maize has been documented for 2 lepidopteran target pests that are not representative of the European fauna: *Busseola fusca* (Van Rensburg, 2007) and *Spodoptera frugiperda* (Matten et al., 2008; Moar et al., 2008; Tabashnik, 2008; Tabashnik et al., 2008b). The first case of field-evolved resistance to Bt-maize has been reported in a population of the African stem borer (*Busseola fusca*) in South Africa, where some larvae were able to survive on maize MON810 plants (Van Rensburg, 2007; Kruger et al., 2009). The second case concerns fall armyworm, *Spodoptera frugiperda*. Larvae surviving on Cry1F expressing maize in 2 fields in the US (Puerto Rico) were collected and exposed to high concentrations of the Cry1F protein in laboratory bioassays, where no mortality was observed (Matten et al., 2008; Moar et al., 2008; Tabashnik, 2008; Tabashnik et al., 2008b).

Lepidopteran target pests of the Cry1Ab expressing maize (such as MON810) have been monitored worldwide for the potential evolution of resistance against specific Cry proteins. A recent analysis of these monitoring data indicates that neither in the EU, nor in the US, have populations of resistant European corn borer (*Ostrinia nubilalis*) or Mediterranean corn borer (*Sesamia nonagrioides*) been found (Tabashnik et al., 2008a). This confirms previous observations (Andow et al., 2000; Bourguet et al., 2003; Farinós et al., 2004; Eizaguirre et al., 2006; Schuphan, 2006; Stodola et al., 2006; Andreadis et al., 2007; Siegfried et al., 2007). In Spain, for instance, after 6 years of field exposure of *Sesamia nonagrioides* to Cry1Ab expressing maize, no indications of resistance evolution were found (Farinós et al., 2004; Eizaguirre et al., 2006; Andreadis et al., 2007). So far, F_2 screenings (Andow and Alstad, 1998) did not detect resistance alleles (dominant or recessive) in corn borer populations (Andow et al., 1998, 2000; Bourguet et al., 2003; Schuphan, 2006; Stodola et al., 2006; Andreadis et al., 2007). Some of these tests were performed on mated females collected from the field across Mediterranean EU countries and their progeny reared under confined conditions. In contrast, laboratory selections for resistance with Cry1Ab proteins have yielded partial resistance levels in some corn borer strains after several generations (Chaufaux et al., 2001; Huang et al., 2002; Farinós et al., 2004; Alves et al., 2006; Schuphan, 2006; Siegfried et al., 2007; Crespo et al., 2009). While resistance levels fluctuated between generations for each strain, Cry1Ab protein susceptibility decreased significantly over generations for all selected strains. However, none of the laboratory-selected resistant corn borer larvae studied by Farinós et al. (2004) survived on Bt-maize seedlings. Nonetheless, the polygenic nature of resistance discovered in tested laboratory strains suggests that major genes for resistance to the Cry1Ab protein are rare in founding populations of the European corn borer (Alves et al., 2006).

Resistance management strategies, relying on a 'high dose/refuge strategy', have been endorsed in several countries (Alstad and Andow, 1995; Bates et al., 2005; Andow, 2008; Bravo and Soberón 2008). The EFSA GMO Panel considers that appropriate insect resistance management strategies are capable of delaying possible evolution of resistance in field conditions (Andow, 2008; Tabashnik et al., 2008a). Even though no resistance has been reported for maize MON810 following several years of extensive cultivation in Spain, the cultivation of Bt-maize in the EU has been on a limited scale in a few geographic regions. Moreover, as potential resistance evolution is dependent upon multiple agronomic, environmental and biological factors, one should be cautious of predicting future responses of corn borer populations in the EU based on experiences elsewhere (Tyutyunov et al., 2008). Therefore, the EFSA GMO Panel advises that the potential evolution of resistance in lepidopteran target pests continues to be monitored in order to detect potential changes in

resistance levels in pest populations, and the high dose/refuge strategy continues to be employed.

In areas where other lepidopteran pests than the European and Mediterranean corn borer are important targets of maize, they might also be subject to resistance evolution due to exposure to the Cry1Ab protein expressed in maize MON810 plants (e.g., *Sesamia cretica*, *Helicoverpa armigera*, *Mythimna unipuncta*) . Therefore, the EFSA GMO Panel recommends these species are considered by the applicant in the context of both case-specific monitoring for insect resistance management strategy (Alcalde et al., 2007) and general surveillance through farm questionnaires (Tinland et al., 2007; Schmidt et al., 2008).

The EFSA GMO Panel agrees with the insect resistance management plan proposed by the applicants' EU working group on insect resistance management (as referred to by Alcalde et al. (2007)). According to this plan, farmers growing more than 5 ha of maize MON810 in the EU need to establish refuge areas with maize not expressing the Cry1Ab protein corresponding to at least 20% of the surface planted with maize MON810. The working group's reasoning for implementing only the *refugia* on farms where the Bt-maize area is greater than 5 ha is based on (1) the high fragmentation of the European agricultural landscape; (2) the lack of economic feasibility for providing *refugia* on farms with less than 5 ha Bt-maize; and (3) the negligible risk of resistance development in Bt-maize areas smaller than 5 ha. In practice, this would mean that non-Bt-maize *refugia* would not be implemented on a considerable proportion of farms in certain EU countries, as the area planted to Bt-maize on these farms would cover less than 5 ha. Considering experiences in Spain and other EU countries, this would not pose a risk, as Bt-maize would not be widely adopted in a given region. The Spanish experience illustrates that only in regions where pest infestation is high (e.g., Cataluña), does the adoption rate of Bt-maize reach approximately 60% (Gómez-Barbero et al., 2008b). Therefore, it is likely that sufficiently large areas of non-Bt-maize will remain providing widely distributed mosaics of both non-Bt and Bt-maize at regional scales. However, if Bt-maize was adopted on a larger scale in a region, the risk of resistance evolution is likely to increase requiring more specific refuge management measures. In the case of a cluster of fields with an aggregate area greater than 5 ha of Bt-maize, there should be *refugia* equivalent to 20% of this aggregate area, irrespective of individual field and farm size. Since risk management is outside the remit of the EFSA GMO Panel, it is the responsibility of appropriate Competent Authorities in Member States to approve insect resistance management plans that are consistent with the environmental protection goals and biodiversity action plans in each Member State.

6.1.4. Interactions between the GM plant and non-target organisms

The applicant reported results of studies on non-target organism previously presented in its market authorisation dossier. Most of these studies were conducted using the purified Cry1Ab protein with the exception of laboratory tests on *Daphnia magna* and *Folsomia candida* where MON810 plant tissues were used. In the application, also a selected number of references from the available scientific literature on laboratory and field experiments conducted with maize MON810 are cited or commented on.

6.1.4.1. Natural enemies: predators and parasitoids

The exposure of natural enemies (predators and parasitoids) to Cry proteins expressed in Bt-plants can occur in different ways: natural enemies can be exposed to the Cry1Ab protein by feeding on plant material or honeydew excreted from sap-sucking species, and/or by feeding on prey/host organisms that have previously been feeding on Bt-maize (Andow et al., 2006; Romeis et al., 2008a,b).

Harwood et al. (2005) and Zwahlen and Andow (2005) studied exposure to the Cry1Ab protein (event Bt11) for several groups of non-target organisms and reported levels of Bt-protein observed in non-target herbivores and their natural enemies under field conditions. The authors showed that significant quantities of the Cry1Ab protein can move into higher trophic levels. Similarly, Obrist et al. (2006a) showed that the Cry1Ab protein from Bt-maize (event Bt176) in some cases accumulated through the arthropod food web in concentrations that were higher than in Bt-maize leaves. The Cry1Ab protein was detected in certain predators (such as *Orius* spp., *Chrysoperla* spp. and *Stethorus* sp.), whilst its presence was negligible in others (e.g., *Hemerobiids*, *Nabis* sp., *Hippodamia* sp., *Demetrias* sp.). Another tritrophic study performed by Obrist et al. (2006b) confirmed protein uptake by larvae of the green lacewing, *Chrysoperla carnea*, via its herbivore preys, *Tetranychus urticae* and *Spodoptera littoralis*, after Bt-maize consumption (see also Dutton et al., 2002), and found that the biological activity of the Cry1Ab protein is maintained after ingestion by both herbivore species. Harwood et al. (2007) showed the presence of the Cry1Ab protein in gut samples of certain predatory coccinellids (e.g., *Coleomegilla maculata, Harmonia axyridis, Cycloneda munda, Coccinella septempunctata*). Spider mites were shown to contain the highest amounts of Cry1Ab protein (on average 5.56 µg/g fw; *Tetranychus urticae*) when kept on maize event Bt11, compared to thrips (0.91 µg/g fw; *Frankliniella tenuicornis*) and leafhoppers (0.20 µg/g fw; *Zyginidia scutellaris*) (Dutton et al., 2004; Obrist et al., 2005). Thus the exposure to Cry1Ab protein differs between predatory taxa due to variability in phenology and feeding habits.

(a) Hazard assessment

Literature data on the susceptibility of natural enemies to Cry proteins are available and have been reviewed. In this respect, Romeis et al. (2006) suggested that there are little or no indications of direct toxic effects of Cry1Ab expressing maize on natural enemies. Based on the current literature, Lövei et al. (2009) reported that the majority of laboratory studies indicate neutral responses of natural enemies to Cry proteins, though more significant non-neutral (either negative or positive) results were detected than expected at random. In addition, Lövei and Arpaia (2005) revealed some shortcomings related to species selection, sample size, statistical power and duration of certain laboratory toxicity studies performed on arthropod natural enemies. Naranjo (2009) conducted a meta-analysis of laboratory studies involving natural enemies and concluded that direct exposure of natural enemies to Bt-proteins generated in predators a slight but significant reduction in developmental rate compared with non-Bt-maize controls. Conversely, Bt-proteins had no effect on survival or reproduction of either predators or parasitoids.

Results obtained in some laboratory studies indicated potential indirect adverse effects on predators due to exposure to the Cry1Ab protein. Meissle et al. (2005), for instance, observed adverse effects on the generalist carabid predator, *Poecilus cupreus*, fed *Spodoptera littoralis* larvae, which had been raised on Bt-maize (event MON810). *Poecilus cupreus* larvae fed

Spodoptera littoralis larvae, raised on Bt-maize, had a higher mortality than those fed larvae raised on conventional maize or 'high quality' *Calliphora* sp. pupae. Since *Spodoptera littoralis* is partly susceptible to the Cry1Ab protein (Dutton et al., 2005; Vojtech et al., 2005), the authors attributed the adverse effects observed to an indirect mechanism due to reduced nutritional prey quality. In contrast, based on the same prey-predator model, Alvarez-Alfageme et al. (2009) did not detect a significant effect on mortality, development time and growth of larvae and pupae of *Poecilus cupreus* fed (*ad libitum*) with *Spodoptera littoralis* larvae reared on Bt176 maize leaves. Moreover, proteolytic digestion in *Poecilus cupreus* was not affected when exposed to the Cry1Ab protein in both laboratory and field assays, indicating that the quality of the prey was not impaired. The differences observed between both studies were attributed by the authors to lower levels of Cry1Ab protein in maize leaves of Bt176 than MON810, and to the lower Cry1Ab protein sensitivity of 10-day old larvae used by Alvarez-Alfageme et al. (2009) as compared with neonate larvae used by Meissle et al. (2005).

Another frequently used predator species in laboratory tritrophic bioassays to investigate potential effects of Bt-crops on predators via dosed prey is lacewing *Chrysoperla carnea* (Lövei and Arpaia, 2005). Hilbeck et al. (1998a,b, 1999) indicated significantly prolonged larval development and increased mortality when immature *Chrysoperla carnea* was fed lepidopteran larvae reared on Cry1Ab expressing maize under laboratory conditions. The authors suggested a possible chronic effect of Cry1Ab protein, while Romeis et al. (2004) indicated possible indirect effects due to poor prey quality. Rodrigo-Simón et al. (2006) reported that the Cry1Ab protein does not show specific binding *in vitro* to brush border membrane vesicles from the midgut of *Chrysoperla carnea* larvae, which is considered as a prerequisite for toxicity. Moreover, no acute adverse effects were reported when *Chrysoperla carnea* larvae were fed non-susceptible *Tetranychus urticae* containing large amounts of biologically active Cry1Ab protein (Dutton et al., 2002). In the field, *Chrysoperla carnea* larvae are known to feed mainly on aphids and lepidopteran larvae are not considered an important prey, especially after their first moult (Romeis et al., 2004). Therefore, the continuous exposure of *Chrysoperla carnea* to diets exclusively based on lepidopteran larvae is unlikely under field conditions where a variety of prey is available (Dutton et al., 2003), though chronic effects cannot be excluded completely.

Schmidt et al. (2009) carried out laboratory toxicity tests with microbially produced trypsin-activated Cry1Ab or Cry3Bb proteins fed to different larval stages (L1-L4) of the coccinellid *Adalia bipunctata*. Bt-protein treatment was performed via 0, 5, 25, or 50 µg/ml Bt-protein spray on *Ephestia* sp. eggs, which were then offered as food in a no-choice test. The authors did not quantify the actual intake of Bt-protein by the larvae, but tested qualitatively the presence of the Bt-proteins in the spray solution by immuno-strip assays. The paper reports that *Adalia bipunctata* larvae fed lepidopteran-active Cry1Ab protein exhibited significantly higher mortality than the control group at the lowest concentration (5 µg/ml) of the protein as well as the higher levels. However, in experiments with the coleopteran-active Cry3Bb toxin, only a concentration of 25 µg/ml resulted in a significantly higher mortality compared to the control. Both experiments revealed a slight decline in mortality at the highest concentration of 50 µg/ml, though this was statistically significant only in the case of Cry1Ab treatment. No differences were detected for development time of larvae and body mass of newly emerged adults. The authors suggest that the increased mortality of larvae in the toxin feeding trials was caused directly by the activated Bt-proteins and raise questions regarding their suggested postulated specificity and their mode of action in *Adalia bipunctata*. However, neither a dose-

response relationship, nor sublethal effects (on developmental time and adult body weight) on surviving specimen were observed; both these features represent a typical response of sensitivity to Cry proteins. The higher toxicity of a Lepidoptera-specific Cry1Ab reported on Coleoptera in comparison to the more Coleoptera-specific Cry3Bb is an outcome that needs to be confirmed based on more quantitative data (both on food intake and actual protein concentration). The EFSA GMO Panel is of the opinion that these data are not sufficient to identify a hazard or indicate a new mode of action of Cry proteins on the coccinellid species tested. Wold et al. (2001) did not find adverse effects on *Adalia bipunctata* in laboratory and field studies (but reported some adverse effects on another coccinellid species in the laboratory test). Dhillon and Sharma (2009) studied the effects of the Cry1Ab and Cry1Ac proteins on the predatory coccinellid *Cheilomenes sexmaculatus* under direct and indirect exposure conditions. Direct exposure of *Cheilomenes sexmaculatus* larvae to Bt-proteins at high concentrations resulted in reduced larval and adult emergence as compared to the controls. However, there were no adverse effects of the Bt-proteins on *Cheilomenes sexmaculatus* when the larvae were reared on *Aphis craccivora* fed on different concentrations of Cry1Ab or Cry1Ac in an artificial diet. A significant and positive correlation was found between the presence of Bt-proteins in aphids, and coccinellid larvae and adults. The authors concluded that direct exposure to Bt-proteins expressed in GM plants or predation on *Helicoverpa armigera* on Bt-plants will have little effect on the activity and abundance of *Cheilomenes sexmaculatus*. Higher tier studies are also available in the literature; in the field, no adverse effects of Bt-maize (different events) were detected on a range of coccinellid species (e.g., Pilcher et al., 1997; Jasinski et al., 2003; Dively and Rose, 2004; de la Poza et al., 2005; Lundgren and Wiedenmann, 2005; Eckert et al., 2006; Alvarez-Alfageme et al., 2008). An important consideration in terms of environmental risk assessment is that it is unlikely that coccinellid larvae will be exposed to biologically relevant amounts of Cry1Ab from maize MON810. The exposure route used by Schmidt et al. (2009) may constitute a useful model for laboratory studies, but the EFSA GMO Panel considers any exposure through egg feeding in the field very unlikely. The Cry1Ab protein content in maize MON810 pollen (which is likely to be the most common source for possible toxin ingestion for coccinellids) is very low and ranges between 1-97 ηg/g fw (Nguyen and Jehle, 2007). Bt-proteins are normally absent in aphids feeding on maize (Head et al., 2001; Raps et al., 2001), which is the main diet of coccinellid larvae.

In general, invertebrate parasitoids appear to be more sensitive than predators to diets containing Cry proteins under laboratory conditions (Lövei et al., 2009), though in most cases neutral results were obtained in tritrophic experiments. The meta-analysis conducted by Naranjo (2009) confirms the higher sensitivity of parasitoids in tritrophic experiments, and the author was able to identify the importance of host quality in determining such results. Parasitoids can be exposed to the Cry1Ab protein through one or more trophic levels (e.g., direct feeding on Bt-plant material or their host organisms feeding on Bt-plant tissue) and therefore host quality has an important effect (e.g., Dutton et al., 2002, 2005; Vojtech et al., 2005). Ramirez-Romero et al. (2007) observed that the exposure to Cry1Ab protein via hosts fed Bt-maize tissue sublethally affected the parasitoid *Cotesia marginiventris*. In experiments where hosts fed maize MON810 were compared with those fed control maize, and where the former accumulated low concentrations of Cry1Ab protein, these effects still occurred. Moreover, host size did not differ between treatments. The authors were able to demonstrate the importance of the plant in causing negative effects at the third trophic level, since negative results were not observed when pure protein-containing diet was used in the tritrophic

experiments. Thus these results suggest an effect on the parasitoid when delivered via the host feeding on plant tissue.

(b) Exposure assessment

Results of a meta-analysis of 42 independent field experiments carried out across different continents by Marvier et al. (2007) indicated that non-target invertebrates are generally more abundant in near-isogenic control fields where no insecticide treatments are applied than in fields cropped with Bt-cotton or Bt-maize (events MON810, Bt176 and MON863). However, when non-Bt-cotton or maize fields are managed conventionally with the application of insecticides, non-target taxa were shown to be less abundant than in fields cropped with Bt-cotton or maize. A more recent meta-analysis of published field studies on non-target effects of Bt-crops (Wolfenbarger et al., 2008) made the differentiation among functional guilds of non-target arthropods. The abundance of predators, parasitoids, omnivores, detritivores and herbivores was compared under scenarios where neither, only the non-Bt-crops, or both Bt and non-Bt-crops received insecticide treatments. Different effects of Bt-maize on functional guilds of non-target arthropods were reported. As expected, fewer specialist parasitoids of the target pest occurred in Bt-maize fields (specifically *Macrocentrus grandii*), as compared to unsprayed non-Bt-maize controls, but no significant reduction was detected for other parasitoids. Higher numbers of the generalist predator *Coleomegilla maculata* were found in Bt-maize compared to non-Bt-maize, with no difference found for other common predatory genera.

In comparison to sprayed non-Bt-crop controls, numbers of predators and of the specialist parasitoid *Macrocentrus grandii* and herbivores were higher in Bt-crops, with the magnitude of the difference being influenced by the type of insecticide (Wolfenbarger et al., 2008).

Nine years of experience of Cry1Ab maize cultivation (events Bt176 and MON810) in Spain revealed no adverse effects on non-target arthropods, including predators and parasitoids (de la Poza et al., 2005; Pons et al., 2005; Eizaguirre et al., 2006; Farinós et al., 2008). In a field monitoring study performed in Germany from 2000 to 2005, field pairs (half-fields) planted with Bt-maize (event MON810) and a conventional maize variety were studied to determine densities of arthropod taxa on plants, activity densities and diversity of ground-dwelling arthropods (Schorling and Freier, 2006). Density comparisons of different taxa (such as aphids, thrips, heteropterans, aphid specific predators, spiders and carabids) revealed a few significant differences for specific taxa between Bt and conventional maize fields, but no general tendencies over the 6 years. No effects due to the growing of maize MON810 on non-target communities were observed during an intensive field study (8 replications for each of the each Bt, chemical insecticide and control treatment) performed in Germany over 3 consecutive years (Eckert et al., 2006; Toschki et al., 2007).

(c) Conclusion

On the basis of the data delivered by the applicant and obtained from a literature survey, the likelihood of adverse effects on non-target natural enemies is foreseen to be very low. Rearrangements of species assemblages at different trophic levels are commonly associated with any pest management practice. The EFSA GMO Panel is of the opinion that maize MON810 will not cause reductions to natural enemies that are significantly greater from those caused by conventional farming where pesticides are used to control corn borers.

6.1.4.2. Non-target Lepidoptera

Since maize is not an important resource of food for indigenous Lepidoptera with the exception of few pest species, exposure to potentially harmful amounts of pollen deposited on host plants in or near maize MON810 fields is expected to be the main risk to non-target Lepidoptera (e.g., BEETLE report, 2009).

(a) Hazard assessment

It is well-documented that larvae of a range of lepidopteran species are susceptible to the Cry1Ab protein and can be affected by the protein after ingestion of significant amounts of it through Bt-maize pollen (Losey et al., 1999; Hansen and Obrycki, 2000; Jesse and Obrycki, 2000; Felke and Langenbruch, 2003, 2005; Hellmich et al., 2001; Felke et al., 2002; Anderson et al., 2004, 2005; Dutton et al., 2005; Mattila et al., 2005; Lang and Vojtech, 2006; Prasifka et al., 2007). Lethal and sublethal effects of Bt-maize pollen consumption by lepidopteran larvae have been reported for several non-target lepidopteran species under laboratory conditions, with the magnitude of these effects varying with the Bt-maize event and lepidopteran species used, the larval stage, the amount of pollen consumed and Cry1Ab protein amounts contained in pollen (e.g., Hellmich et al., 2001; Felke et al., 2002; Mendelsohn et al., 2003; Felke and Langenbruch, 2005; Wolt et al., 2005). A laboratory assay revealed toxicity to monarch butterfly (*Danaus plexippus*) larvae that consumed maize Bt11 pollen deposited on milkweed (*Asclepias* spp.) leaves compared to those reared on leaves dusted with non-GM maize pollen or on leaves without pollen (Losey et al., 1999). Larvae of the common species, *Pieris brassicae*, *Pieris rapae* and *Plutella xylostella* also fed less, grew more slowly and showed a higher mortality when they ingested food plant material containing pollen of maize Bt176, compared to larvae of an untreated control group (Felke et al., 2002).

In no-choice feeding studies with diets containing maize Bt176 pollen, Felke and Langenbruch (2005) revealed that based on LD_{50}-values *Plutella xylostella* is the most sensitive lepidopteran species of the 7 species tested. 50% of the fourth instar larvae of *Plutella xylostella* died (LD_{50}-values) when they consumed on average 8 pollen grains of maize Bt176 placed on a 0.071cm² host plant leaf disk, as compared with 39.0 pollen grains for second instar larvae of *Pieris rapae* and 139.3 pollen grains for second instar larvae of *Pieris brassicae* (Felke et al., 2002). Concentrations of Cry1Ab protein in pollen of maize MON810 are approximately 40 times less than in maize Bt176 (Hellmich et al., 2001; Sears et al., 2001; Mendelsohn et al., 2003). Larvae of *Plutella xylostella* remained unaffected by the consumption of up to 80 pollen grains of maize MON810 in no-choice feeding studies (Felke and Langenbruch, 2005). However, since anthers have a higher concentration of Cry1Ab protein as compared to pollen (in a range similar to maize leaves e.g., between 0.30-6.65 µg/g as reported in Nguyen and Jehle, 2007), the consumption of anther fragments of maize MON810 caused in a laboratory experiment a significant increase in mortality (Felke and Langenbruch, 2005), confirming previous observations made on monarch butterfly larvae (Hellmich et al., 2001).

Based on concentrations of Cry1Ab protein in pollen of maize Bt176 and MON810, the applicant estimated the acute sensitivity (LD_{50}-values) of certain lepidopteran species to Cry1Ab-containing pollen and concluded that depending upon the lepidopteran species and larval stage relatively high amounts of maize MON810 pollen grains are needed to adversely affect significant proportions of larvae.

The EFSA GMO Panel notes that acute toxicity is often not considered a reliable predictor of sublethal effects (Suter, 2007) and that sublethal effects of Cry1Ab protein are anticipated for non-target Lepidoptera (e.g., Dively et al., 2004; Lang and Vojtech, 2006). Sublethal effects are likely to be more frequent under field conditions than lethal effects, as densities of pollen grains decline rapidly with increasing distance from the maize source (Eastham and Sweet, 2002; Jarosz et al., 2004; Devos et al., 2005; Hofmann, 2007, 2009; Boehm et al., 2008). In summary, the potential lethal and sublethal effects of pollen from maize MON810 represent a potential hazard to non-target European Lepidoptera.

(b) Exposure assessment

The report of the hazard of Bt-maize pollen ingestion for the monarch butterfly in laboratory experiments (Losey et al., 1999) triggered extensive field studies to determine whether monarch butterfly populations would be at risk under real exposure conditions in the US. These field studies indicated that the proportion of monarch butterfly populations exposed to toxic levels of Bt-pollen is small due to limited spatial distribution of pollen (Pleasants et al., 2001) and the limited temporal overlap between larval development and pollen shed (Oberhauser et al., 2001). Exposure to potentially harmful quantities of pollen was shown to be largely restricted to pollen deposited on host plants in the area of field margins as the highest maize pollen concentrations occur in and near maize fields and as weed food plants are generally not abundant in maize fields. Hence, pollen concentrations exceeding toxicity levels mainly occur on leaf surfaces in Bt-maize fields and within 1-5 m of the edge of the Bt-maize field (events Bt176, Bt11 and MON810) for a range of lepidopteran species (Jesse and Obrycki, 2000; Pleasants et al., 2001; Zangerl et al., 2001; Jarosz et al., 2003; Wolt et al., 2003; Dively et al., 2004; Felke and Langenbruch, 2005; Gathmann et al., 2006b; Lang and Vojtech, 2006). Based on the US data, a risk assessment model estimated that 50% of the breeding population of the monarch butterfly was potentially exposed to Cry1Ab expressing pollen in the US corn belt (Sears et al., 2001) and that only an additional 0.6-2.5% mortality would be generated due to Bt-maize cultivation (Dively et al., 2004).

In addition, 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 (event 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). However, an examination of anthers in and near maize fields showed that toxic levels of anthers rarely occur under normal field conditions, so that exposure of monarch butterflies to toxins from intact anthers from Bt-maize alone or in combination with pollen from Bt-maize is likely to be very low (Anderson et al., 2004). 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 EFSA GMO Panel notes that similar extensive pollen and anther exposure assessment studies as those performed in the US have not been conducted under European environmental conditions, but that some studies provide relevant information for the exposure of European lepidopteran species in agricultural landscapes on a population level (Schmitz et al., 2003; Lang et al., 2004; Anonymous, 2006; Gathmann et al., 2006a,b). The EFSA GMO Panel does not consider anthers to contribute significantly to European lepidopteran larvae exposure of Cry1Ab since anthers show a much lower spatial and temporal distribution compared to pollen.

In a theoretical exposure assessment, Schmitz et al. (2003) estimated that approximately 7% of German macrolepidopteran species (butterflies and nocturnal species) mainly occur in plant communities (as habitat) in or near maize fields in farmland areas and thus could be potentially affected by exposure to Bt-maize pollen. Of these species being potentially affected by Bt-maize pollen exposure, 14% were found to be potentially exposed on a regional scale (Schmitz et al., 2003). Traxler et al. (2005) reported that, of the 215 butterfly species occurring in Austria, 152 appear in agricultural landscapes and a proportion of these are in potential contact with maize pollen. In the case of 29 butterfly species, it was estimated that their development would coincide with the time of pollen shed of maize for 75-100% of the time, whilst shorter overlapping timeframes are seen with other butterfly species (ranging from 25 to 75%). According to Darvas et al. (2004), 16% of the 187 protected Lepidoptera species might feed on weeds growing at the edge of maize fields in Hungary, of which 2 species (*Inachis io* and *Vanessa atalanta*) might be affected by Bt-maize pollen when feeding on great nettle (*Urtica dioica*) in maize field margins. However, no exposure analysis is available from this study.

During a 3-year field study performed in Germany, no difference in abundance of larvae of the butterfly species *Pieris rapae* and *Plutella xylostella* were observed between the Bt-based treatment (event MON810) and control treatment on weed strips artificially sown in maize field plots (Gathmann et al., 2006b). Although 7 other butterfly species were observed in the study, their low abundance did not enable suitable statistical analysis, confirming the practical difficulty of detecting small effects where they exist on all lepidopteran species that could be potentially exposed to Bt-maize pollen (Lang, 2004; Gathmann et al., 2006b; Aviron et al., 2009).

Hence, extrapolating observations made on certain non-target lepidopteran species to others remains difficult due the variability in acute sensitivity to the Cry1Ab protein and due to the different biology among lepidopteran species. Moreover, event MON810 is occurring in an increasing number of commercial varieties with a range of flowering dates so that temporal variability in exposure to the Cry1Ab protein might be expected (e.g., van Hout et al., 2008). Currently, more than 90 maize varieties derived from the transformation event MON810 are registered in the EU common seed catalogue.

The large-scale cultivation of maize MON810 within Europe has been restricted to Spain, an environment where Lepidoptera populations are rather low. Therefore, data on some aspects of exposure, such as phenology, are rare within Europe. In order to explore possible scenarios for the exposure of European species of butterflies to maize MON810 pollen, the EFSA GMO Panel built a simulation model to help quantify the risk assessment. The parameter values chosen for the model were informed by field data where available, supplemented by the estimates of a range of environmental experts from various EU regions.

Exposure was modelled for 3 combinations of lepidopteran species and their host-plants, all of which occur widely throughout the EU. These were:

(1) the butterfly *Inachis io* feeding on its host-plant *Urtica dioica*;

(2) the butterfly *Vanessa atalanta* and its host-plant *Urtica dioica*;

(3) the moth pest species *Plutella xylostella* and its host-plant species within the family Brassicaceae; *Plutella xylostella* has been shown to be a species very sensitive to the Cry1Ab protein.

Analysis was based on an 11-parameter deterministic mathematical model, of which 7 parameters were specific to particular regions within Member States and 4 parameters were more generic to the particular species/host-plant combination.

The 7 regional parameters concerned the following measures:

(1) the proportion of the host-plant species found within arable fields and their margins;

(2) the proportion of arable fields cropped with maize;

(3) the proportion of maize cropped that is (within Spain, currently) or might be (in other Member States, in the future) Bt-maize;

(4) the average area of fields cropped with maize;

(5) the average width of field margins of fields cropped with maize;

(6) the average within-field density of the host-plant species;

(7) the average within-margin density of the host-plant species.

The 4 more generic parameters were:

(8) the degree to which physical effects (such as rain washing pollen off leaves, larvae feeding on the underside of leaves where pollen densities are smaller, etc.) reduces exposure;

(9) the degree to which exposure is reduced due to a lack of temporal coincidence between the susceptible larval stage concerned and the period over which maize MON810 pollen is shed;

(10) the average proportion of susceptible larvae within the field margin of maize MON810 fields that suffer mortality before allowance for other effects listed above;

(11) the average proportion of susceptible larvae within a maize MON810 field that suffer mortality before allowance for other effects listed above.

Essentially, the model operates by estimating the total mortality possible from parameters 10 and 11, and reducing it successively and proportionally according to the values of parameters 1, 2, 3, 8 and 9. The model estimates mortality for the most susceptible larval stage of the

lepidopteran species concerned. For areas outside of Spain, where maize MON810 is currently cropped extensively, a worst-case assumption was made by setting parameter 3 to the maximal likely uptake of MON810; for most areas this value was set at 0.8, given that 0.2 is obligatory for refuge areas with non-Bt-maize (see section 6.1.3). Sublethal effects were modelled on the basis that the dose, *d*, of maize MON810 pollen sufficient to cause mortality in a proportion, *p*, of the larval population would give rise to sublethal effects in a proportion of 4*p* of the population (for $p<0.25$). It was estimated that the dose of maize MON810 pollen on host-plant leaves would be insufficient to cause mortality within the field margin at distances from the edge of the field greater than 2 m for the 2 butterfly species and 5 m for the moth species (Sears et al., 2001; Felke and Langenbruch, 2005). Further, it was estimated that the dose of maize MON810 pollen on host-plant leaves would be insufficient to cause sublethal effects within the field margin at distances from the edge of the field greater than 5 m for the 2 butterfly species and 9 m for the moth species.

Ten areas were modelled in 4 Member States: in Germany, 6 areas near Bonn, Oderbruch, Aachen, Grebbin, Berkatal and the Upper Rhine Valley; in Italy, in the Po valley; in Hungary, in Tolna county; and in Spain, an area near Madrid and another in Cataluña. Regional experts set parameters 1-7 for the particular areas within which they had local knowledge. For parameters 8-11, estimates were provided by 7 members of the EFSA GMO Panel Working Group on Environment. Variability in estimated mortality and sublethality resulted from: (1) natural variation between areas, reflecting expected agronomic and environmental heterogeneity; (2) differences between experts' interpretation; and (3) uncertainties arising from incomplete data available. One example for region-specific results relates to the varying abundance of host plants in European regions. In areas where *Urtica* spp. are poor or completely missing as around Madrid and in Cataluña, no estimations for effects of Bt-maize on *Vanessa atalanta* and *Inachis io* can be drawn.

A good agreement among the experts was achieved for parameters 9, 10 and 11; there was fair agreement for parameter 8. The best estimates for parameters 8-11 were defined as the median values of the estimates of the 7 experts. Had the maximum values for each of the parameters 8-11 been selected instead, this would have given a worst-case (greatest) estimated mortality and sublethality, and have resulted in an approximately 3-fold increase in proportional estimated mortality or sublethality for *Inachis io*, a 5-fold increase for *Vanessa atalanta* and a 2.5-fold increase for *Plutella xylostella*. None of the experts provided the maximum value for all 4 parameters, so such worst-case estimates should be considered as very conservative.

For *Inachis io*, the best estimate of proportional mortality ranged from zero in Madrid and Cataluña (where the host-plant *Urtica* is not found), to 0.0024 (one individual in every 417) in the Upper Rhine Valley; excluding Spain, the median estimated mortality over the areas was one individual in every 1853. For *Vanessa atalanta*, the best estimate of proportional mortality ranged from zero in Madrid and Cataluña, to one individual in every 694 in the Upper Rhine Valley; excluding Spain, the median over the areas was one individual in every 3603. For *Plutella xylostella*, the best estimate of proportional mortality ranged from one individual in every 21.2 million in Madrid, to one individual in every 94 in the Upper Rhine Valley; the median over the areas was one individual in every 333.

For *Inachis io*, the best estimate of proportional sublethality ranged from zero in Madrid and Cataluña, to one individual in every 167 in the Upper Rhine Valley; excluding Spain, the median estimated sublethality over the areas was one individual in every 553. For *Vanessa*

atalanta, the best estimate of proportional sublethality ranged from zero in Madrid and Cataluña, to one individual in every 278 in the Upper Rhine Valley; excluding Spain, the median over the areas was one individual in every 1080. For *Plutella xylostella*, the best estimate of proportional sublethality ranged from one individual in every 4.7 million in Madrid, to one individual in every 33 in the Upper Rhine Valley; the median over the areas was one individual in every 111.

The results obtained were least sensitive to perturbations in parameter 11, because the ratio between parameters 7 to 6 was never less than 80 for *Urtica* spp. and 10 for Brassicaceae; this reflects normal agricultural practice in within-field weed suppression. The results obtained were most sensitive, and varied proportionally, to perturbations in parameter 8.

It is understood that pollen deposition may vary greatly spatially depending upon weather conditions. Due to vertical wind movements or gusts (Bannert and Stamp, 2007; Delage et al., 2007; Hofmann, 2009; Kuparinen et al., 2009), a certain small area may experience a larger than average concentration of pollen, even tens of meters away from a maize field. However, such larger than average pollen concentrations are balanced by smaller than average values elsewhere, where effects are diluted. The calculations of the EFSA GMO Panel are deterministic and relate to average outcome; they are robust to the above examples of heterogeneity.

The conclusions from this modelling exercise are that a full exposure assessment is possible for several lepidopteran species, but it requires many factors to be taken into account, some of which had to be modelled with little available data. However, these predictions are relatively robust, as the difference between the best and most conservative (worst-case scenario) estimates led to no more than a 2.5 to 5-fold increase in the predicted mortality and sublethality. For the majority of areas where *Urtica* occurred, for both the butterfly species considered here, the best estimate for mortality was less than one individual in every 1800, and of sublethality was less than one individual in every 550. Corresponding worst-case estimates for mortality and sublethality were less than one individual in every 650 and 190, respectively. For the majority of areas and the pest moth species considered here, the best estimate for mortality was less than one individual in every 300, and of sublethality was less than one individual in every 100. Corresponding worst-case estimates for mortality and sublethality were less than one individual in every 130 and 40, respectively. All these estimates were made under the scenario of maximum uptake of maize MON810 by growers for the areas in Germany, Hungary and Italy.

(c) Conclusion

On the basis of the data provided by the applicant and obtained from a literature survey and a modelling exercise, the EFSA GMO Panel considers that the amounts of maize MON810 pollen grains found in and around maize fields are unlikely to adversely affect a significant proportion of non-target lepidopteran larvae.

The EFSA GMO Panel is aware that all modelling exercises are subject to uncertainties; as with any ecological model, further data would refine the estimates reported here. The EFSA GMO Panel considers it advisable that, especially in areas of abundance of non-target Lepidoptera populations, the adoption of the cultivation of maize MON810 be accompanied by management measures in order to mitigate the possible exposure of these species to

MON810 pollen. As an example, the planting of border rows of non-Bt-maize adjacent to uncultivated field margins of maize MON810 fields, could limit the exposure to those individuals feeding on weeds present within maize field borders and also could contribute to the required percentage of non-Bt-maize necessary to constitute refuge areas for lepidopteran target pests in the framework of resistance management plans.

6.1.4.3. Pollinating insects: honeybees

Maize pollen can be collected, stored and consumed by honeybees, especially in regions where there are limited sources of pollen when maize is flowering. Pollen feeding is a route of exposure of honeybees to Cry1Ab protein expressed in maize MON810 (e.g., BEETLE report, 2009).

(a) Hazard assessment

Reviewing available scientific data on potential adverse effects on honeybees of the Cry1Ab protein or Bt-pollen of maize gathered either under laboratory or semi-field conditions, Malone and Burgess (2009) concluded that none of the Bt-plants commercially available at the time of the publication have significant impacts on the health of honeybees. Other feeding studies performed in controlled conditions with honeybees being fed either with Bt-pollen or mixtures of honey or sugar syrup containing purified Cry1Ab protein have indicated no direct adverse effects on larvae and adult survival (Ramirez-Romero et al., 2005, 2008; Rose et al., 2007). 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, Duan et al. (2008) considered that in field settings, honeybees might face additional stresses, which could theoretically affect their susceptibility to Cry proteins and generate indirect effects.

Since exposure to Bt-pollen could have potential indirect adverse effects on the development of the whole honeybee colony, some studies focused on the hypopharyngeal gland development in honeybees. Hypopharyngeal glands are considered an important indicator of bee life history and thus for colony development, as worker (nurse) bees use their hypopharyngeal gland to prepare brood food (jelly) for the larvae. In this respect, Babendreier et al. (2005) fed young adult bees for 10 days with Bt-maize pollen expressing Cry1Ab protein (event MON810) or with purified Cry1Ab protein solubilized in sugar solutions. No significant differences either in diameter or weight development of hypopharyngeal glands of control bees and bees fed Bt-pollen or Bt-containing sugar solutions were found. By contrast, protease inhibitors caused significant differences which indicated the sensitivity of the method.

In a field study where colonies foraged on Cry1Ab expressing maize (event Bt11) and were fed Bt-pollen cakes for 28 days, Rose et al. (2007) did not observe adverse effects on bee weight, foraging activity, and colony performance. 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 Cry1Ab protein (5000 µg/kg), which do not occur under normal apicultural or field conditions (Ramirez-Romero et al., 2008).

(b) Exposure assessment

In most cases, the proportion of maize pollen as a total of all pollen collected and fed to larvae during a summer will be low. Babendreier et al. (2004), for instance, reported that fully grown worker bee larvae contain between 1720 and 2310 maize pollen grains in their gut before defecation, corresponding to 1.52-2.04 mg of pollen consumed per larva. On average, 74.5% of pollen grains were completely digested, while 23.3% were partially digested and 2.2% remained undigested. Since pollen consumption of honeybee larvae is minimal when compared to adults, larval stages are far less exposed to Bt-proteins: Babendreier et al. (2004) indicated that the contribution of the protein by directly feeding larvae with pollen is less than 5% in relation to the total amount of protein necessary for complete larval development. Moreover, due to the low concentration of Cry1Ab in MON810 pollen, honeybees will only be exposed to very low concentrations of the protein.

(c) Conclusion

While the EFSA GMO Panel agrees that in field settings honeybees might face additional stresses that could theoretically affect their susceptibility to Cry proteins or generate indirect effects, it concludes that the likelihood of adverse effects on honeybees is expected to be very low. The EFSA GMO Panel has no reason to consider that maize MON810 will cause reductions to pollinating insects that are significantly greater from those caused by conventional farming.

6.1.4.4. Water-dwelling organisms

Based on findings reported by Rosi-Marshall et al. (2007) and Bøhn et al. (2008), concerns have been expressed about the transport of Bt-maize byproducts (e.g., pollen, detritus) to downstream water bodies and their potential toxic effects on non-target aquatic organisms following consumption (e.g., BEETLE report, 2009).

(a) Hazard assessment

A laboratory experiment performed by Bøhn et al. (2008) revealed that *Daphnia magna* fed a suspension of maize MON810 flour had a higher mortality and lower proportion of females reached sexual maturity, as compared to the non-Bt-maize treatment, suggesting toxic effects of Bt-maize. However, since maize flour is not part of the natural diet of *Daphnia*, the unusual delays in development of *Daphnia* fed non-Bt-maize might have been caused by nutritional deficiencies related to the maize-based diet. Moreover, internationally accepted guidelines for toxicity and reproduction testing of *Daphnia* were not followed.

Rosi-Marshall et al. (2007) reported that byproducts of Cry1Ab expressing maize entered headwater streams and claimed on the basis of experimental data obtained under laboratory conditions that this would reduce growth and increase mortality of some non-target stream insects such as Trichopterans. This study quantified maize biomass (Bt or non-Bt) in headwater streams, measured degradation rates in aquatic systems, but found no difference

between Bt and non-Bt-maize plant material. Concentrations of the Cry1Ab protein in leaves and pollen were not measured, so no dose-response relationship with the Bt-protein can be made. It is thus unclear how the degradation rate of the Bt-protein is related to that of plant material. In addition, the identity of the Bt-maize event used in the feeding test is not clear and no isogenic controls to compare with the GM material were used. Also, there is no detailed information given on the amount of maize material fed to test organisms.

(b) Exposure assessment

The EFSA GMO Panel considers that important background information on levels of exposure and plant material used is missing and that the conclusions made by Rosi-Marshall et al. (2007) are not supported by the data presented in the paper (EFSA, 2007a). Similar views were also expressed by ACRE (2007), Beachy (2008) and Parrott (2008). Hence, it could be concluded that a potential hazard for Trichopterans has been identified under laboratory conditions when exposed to high doses of Cry proteins. However, due to the low level of Cry proteins in aquatic systems, as reported by Douville et al. (2005, 2007), exposure of Trichopterans in aquatic ecosystems is likely to be very low (Chambers et al., 2007).

(c) Conclusion

The EFSA GMO Panel is of the opinion that it is unlikely that the Cry1Ab protein in maize MON810 products would cause adverse effects on non-target water-dwelling organisms in the context of its proposed use.

6.1.4.5. Soil organisms: earthworms

Earthworms play an important role in decomposing plant litter, and are responsible for numerous physical changes that affect the biological properties and processes in soil (e.g., structure, fertility). They are considered important organisms in the regulation of nutrient cycling processes (Icoz and Stotzky, 2008). Since the Cry1Ab protein from Bt-maize enters the soil by root exudates (Saxena et al., 2002, 2004) as well as by plant material (Webster et al., 2008) and residues (Stotzky, 2004), earthworms can be exposed to the protein (e.g., BEETLE report, 2009).

(a) Hazard assessment

Laboratory studies performed on some earthworm species, such as *Aporrectodea caliginosa* (Vercesi et al., 2006; Schrader et al., 2008), *Eisenia foetida* (Clark and Coats, 2006) and *Lumbricus terrestris* (Saxena and Stotzky, 2001a; Zwahlen et al., 2003b; Schrader et al., 2008) did not reveal significant adverse effects on earthworm survival, growth and reproduction following Cry1Ab ingestion.

Saxena and Stotzky (2001a) concluded that the uptake of the Cry1Ab protein (event MON810) by earthworms is of no safety concern, since no adverse effects on mortality or weight were observed on *Lumbricus terrestris* exposed to soil planted to or amended with plant material from the Cry1Ab expressing maize after 40 or 45 days, respectively, compared to non-Bt-maize. However, as pointed by Clark et al. (2005), growth is probably not an appropriate assessment endpoint: individuals used by Saxena and Stotzky (2001a) were already mature, with fully developed clitella, and thus less likely to exhibit changes in growth. Zwahlen et al. (2003b) investigated mortality and growth of *Lumbricus terrestris* in laboratory

and field experiments by exposing juveniles and adults to maize Bt11 (expressing the Cry1Ab protein) during a period of 200 days. Even though earthworms were not affected lethally by the exposure to Bt-maize, sublethal long-term effects were observed in the laboratory study: the growth of adults, expressed as mean fresh weight, was similar for 160 days, but significantly declined thereafter in Bt-exposed earthworms up to 200 days. In the field study with immature *Lumbricus terrestris*, no adverse effects of Bt-maize exposure were found (Zwahlen et al., 2003b). The EFSA GMO Panel notes that the experimental conditions in the laboratory were quite different from those encountered under field conditions, and it is difficult to attribute this biological effect to the life stage, Cry protein or to unanticipated changes in plant characteristics that could have altered microbial composition in such confined soil samples. Moreover, earthworm reproductive activity was recorded, but not quantified and therefore it is not possible to make any inference on long-term effects on natural populations. Lower earthworm biomass could have been attributed to, for instance, differences in timing or production of cocoons in the Bt-maize treatment.

Laboratory toxicity studies, in which *Eisenia foetida* were fed leaf material from Bt-maize (events Bt11 and MON810) or the isogenic counterpart in a soil system and monitored for 28 days, did not reveal adverse effects on survival or reproduction due to the ingestion of Bt-maize leaf material. However, differences in nutritional parameters of Bt-maize lines and isolines were anticipated to lead to differences in effects on earthworms (Clark and Coats, 2006).

Vercesi et al. (2006) studied effects of maize MON810 on important life-history traits (survival, reproduction and growth) of *Aporrectodea caliginosa* under various experimental conditions. In a series of experiments, the authors investigated the growth of juveniles until maturity as well as cocoon production and hatchability. Finely ground leaves of maize MON810 added to soil had no adverse effects on these life-history traits in *Aporrectodea caliginosa*, even if they were exposed to high worst-case scenario concentrations. In addition, growth of juvenile *Aporrectodea caliginosa* was unaffected when they were kept in pots with a growing Bt-maize plant for 4 weeks. Only when considering cocoon hatchability, a slight, but statistically significant, negative effect of high concentration of finely ground Bt-maize residues was observed. However, due to the addition of high concentrations of finely ground Bt-maize residues, Vercesi et al. (2006) questioned whether the negative effect would have any ecological significance under field conditions. In experiments performed by Schrader et al. (2008), the 2 tested earthworm species, *Aporrectodea caliginosa* and *Lumbricus terrestris*, survived incubation for 5 weeks, irrespective of whether they received MON810 or non-transgenic maize material.

(b) Exposure assessment

In the field, earthworms can be exposed to the Cry1Ab protein through root exudates and decomposing plant material. The ingestion of the Cry1Ab protein by earthworms was confirmed through the detection of the protein in their gut and faeces (e.g., Zwahlen et al., 2003b). However, so far, no adverse effects on earthworms were detected in field surveys during the cultivation of Bt-maize expressing the Cry1Ab protein (e.g., Zwahlen et al., 2003b; Anonymous, 2006; Krogh et al., 2007). While the growth of adult *Lumbricus terrestris* was shown to decline after 160 days in Bt-exposed earthworms under laboratory conditions, no differences were detected in growth rate between Bt-based and near-isogenic maize material in the field exposure experiment (Zwahlen et al., 2003b). No significant differences were

reported in the population density or biomass of *Lumbricidae* between soils with Bt (events MON810 and Bt176) and non-Bt-maize and between soils with maize treated with or without insecticide at 5 sites during 4 years of maize cultivation in field, though both the site and sampling years had a significant influence on both assessment endpoints (Anonymous, 2006). In the frame of the ECOGEN project, Krogh et al. (2007) concluded that earthworms are not affected by maize MON810 as no effects of Bt-maize were detected when compared with the near-isogenic variety.

(c) Conclusion

The EFSA GMO Panel is of the opinion that there is no evidence to indicate that the placing of maize MON810 and derived products on the market is likely to cause adverse effects on earthworms in the context of its proposed use.

6.1.4.6. Soil organisms: enchytraeid worms

Through their feeding activities, enchytraeid worms support mineralisation processes and improve the fine structure of soil, and can be exposed to the Cry1Ab protein.

(a) Hazard assessment

In a laboratory feeding experiment, the effects of diets containing leaf material of Bt-maize (events Bt11 and MON88017) were analysed on the survival and reproduction of *Enchytraeus albidus* (Hönemann and Nentwig, 2009). Significantly more individuals were reported to survive in the treatment with maize Bt11 than in the control treatment with the corresponding near-isoline. In contrast, a significantly higher number of offspring was shown for the control treatment, as compared with the Bt11 containing diet. The authors attributed the differences in survivorship and offspring to differences in plant components amongst maize varieties, rather than to the Bt-maize leaf material in the food diet (Hönemann and Nentwig, 2009). This variability in plant components was expected to have influenced the degradability and consequently the quality of plant material as food resource (Saxena and Stotzky, 2001b; Flores et al., 2005; Poerschmann et al., 2005; Fang et al., 2007; Griffiths et al., 2007b; Raubuch et al., 2007; Lehman et al., 2008; Tarkalson et al., 2008).

(b) Exposure assessment

Enchytraeid worms were observed to feed on diets that contained leaf material of Bt-maize, though no Cry1Ab proteins were detected in adults after 3 weeks feeding on these diets (Hönemann and Nentwig, 2009). So far, no field studies have been performed on enchytraeid worms to analyse the potential consequences of ingestion of Bt-maize plant material. However, Hönemann and Nentwig (2009) do not expect Cry1Ab expressing maize to endanger the survival or reproduction *Enchytraeus albidus*, provided that organic matter of sufficient quality is available in the soil. Generally, enchytraeid worms do not feed on a single food source, but take up all degradable organic matter of adequate size in the field.

(c) Conclusion

The EFSA GMO Panel is of the opinion that there is no evidence to indicate that the placing of maize MON810 and derived products on the market is likely to cause adverse effects on enchytraeid worms in the context of its proposed use.

6.1.4.7. Soil organisms: nematodes

Nematodes are considered useful indicators of soil quality, due to their great diversity and participation in many functions at different levels of food webs in soil and due to their presence in almost all soils with a high population density and a large number of species (Anonymous, 2006; Icoz and Stotzky, 2008). Several studies have been performed to assess potential consequences on nematodes of exposure to the Cry1Ab protein.

(a) Hazard assessment

A recent review on the effects of Bt-crops on soil ecosystems illustrated that, depending upon experimental conditions, the Cry1Ab protein might have different effects on nematodes (Icoz and Stotzky, 2008). Saxena and Stotzky (2001a) found no significant differences in the number of nematodes in the rhizosphere soil of Bt and non-Bt-maize grown in a plant-growth chamber or between soil amended with biomass of Bt and non-Bt-maize. However, Griffiths et al. (2006) reported significantly higher nematode populations of *Acrobeloides* spp. and *Pratylenchus* spp. under Bt-maize (event MON810) than non-Bt-maize in a greenhouse study. There was an overall increase in nematode numbers under Bt-maize when all data were pooled, but no significant effect at any individual plant growth stage or in any particular soil type. In addition, based on a glasshouse study involving 8 different paired varieties of maize (Bt – including event MON810 – and near-isogenic), Griffiths et al. (2007b) reported that (1) nematode abundance varied mainly between maize varieties, rather than between Bt and non-Bt-maize, and that (2) differences in previously published soil nematode studies under Bt-maize were smaller than varietal effects.

In a laboratory bioassay, Höss et al. (2008) studied potential toxic effects of the Cry1Ab protein on *Caenorhabditis elegans* either by exposing *Caenorhabditis elegans* to rhizosphere and bulk soil from experimental fields cultivated with Bt-maize (event MON810) or to different solutions of the Cry1Ab protein expressed in *Escherichia coli*. Nematode reproduction and growth were significantly reduced in rhizosphere and bulk soil of Bt-maize as compared with soil from isogenic maize, and were significantly correlated with concentrations of the Cry1Ab protein in soil samples. However, because concentrations of the Cry1Ab protein measured in soil samples from Bt-maize were low and not sufficiently high to produce direct toxic effects on *Caenorhabditis elegans* (see also Baumgarte and Tebbe, 2005), adverse effects on the reproduction and growth of *Caenorhabditis elegans* were assigned to indirect effects. Höss et al. (2008) concluded that further investigations are needed to assess whether there are potential indirect effects of the protein on reproduction and growth of *Caenorhabditis elegans* and to clarify the causes. Any observed effects would then have to be compared with other factors limiting populations such as cultivation and other fluctuations in the physical soil environment (e.g., Priestley and Brownbridge, 2009).

(b) Exposure assessment

Fields experiments conducted in the context of the ECOGEN project showed that changes to nematode communities due to Bt-maize (event MON810) were small and transient, and smaller than those induced by seasonal, soil type, tillage, crop type or varietal effects (Griffiths et al., 2007a). Nematode community structure was different at each site and the effect of Bt-maize was not confined to specific nematode taxa (Griffiths et al., 2005). The authors concluded that the effect of Bt-maize was small and within the normal variation range expected in the considered agricultural systems.

Effects of Bt-maize (events MON810 and Bt176) on 2 nematode species, plant-parasitic *Pratylenchus* spp. and the bacteriovorious *Caenorhabditis elegans*, have also been studied in field trials in Germany (Anonymous, 2006). No adverse Bt-effects were observed with respect to population density of *Pratylenchus* spp., whilst growth, number of eggs and reproduction rate of *Caenorhabditis elegans* were negatively affected.

(c) Conclusion

The EFSA GMO Panel is of the opinion that there is no evidence to indicate that the placing of maize MON810 and derived products on the market is likely to cause adverse effects on nematodes in the context of its proposed use. Any effects on nematodes by Bt-maize and their products are likely to be minor compared with effects of agricultural practices, environmental stresses or differences between localities and maize varieties. Rearrangements of nematodes occur frequently in the agricultural environment, are associated to several sources of variation, and are not necessarily an indication of environmental harm.

6.1.4.8. Soil organisms: isopods

Woodlouse (*Porcellio scaber*), considered a model decomposer organism, have been used in laboratory feeding studies for detecting potential adverse impacts related to exposure to plant material from Cry1Ab expressing maize and the protein itself.

(a) Hazard assessment

No adverse effects of the Cry1Ab protein on consumption, survival and growth of *P. scaber* were observed when fed plant material of Bt-maize expressing the Cry1Ab protein and non-Bt-maize (Escher et al., 2000). The survival and growth of *Trachelipus rathkii* and *Armadillidium nasatum*, 2 abundant isopods in maize growing regions, were not adversely affected after exposure to the purified Cry1Ab protein or leaves of Bt-maize (events Bt11 and MON810) under laboratory conditions for 8 weeks (Clark et al., 2006). Detected differences in mortality, weight gain and consumption by isopods and in digestibility of plant material were generally attributed to differences in the nutritional quality of maize varieties used (Escher et al., 2000; Wandeler et al., 2002; Pont and Nentwig, 2005; Clark et al., 2006).

(b) Exposure assessment

Exposure to and assimilation of the Cry1Ab protein by *P. scaber* were demonstrated by the detection of lower concentrations of the protein in faeces than in the consumed plant material (Wandeler et al., 2002; Pont and Nentwig, 2005).

(c) Conclusion

The EFSA GMO Panel is of the opinion that there is no evidence to indicate that the placing of maize MON810 and derived products on the market is likely to cause adverse effects on idospods in the context of its proposed use.

6.1.4.9. Soil organisms: collembolans

Because collembolans are important in the breakdown and recycling of crop residues, they are key indicator species of soil fertility and health, and have been used to detect the potential impact of Cry1Ab protein.

(a) Hazard assessment

In general, no negative effects of the Cry1Ab protein on collembolans have been observed (reviewed by Icoz and Stotzky, 2008). The addition of 4 purified Bt insecticidal proteins (Cry1Ab, Cry1Ac, Cry2A, and Cry3A) at concentrations of 200 mg/g to the diet of the collembolans, *Folsomia candida* and *Xenylla grisea*, for 21 days did not affect their survival or reproduction compared with the unamended diet (Sims and Martin, 1997). No deleterious effects on survival and reproduction of *Folsomia candida* were observed when fed leaves of Bt-maize expressing the Cry1Ab protein compared with leaves of non-Bt-isolines (Clark and Coats, 2006). While Bakonyi et al. (2006) showed that Bt-maize was less preferred as food by *Folsomia candida* than near-isogenic non-Bt-maize, this effect was not observed for *Heteromurus nitidus* and *Sinella coeca*. *Folsomia candida* defecated 30% less around Bt-maize, but did not show preference to stay on any plant material. Preference was not linked to consumption, so the tendency to stay on the plant material was not linked to palatability. For well-fed *Folsomia candida*, the consumption was 30% less on Bt-diet, but when they were starved, they indiscriminately consumed both diets. An interpretation of the study in toxicological terms relies on the value of an avoidance of toxic substances for predicting the toxic potential in a realistic field situation. Hitherto the Cry1Ab protein has not been shown to be toxic to Collembola. In addition to the presence of the assumed toxicant (cf., the Cry1Ab protein), there were differences in C/N ratio in the plant material. Such differences are common because Bt-maize is a F_1 hybrid and comparators are of similar hybrid origin or single lines and therefore not fully isogenic. Different varieties have been shown previously to elicit various responses related to their background genetic composition and not to the GM event or its products (Griffiths et al., 2007b). The different consumption of Bt-maize may be due to nutritional differences, as suggested by the C/N ratio. The study shows that *Folsomia candida*, which responded with a lower consumption of the Bt-protein, did not discriminate between the 2 diets under starved condition. Heckmann et al. (2006) reported that the growth and reproduction of the collembolan, *Protaphorura armata*, reared on ground roots of Bt-maize expressing the Cry1Ab protein were not significantly different from those reared on ground roots of non-Bt-maize for 4 weeks. *Protaphorura armata* performed significantly better on a diet of yeast amended with purified Cry1Ab protein than on ground root tissue of Bt and non-Bt-maize.

(b) Exposure assessment

Collembola are usually exposed to the Cry1Ab protein in the Bt-maize field environment. However, so far, no significant differences in the population density of collembolans were found in soils cultivated with Bt and non-Bt-maize, and between the application of an insecticide (Baythroid) and no insecticide (Anonymous, 2006). Moreover, concentrations of Cry proteins in plant material in soil in the field are usually low and estimated to be less than 30 µg/g of fresh weight, suggesting that these concentrations will not pose a risk to soil collembolans (Sims and Martin, 1997).

(c) Conclusion

The EFSA GMO Panel is of the opinion that there is no evidence to indicate that the placing of maize MON810 and derived products on the market is likely to cause adverse effects on Collembolla in the context of its proposed use.

6.1.4.10. Soil organisms: diplopods

Even though diplopods are not the most important group of macrodecomposers of plant litter in soil, they are widely spread in the agricultural landscape and regularly occur in maize fields. Hence, diplopods can be exposed to the Cry1Ab protein.

(a) Hazard assessment

Laboratory studies have been performed on *Allajulus latestriatus* to analyse the effects of Bt-maize (event Bt11) on mortality, consumption, weight gain, and faeces production. No significant differences were found in mortality, consumption and weight gain when animals where fed on Bt-maize compared to its isoline and 2 other varieties. In the toxicity test, exposure to very high Cry1Ab concentrations (more than 100 times those found in Bt-maize leaves) did not result in a significant higher mortality of diplopods. The faeces production per diplopod was significantly increased when animals were kept on Bt-maize as compared with the isoline and another maize variety. Since the Cry1Ab protein in faeces was shown to be insecticidally active, other soil organisms can be exposed to it (Weber and Nentwig, 2006).

(b) Exposure assessment

Since diplopods prefer feeding on partly degraded plant material which usually contains lower Cry1Ab concentrations (Weber and Nentwig, 2006), they will be exposed only to low levels of the Cry1Ab protein under field conditions.

(c) Conclusion

The EFSA GMO Panel is of the opinion that there is no evidence to indicate that the placing of maize MON810 and derived products on the market is likely to cause adverse effects on diplopods in the context of its proposed use.

6.1.5. Effects on human and animal health

No adverse effects on human and animal health are indicated by the molecular analysis and compositional and toxicological data supplied (see sections 3 and 4).

6.1.6. Effects on biogeochemical processes and interactions with the abiotic environment

6.1.6.1. Fate of Bt-proteins in soil

It is well-documented that during plant growth maize MON810 can contribute to the presence and persistence of plant-produced Cry1Ab protein in soil via root exudation (e.g., Saxena et al., 2002, 2004; BEETLE report, 2009). A second route for potential accumulation and persistence of Bt-proteins in soil relates to dead plant material remaining in fields after harvest which is incorporated into the soil during tillage operations (Stotzky, 2004; BEETLE report, 2009).

The persistence of the Cry1Ab protein in soil is dependent upon multiple factors, varying among experimental conditions (e.g., type of crop, soil characteristics, microbial activity, temperature) and the method used for quantification of the protein. In a recent review paper, Icoz and Stotzky (2008) discuss the variability in persistence of the Cry1Ab protein in soils. Half-lives (the time until the amount of a substance remaining is 50% of the original amount) of the Cry1Ab protein ranged from 1.6 days in a soil amended with biomass of Bt-maize (Sims and Holden, 1996) up to 34 days in soil amended with biomass of and planted to Bt-rice (Wang et al., 2006). Schrader et al. (2008) observed a strong decline of immunoreactive Cry1Ab in plant residues of maize MON810 in microcosm experiments: after 5 weeks, in leaf material, it was reduced to 14.1% and in root material to 12.8% of the initial concentration, which was approximately 5 µg/g.

Although Bt-proteins are degraded or inactivated in soil within weeks, a small fraction can persist far longer under certain conditions. Laboratory studies have shown that the Cry1Ab protein can bind on clay minerals and humic substances in soil, thereby reducing its availability to microorganisms. This reduced availability decreases degradation of the Cry1Ab protein, so the insecticidal activity is retained during the growing season (e.g., Tapp et al., 1994; Tapp and Stotzky, 1995, 1998; Crecchio and Stotzky, 1998, 2001; Pagel-Wieder et al., 2007). In this respect, Zwahlen et al. (2003a) showed that the Cry1Ab protein is still detectable in decaying maize material after a soil exposure in litter bags for 200-240 days. Cry1Ab protein in low concentrations was detected for up to 56 days in soil amended with purified protein or biomass of Bt-cotton (Donegan et al., 1995), 234 days in soil amended with purified protein (Tapp and Stotzky, 1998) or for up to 180 to 350 days in soil amended with biomass of or planted to Bt-maize residues of Bt-maize (Saxena and Stotzky, 2002). Stozky (2004) reported that Cry1Ab protein released in root exudates and from biomass of Bt-maize persisted in low concentrations in soil microcosms for at least 180 days and 3 years, respectively.

The potential accumulation of plant-produced Cry1Ab proteins in soil following repeated and large-scale cultivation of Bt-maize has been studied. Unbound Cry1Ab protein was recorded in soil during 4 consecutive years of Bt-maize cultivation, and no accumulation was observed (Icoz et al., 2008). In addition, Baumgarte and Tebbe (2005) and Andersen et al. (2007) reported that concentrations of the Cry1Ab protein found in soil were higher in a given season for plots planted with varieties derived from the maize MON810 in comparison with non-Bt-maize varieties, but concentrations did not seem to increase from year to year. Hopkins and Gregorich (2003, 2005) and Dubelman et al. (2005) also reported that Cry1Ab proteins from GM plants do not persist in biologically relevant concentrations in soil 3 months after harvest, and they found no evidence of accumulation of the Cry1Ab protein in soil from fields planted for at least 3 consecutive years with Bt-maize, regardless of soil type, geographic regions and climatic conditions (Dubelman et al., 2005). Despite the fact that Cry proteins can bind rapidly on clay minerals and humic substances, there is no evidence for accumulation of the Cry1Ab protein in soils in the field, even after 3 years of continuous cultivation of Bt-crops (e.g., Baumgarte and Tebbe, 2005; Marchetti et al., 2007; Gruber et al., 2008; Hönemann et al., 2008).

Reviews of the literature indicate that exposure of non-target soil organisms to Cry1Ab protein is likely to be variable and case-specific (see section 6.2.3). In this respect, the focus of the EFSA GMO Panel is on the assessment of the susceptibility of non-target soil fauna to

the Cry1Ab protein, effects on microorganisms and impacts on soil organism diversity that would affect biogeochemical cycles. These aspects are discussed in the following sections.

6.1.6.2. Effects on soil microorganisms

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 (e.g., BEETLE report, 2009). Some studies demonstrated consistent significant differences in relation to microorganisms between soils with Bt and non-Bt-maize. Root exudates of Bt-maize (event Bt176) were shown to reduce presymbiotic hyphal growth of the arbuscular mycorrhizal fungus, *Glomus mosseae*, as compared with those of another Bt-maize (event Bt11) and control maize (Turrini et al., 2004). Castaldini et al. (2005) also reported consistent differences in rhizosphere heterotrophic bacteria and mycorrhizal colonization (including *Glomus mosseae*) between Bt-maize (event 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 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; Filion, 2008; Icoz and Stotzky, 2008), performed under laboratory, glasshouse or field conditions 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; Griffiths et al., 2006; Mulder 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 microorganisms or microorganism-mediated processes (Saxena and Stotzky, 2001a; Flores et al., 2005; Anonymous, 2006; Hönemann et al., 2008; Icoz et al., 2008). Where 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 and soil type (Fang et al., 2005, 2007; Griffiths et al., 2005, 2006; Lilley et al., 2006; Filion, 2008; Icoz and Stotzky, 2008). Factors such as plant growth stage and field heterogeneity produced larger effects on soil microbial community structure than maize MON810 (Baumgarte and Tebbe, 2005; Griffiths et al., 2007b).

Mulder et al. (2006) reported short-term effects of maize MON810 which induced ecological shifts in microbial communities of cropland soils in laboratory tests. However, differences in agronomic and compositional characteristics between the tested Bt-maize and the near-isogenic control 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, soluble sugar content (Biavati and Sorlini, 2007) rather than 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 reported by Mulder et al. (2006) during the first 72 hours after the addition of Bt-maize residues can be interpreted as being related to the presence of other macronutrient in crop residues. However, 3 weeks after the addition of the maize residues to the soil, no differences were detected between the activity of specific bacterial guilds in soils amended with transgenic maize and in soils amended with conventional maize.

Studies in which the decomposition of Bt-maize was compared with that of non-Bt-isogenic lines mostly showed that Cry1Ab expressing maize does not affect 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 (event Bt11) reported by Zwahlen et al. (2007) did not reveal major changes in the decomposition rate of Bt-maize residues. Similarly, various studies on maize MON810 found no evidence of effects related to the genetic modification when examining the decomposition rate of Bt-maize (Griffiths et al., 2007b; 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 inhibition of soil microorganisms by the Cry1Ab protein, but more likely from increased lignin contents in certain maize varieties. Altered lignin content in maize varieties has been shown not to be a generic effect of the *cry1Ab* gene insertion (Griffiths et al., 2007b).

6.1.6.3. Biological effects in soil

Multi-year experiments conducted within the EU-funded ECOGEN project (Andersen et al., 2007; Krogh and Griffiths, 2007) on GM maize at 4 sites across 3 European climatic zones, showed that no or only few effects on microarthropods, mycorrhizal fungi or on snails could be attributed to Bt-maize (event MON810) (Cortet et al., 2007; de Vaufleury et al., 2007; Griffiths et al., 2007a; Kramarz et al., 2007a,b; Krogh et al., 2007). Field experiments revealed that Bt-maize could have a significant, but small and transient, effect on soil protozoa, nematodes and microorganisms (Griffiths et al., 2005, 2007a). No direct effects on the snail, *Cantareus aspersus* (synonym: *Helix aspersa*), were detected after exposure to purified Cry1Ab protein for 4 weeks (Kramarz et al., 2007a) or to growing Bt and non-Bt-maize for 3 months in microcosm experiments, though the Cry1Ab protein was detected in snail faeces and is a route of exposure for soil microorganisms (de Vaufleury et al., 2007). In a no-choice feeding experiment, a reduction in body mass (expressed in growth coefficient) of *Cantareus aspersus* was observed both after 47 and 68 weeks of exposure to the Cry1Ab protein via food and soil in comparison with the non-Bt-treatment, whilst after 88 weeks of exposure no significant differences in body mass were observed between treatments (Kramarz et al., 2009). The ECOGEN experiments allowed for a comparison of results ensuing from different scales and for an assessment of their utility since the same organisms and soils were studied in laboratory, glasshouse and field. Although useful information and insights from each of the experimental approaches and scales were gathered, predicting outcomes to one scale from results obtained from another still remains difficult (Birch et al., 2007). Based on the ECOGEN analyses, the authors concluded that Bt-maize does not have adverse effects on soil biota, since effects observed were most likely to be caused by season, soil type, tillage, crop type or variety (Cortet et al., 2007; de Vaufleury et al., 2007; Griffiths et al., 2007a; Krogh et al., 2007). Similarly, effects on soil microbial community structure, microarthropods and larvae of a non-target root-feeding Diptera (*Delia radicum*) observed in a glasshouse experiment were most likely due to soil type and plant growth stage, rather than Bt-maize (event MON810). Although statistically significant effects of Bt-maize on soil microfauna populations (e.g., overall increase in protozoa (amoebae) and nematode numbers) were observed, these effects were relatively small, especially when compared with effects of soil type, plant growth stage, insecticide application and variety (Griffiths et al., 2006, 2007b).

Several other studies did not show any consistent effect of Bt-maize on soil species. For example, in an 8-month field study consisting of litter-bag experiments with Bt-maize (Bt11), Zwahlen et al. (2007) did not detect major changes in the composition of the soil fauna community, collembolans, mites and annelids, during the experiment. Similar conclusions were drawn by Hönemann et al. (2008) who observed similar meso and macrofauna soil communities between the tested maize varieties (including 2 varieties containing event MON810).

6.1.6.4. Conclusion

The EFSA GMO Panel is of the opinion that potential effects on soil microorganisms and microbial communities due to maize MON810 if they occur, will be transient, minor and localised in different field settings and are likely to be within the range currently caused by other agronomic and environmental factors.

6.1.7. Impacts of the specific cultivation, management and harvesting techniques

No new specific cultivation practices, management or harvesting techniques are associated to the cultivation of maize MON810. The only difference between maize MON810 and its conventional counterpart is due to fewer insecticide treatments needed to control lepidopteran target pests such as *Ostrinia nubilalis* and *Sesamia nonagrioides* (Gómez-Barbero et al., 2008a). As discussed above, the implementation of insect resistance management strategies is desirable to delay or prevent the potential evolution of insect resistance to Cry1Ab in lepidopteran target pest populations.

6.1.8. Conclusion

Since the scope of one of the current renewal applications includes the use of seed for cultivation, the environmental risk assessment considered the environmental impact of full-scale commercialisation.

In line with the conclusions of the Spanish Competent Authority and its Biosafety Commission, the EFSA GMO Panel is of the opinion that no significant risk has been identified in the environmental risk assessment with the exception of resistance evolution in lepidopteran target pests.

Maize MON810 has no altered survival, multiplication or dissemination characteristics. The EFSA Panel agrees with the assessment that the likelihood of unintended environmental effects due to the establishment and spread of maize MON810 will be no different from that of conventional maize varieties.

On the basis of the data provided by the applicant and obtained from a literature survey, the likelihood of adverse effects on non-target natural enemies is foreseen to be very low. Rearrangements of species assemblages at different trophic levels are commonly associated with any pest management practice. The EFSA GMO Panel is of the opinion that maize MON810 will not cause reductions to natural enemies that are significantly greater from those caused by conventional farming where pesticides are used to control corn borers.

On the basis of the data provided by the applicant and obtained from a literature survey and a modelling exercise, the EFSA GMO Panel considers that the amounts of maize MON810 pollen grains found in and around maize fields are unlikely to adversely affect a significant proportion of non-target Lepidoptera larvae.

While the EFSA GMO Panel agrees that in field settings honeybees might face additional stresses that could theoretically affect their susceptibility to Cry proteins or generate indirect effects, it concludes that the likelihood of adverse effects on honeybees is expected to be very low. The EFSA GMO Panel has no reason to consider that maize MON810 will cause reductions to pollinating insects that are significantly greater from those caused by conventional farming.

The EFSA GMO Panel is of the opinion that it is unlikely that the Cry1Ab protein in maize MON810 products would cause adverse effects on non-target water-dwelling organisms in the context of its proposed use. Even though a hazard has been identified for non-target Trichoptera species, the EFSA GMO Panel does not believe that high exposure to maize MON810 pollen or maize plant residues is likely to occur.

Based on a literature survey and in line with the applicant's environmental risk assessment and EFSA GMO Panel's previous scientific opinions, the EFSA GMO Panel concludes that any effects on non-target soil organisms by maize MON810 and its products are likely to be minor compared with effects of agricultural practices, environmental stresses or differences between localities and maize varieties. Rearrangements of non-target soil populations occur frequently in the agricultural environment, are associated to several sources of variation, and are not necessarily an indication of environmental harm. Therefore, the EFSA GMO Panel concludes that there is no evidence to indicate that the placing of maize MON810 and derived products on the market is likely to cause adverse effects on non-target soil organisms in the context of its proposed use.

In relation to soil microorganisms and microbial communities, the EFSA GMO Panel is of the opinion that potential effects on soil microorganisms and microbial communities due to maize MON810 if they occur, will be transient, minor and localised in different field settings and are likely to be within the range of change currently caused by other agronomic and environmental factors.

6.2. Post-market environmental monitoring

In its environmental risk assessment report, the Spanish Competent Authority and its Biosafety Commission (see Annex H) request case-specific monitoring for "*the development of resistance of the corn borers, Ostrinia nubilalis and Sesamia spp., to the newly introduced protein Cry1Ab expressed in the plant*" and for "*potential effects on non-target Lepidoptera representative of EU maize growing regions*". The Spanish Competent Authority and its Biosafety Commission consider that "*the use of farm questionnaires as the only method for general surveillance is not considered suitable for the assessment of unexpected environmental effects of maize MON810*".

6.2.1. General aspects of monitoring

The objectives of a post-market environmental monitoring plan according to Annex VII of Directive 2001/18/EC are (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the environmental risk assessment.

The EFSA GMO Panel notes that it only gives its opinion on the scientific quality of the post-market environmental monitoring activities proposed by applicants, whilst the final endorsement thereof is done by risk managers.

The EFSA GMO Panel is of the opinion that the structure of the post-market environmental monitoring plan provided by the applicant complies with the requirements defined in Directive 2001/18/EC and the EFSA GMO Panel scientific opinion on post-market environmental monitoring (EFSA, 2006b). Although the applicant has placed insect resistance monitoring within their Stewardship Plan, the EFSA GMO Panel considers this monitoring and management of an identified risk as appropriate under case-specific monitoring (see section 6.2.3).

6.2.2. Interplay between environmental risk assessment and monitoring

Since the scope of one of the current applications covers the use of seed for cultivation, the post-market environmental monitoring plan has to consider the environmental impact of full-scale commercialisation of maize MON810. The EFSA GMO Panel is of the opinion that no significant risk has been identified in the environmental risk assessment with the exception of resistance evolution in lepidopteran target pests.

6.2.3. Case-specific monitoring

In line with the recommendations of the Spanish Competent Authority and its Biosafety Commission, the EFSA GMO Panel advises that the evolution of resistance in lepidopteran target pests continues to be monitored in order to detect potential changes in resistance levels in pest populations. In areas where other lepidopteran pests are important targets of maize MON810, they might also be subject to resistance evolution due to exposure to the Cry1Ab protein expressed in plants. Therefore, the EFSA GMO Panel also recommends these species to be considered by the applicant in the context of both case-specific monitoring for insect resistance management strategy and general surveillance through farm questionnaires.

The Spanish Competent Authority and its Biosafety Commission are of the opinion that the potential effects of maize MON810 on non-target Lepidoptera have to be considered more deeply in the post-market environmental monitoring plan as the applicant failed to provide the data requested. However, after an additional request from the EFSA GMO Panel, the applicant provided more information on the environmental risk assessment of non-target Lepidoptera. Based on the additional information package provided by the applicant and data obtained from a modelling exercise, the EFSA GMO Panel considers that the amounts of maize MON810 pollen grains found in and around maize fields are unlikely to adversely affect a significant proportion of non-target lepidoptera larvae (see section 6.1.4.2). Moreover,

an analysis of an existing dataset on butterfly communities in Switzerland (Aviron et al., 2009) have shown that case-specific monitoring would at best detect large effects in ubiquitous butterfly populations. These authors and Lang (2004) also indicated that monitoring butterfly populations, particularly of infrequent species, is unlikely to achieve the level of sensitivity commensurate with the effects that are anticipated by the EFSA GMO Panel, unless thousands of samples are taken. Thus the EFSA GMO Panel is of the opinion that case-specific monitoring would not detect minor shifts in non-target Lepidoptera and is therefore not appropriate.

6.2.4. General surveillance

The objective of general surveillance is to identify unforeseen adverse effects of the GM plant or its use on human health and the environment that were not predicted in the risk assessment.

The general surveillance proposed by the applicant is based on 4 pillars: (1) the use of annual farm questionnaires to feed a general surveillance database; (2) the review of scientific information provided by existing observation networks; (3) the implementation of company stewardship programs; and (4) the follow-up of various information sources such as official websites, scientific publications and expert reports on GMOs to identify potential adverse effects associated with the intended uses of maize MON810.

The EFSA GMO Panel considers general surveillance for the environmental effects of maize MON810 cultivation to be in line with the general recommendations of its scientific opinion on post-market environmental monitoring (EFSA, 2006b).

The EFSA GMO Panel welcomes the approach of the applicant to establish farm questionnaires as a reporting format. The questionnaires to farmers exposed to or using maize MON810 provided by the applicant are regarded as an adequate tool for addressing several aspects of general surveillance. While the EFSA GMO Panel considers the format of the farm questionnaires as comprehensive, it proposes the following modifications:

- Reconsideration whether the alternative response "*don't know*" or similar ones should be added to the answering options to prevent false answers;

- Questions should be added on the occurrence/observation of (GM) feral plants and/or (GM) volunteers in previous and current seasons (for the consideration of persistence or selection);

- Independent from the occurrence/abundance of wildlife an open question/answer should address "*unexpected observations*" (... "*if – please specify*" for the consideration of effects on non-target organisms);

- The questionnaire should be designed to allow for the input of general farm information (e.g., crop rotations, crop performance, crop yields) and field-specific information (e.g., data on fertilizer usage, pests and diseases, pesticide use and weed abundance) for each Bt-maize field that is being monitored (e.g., Schmidt et al., 2008). In addition, the questionnaire should include an advisory note explaining that separate data sets are required for each maize MON810 field to be monitored on a single farm;

- Farm questionnaire sent to the farmers for the year(s) after the GM maize cultivation needs to be adapted for the monitoring of the specific crops (maize or different) that follow the maize MON810 cultivation. It should be in a format that is statistically compatible with the questionnaires supplied for the maize MON810 growing season.

The EFSA GMO Panel agrees with the proposal of the applicant to describe the generic approaches for using other existing surveillance networks. The applicant has also given consideration to the use of any future surveys of conservation goals as defined in Directive 2004/35/EC on environmental liability in farming regions where maize MON810 will be cultivated, and intends to investigate their suitability for providing data on potential changes in biota. However, the EFSA GMO Panel would welcome more clarifications on the following points:

- The applicant should commit more explicitly to take into account data collected and published from existing surveillance networks;

- The applicant should be willing to work, within an appropriate time of establishment of the new monitoring commitments, with the Competent Authorities under Directive 2001/18/EC of the different Member States where the maize MON810 will be grown, to review the existing monitoring networks.

The EFSA GMO Panel also recommends some improvements of general surveillance for the following issues:

- The role and interplay of all intended actors on behalf of recording, analysis, evaluation and reporting of monitoring data should be specified and clarified transparently;

- The current monitoring plan describes the distribution and analysis of farm questionnaires by the applicant, whilst the obligation for further data collection and analysis is assigned to third parties and the Competent Authorities in the Member States. However, at this stage, no agreement on the procedure is seemingly achieved with these institutions. Moreover, it is not clear which kind of data will be collected to allow further assessment. Hence, stating to evaluate some annual reports from third parties provides no insight of what is actually intended. Therefore, this aspect should be clarified by the applicant before market consent is given.

6.2.5. Reporting results of monitoring

The applicant will submit a general surveillance report on an annual basis. In case of adverse effects altering the conclusions of the environmental risk assessment, the applicant will immediately inform the European Commission and Member States. The EFSA GMO Panel agrees with the proposal made by the applicant on the reporting intervals. The EFSA GMO Panel recommends that effective reporting procedures are established with the Competent Authorities and the European Commission as required under the 2002/811/EC of Council Decision on monitoring.

6.2.6. Conclusion

In line with the recommendations of the Spanish Competent Authority and its Biosafety Commission, the EFSA GMO Panel advises that the evolution of resistance in lepidopteran target pests continues to be monitored in order to detect potential changes in resistance levels in pest populations, and the high dose/refuge strategy continues to be employed. In areas where other lepidopteran pests than the European and Mediterranean corn borer are important targets of maize MON810, the EFSA GMO Panel recommends these species are considered by the applicant in the context of both case-specific monitoring for insect resistance management strategy and general surveillance through farm questionnaires.

The EFSA GMO Panel considered if case-specific monitoring should be recommended to measure potential adverse effects on European lepidopteran species due to the indirect exposure to the Bt-protein when feeding on host plant leaves naturally dusted with pollen grains and anther fragments of Bt-maize during anthesis. Considering the additional information provided by the applicant, and the data obtained from a literature survey and a modelling exercise, the EFSA GMO Panel concludes that the amounts of MON810 pollen grains in and around maize fields are unlikely to adversely affect a significant proportion of non-target Lepidoptera larvae. Therefore, no case-specific monitoring plan for non-target Lepidoptera is deemed necessary. The EFSA GMO Panel is aware that all modelling exercises are subject to uncertainties; as with any ecological model further data would refine the estimates reported here. The EFSA GMO Panel considers it advisable that, especially in areas of abundance of non-target Lepidoptera populations in field margins, the adoption of the cultivation of maize MON810 be accompanied by management measures in order to mitigate the possible exposure of these species to MON810 pollen. As an example, the planting of border rows of non-Bt-maize adjacent to uncultivated field margins of maize MON810 fields, could limit the exposure to those individuals feeding on weeds present within maize field borders and also could contribute to the required percentage of non-Bt-maize necessary to constitute refuge areas for lepidopteran target pests in the framework of resistance management plans.

In the frame of general surveillance, the Spanish Competent Authority and its Biosafety Commission consider the use of farm questionnaires as sole monitoring means insufficient for the detection of unexpected environmental effects related to the cultivation of maize MON810. The EFSA GMO Panel notes that the general surveillance plan not only relies on farm questionnaires, but also on other sources of data input (such as stewardship programs, literature screenings). In principle, the EFSA GMO Panel agrees with the general methods and approaches of the general surveillance plan, but advises the applicant to describe in more detail how information will be collected that could be used to assess if the intended uses of maize MON810 are having unanticipated adverse environmental effects. The EFSA GMO Panel is content with the generic plan of the applicant to liaise with Competent Authorities to implement an EU-wide post-market environmental monitoring plan on a national level.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The EFSA GMO Panel assessed maize MON810 with reference to the intended uses and appropriate principles described in the guidance document of the EFSA GMO Panel for the risk assessment of GM plants and derived food and feed.

The EFSA GMO Panel is of the opinion that the molecular characterisation provided for maize MON810 is sufficient for the safety assessment. The bioinformatics analysis of the inserted DNA and the flanking regions of maize MON810 does not raise any safety concern. The expression of the genes introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The updated molecular and bioinformatic analyses provided for the maize MON810 do not indicate any safety concerns.

Analyses carried out on materials from maize MON810, including stacked GM maize events where maize MON810 was one of the parental lines, and their comparators indicate that maize MON810 is compositionally, phenotypically and agronomically equivalent to the non-GM counterparts and conventional maize, except for the newly expressed protein.

The Cry1Ab protein shows no homology with proteins known to be toxic for humans and other mammals and/or allergens. In addition, this protein is rapidly degraded under simulated gastric conditions. Furthermore, the Cry1Ab protein has been extensively assessed in previous opinions of the EFSA GMO Panel. No concerns for humans and animals were identified regarding the safety of the Cry1Ab protein.

Maize MON810 and derived products are unlikely to have any adverse effect on human and animal health in the context of the intended uses.

On 8 November 2008, the Spanish Competent Authority and its Biosafety Commission provided to EFSA its environmental risk assessment report in line with Articles 6.3(e) and 18.3(e) of Regulation (EC) No 1829/2003. The Spanish Competent Authority and its Biosafety Commission conclude that "*according to the current state of scientific knowledge and after examining the existing information and the data provided by the Monsanto Company, the Spanish Commission on Biosafety could give a favourable opinion to the renewal of commercialisation in the EU of maize MON810 if the proposals and conditions defined in this environmental risk assessment report are implemented*" (see Annex H of the overall opinion).

The EFSA GMO Panel specifically considered regional differences, especially when addressing the possible effects of vertical gene flow and the interactions with non-target organisms.

Maize MON810 has no altered survival, multiplication or dissemination characteristics. The EFSA GMO Panel agrees with the assessment that the likelihood of unintended environmental effects due to the establishment and spread of maize MON810 will be no different from that of conventional maize varieties.

On the basis of the data provided by the applicant and obtained from a literature survey and a modelling exercise on the effects of maize MON810 cultivation on non-target lepidopteran species representative of EU maize cultivation regions, the EFSA GMO Panel concludes that the likelihood of adverse effects on non-target organisms or on ecological functions very low, especially if appropriate management measures are adopted to mitigate exposure. In

agreement with the ERA conducted by the Spanish Competent Authority and its Biosafety Commission, the EFSA GMO Panel identifies the possible evolution of resistance in target species, as the main potential risk linked to the cultivation of maize MON810.

In conclusion, the EFSA GMO Panel considers that the information available for maize MON810 addresses the scientific comments raised by Member States and that maize MON810 is as safe as its conventional counterpart with respect to potential effects on human and animal health. The EFSA GMO Panel also concludes that maize MON810 is unlikely to have any adverse effect on the environment in the context of its intended uses, especially if appropriate management measures are put in place in order to mitigate possible exposure of non-target Lepidoptera.

RECOMMENDATIONS

The EFSA GMO Panel recommends that resistance management strategies continue to be employed and case-specific monitoring is conducted by the applicant under Directive 2001/18/EC.

Moreover, the EFSA GMO Panel advises that measures are established in agreement with risk managers in different European zones with the aim of mitigating the possible exposure of non-target Lepidoptera species.

DOCUMENTATION PROVIDED TO EFSA

Application EFSA-GMO-RX-MON810[8.1.a]

- Letter from the European Commission, dated 29 June 2007, concerning a request for renewal of the authorisation for continued marketing of existing food and food ingredients produced from maize MON810 that were previously notified, according to Articles 8(1)(a) Regulation (EC) No 1829/2003 on genetically modified food and feed.
- Acknowledgement letter, dated 20 July 2007, from EFSA to the European Commission (Ref SR/DC/eb (2007) 2266328).
- Letter from EFSA to applicant, dated 20 December 2007, with a request for clarifications under completeness check (Ref SR/KL/shv (2007) 2589457).
- Letter from applicant, dated 16 January 2008, providing EFSA with an updated version of the application.
- Letter from EFSA to applicant, dated 29 January 2008, delivering the 'Statement of Validity' for the application (Ref SR/KL/shv (2008) 2648282).
- Letter from applicant to EFSA, dated 11 February 2008, providing the valid application.
- Letter from EFSA to applicant, dated 24 April 2008, requesting additional information and stopping the clock (Ref SR/KL/md (2008) 2974621).
- Letter from applicant to EFSA, dated 15 May 2008, providing additional information.

- Letter from EFSA to applicant, dated 22 July 2008, requesting additional information and stopping the clock (Ref PB/KL/md (2008) 3185439).
- Letter from applicant to EFSA, dated 2 September 2008, providing a timeline for submission of response.
- Letter from applicant to EFSA, dated 30 September 2008, providing additional information.
- Letter from EFSA to applicant, dated 12 November 2008, requesting additional information and maintaining the clock stopped (Ref PB/KL/md (2008) 3446577).
- Letter from applicant to EFSA, dated 5 December 2008, providing additional information.
- Letter from EFSA to applicant, dated 11 February 2009, requesting additional information and maintaining the clock stopped (Ref PB/KL/md (2009) 3664892).
- Letter from applicant to EFSA, dated 27 February 2009, providing additional information.
- Letter from EFSA to applicant, dated 7 April 2009, restarting the clock (Ref PB/KL/md (2009) 3860739).

Application EFSA-GMO-RX-MON810[20.1.a]

- Letter from the European Commission, dated 29 June 2007, concerning a request for renewal of the authorisation for continued marketing of existing feed consisting of and/or containing maize MON810 and maize MON810 for feed use (including cultivation) that were authorised under Directive 90/220/EEC (Decision 98/294/EC) and subsequently notified according to Article 20(1)(a) of Regulation (EC) No 1829/2003 on genetically modified food and feed.
- Acknowledgement letter, dated 20 July 2007, from EFSA to the European Commission (Ref SR/DC/eb (2007) 2266328).
- Letter from EFSA to applicant, dated 20 December 2007, with a request for clarifications under completeness check (Ref SR/KL/shv (2007) 2589457).
- Letter from applicant, dated 16 January 2008, providing EFSA with an updated version of the application.
- Letter from EFSA to applicant, dated 29 January 2008, delivering the 'Statement of Validity' for the application (Ref SR/KL/shv (2008) 2648332).
- Letter from applicant to EFSA, dated 11 February 2008, providing the valid application.
- Letter from EFSA to applicant, dated 17 March 2008, requesting an additional application copy (Ref /KL/shv (2008) 2869298).
- Letter from applicant to EFSA, dated 26 March 2008, providing the additional application copy.
- Letter from EFSA to applicant, dated 24 April 2008, requesting additional information and stopping the clock (Ref SR/KL/md (2008) 2974621).

- Letter from EFSA (Spanish CA) to applicant, dated 13 May 2008, requesting additional information and maintaining the clock stopped (Ref PB/KL/md (2008) 3010483).
- Letter from applicant to EFSA, dated 15 May 2008, providing additional information.
- Letter from applicant to EFSA (Spanish CA), dated 9 June 2008, providing additional information.
- Letter from EFSA (Spanish CA) to applicant, dated 22 July 2008, requesting additional information and maintaining the clock stopped (Ref PB/KL/md (2008) 3185669).
- Letter from applicant to EFSA (Spanish CA), dated 20 August 2008, providing additional information.
- Letter from EFSA to applicant, dated 13 October 2008, restarting the clock (Ref PB/KL/shv (2008) 3372902).
- Letter from EFSA to applicant, dated 12 November 2008, requesting additional information and stopping the clock (Ref PB/KL/md (2008) 3446447).
- Letter from the Spanish Competent Authority to EFSA, dated 18 November 2008, with the Environmental risk assessment report of the Spanish Biosafety Commission.
- Letter from applicant to EFSA, dated 2 December 2008, providing additional information.
- Letter from applicant to EFSA, dated 5 December 2008, providing additional information.
- Letter from EFSA to applicant, dated 11 February 2009, requesting additional information and maintaining the clock stopped (Ref PB/KL/md (2009) 3664792).
- Letter from applicant to EFSA, dated 27 February 2009, providing additional information.
- Letter from EFSA to applicant, dated 7 April 2009, restarting the clock (Ref PB/KL/md (2008) 3868201).

Application EFSA-GMO-RX-MON810[8.1.b/20.1.b]

- Letter from the European Commission, dated 29 June 2007, concerning a request for renewal of the authorisation for continued marketing of existing food additives, feed material and feed additives produced from maize MON810 that were previously notified , according to Articles 8(1)(b) and 20(1)(b) of Regulation (EC) No 1829/2003 on genetically modified food and feed.
- Acknowledgement letter, dated 20 July 2007, from EFSA to the European Commission (Ref SR/DC/eb (2007) 2266328).
- Letter from EFSA to applicant, dated 20 December 2007, with a request for clarifications under completeness check (Ref SR/KL/shv (2007) 2589457).
- Letter from applicant, dated 22 January 2008, providing EFSA with an updated version of the application.

- Letter from EFSA to applicant, dated 29 January 2008, delivering the 'Statement of Validity' for the application (Ref SR/KL/shv (2008) 2648498).
- Letter from applicant to EFSA, dated 11 February 2008, providing the valid application.
- Letter from EFSA to applicant, dated 24 April 2008, requesting additional information and stopping the clock (Ref SR/KL/md (2008) 2974621).
- Letter from applicant to EFSA, dated 15 May 2008, providing additional information.
- Letter from EFSA to applicant, dated 22 July 2008, requesting additional information and stopping the clock (Ref PB/KL/md (2008) 3185669).
- Letter from applicant to EFSA, dated 2 September 2008, providing a timeline for submission of response.
- Letter from applicant to EFSA, dated 30 September 2008, providing additional information.
- Letter from EFSA to applicant, dated 12 November 2008, requesting additional information and maintaining the clock stopped (Ref PB/KL/md (2008) 3446580).
- Letter from applicant to EFSA, dated 5 December 2008, providing additional information.
- Letter from EFSA to applicant, dated 11 February 2009, requesting additional information and maintaining the clock stopped (Ref PB/KL/md (2009) 3665855).
- Letter from applicant to EFSA, dated 17 February 2009, providing additional information.
- Letter from applicant to EFSA, dated 27 February 2009, providing additional information.
- Letter from EFSA to applicant, dated 7 April 2009, restarting the clock (Ref PB/KL/md (2009) 3860966).

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