SCIENTIFIC OPINION

Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize MON 810[1]

EFSA Panel on Genetically Modified Organisms (GMOs)[2, 3]

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ABSTRACT

Following a request from the European Commission, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) compiled its previous risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize MON 810, and considered their validity in the light of new relevant scientific publications published from 2009 onwards. Following a search of the scientific literature published between 2009 and October 2012, the EFSA GMO Panel identified 165 peer-reviewed publications containing evidence specific to the risk assessment and/or management of maize MON 810, of which 68 publications were discussed and/or cited in previous EFSA GMO Panel scientific outputs. From the remaining 97 publications, eight were relevant for the molecular characterisation, 27 for food and feed safety assessment, 55 for the environmental risk assessment and/or risk management, two for the molecular characterisation and the environmental risk assessment and/or risk management and five for the food and feed safety assessment and the environmental risk assessment and/or risk management of maize MON 810. None of these publications reported new information that would invalidate the previous conclusions on the safety of maize MON 810 made by the EFSA GMO Panel. Therefore, the EFSA GMO Panel considers that its previous risk assessment conclusions on maize MON 810, as well as its previous recommendations on risk mitigation measures and monitoring, remain valid and applicable.

KEY WORDS

GMO, maize (*Zea mays*), MON810, MON 810, insect resistance, Cry1Ab, risk assessment, food and feed safety, environment, food and feed uses, import and processing, cultivation

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1 On request from the European Commission, Question No EFSA-Q-2012-00711, adopted on 6 December 2012

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3 Acknowledgement: The Panel wishes to thank the members of the Working Groups on Molecular Characterisation, Food/Feed and Environment of GMO applications for the preparatory work on this scientific output, and Anna Christodoulidou, Yann Devos, Antonio Fernández Dumont, Andrea Gennaro, Ana Gomes, Sylvie Mestdagh and Stefano Rodighiero for the support provided to the development of this EFSA scientific output

Suggested citation: EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize MON 810. EFSA Journal 2012; 10(12):3017. [98 pp.] doi:10.2903/j.efsa.2012.3017. Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) compiled its previous risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize MON 810, and considered their validity in the light of new relevant scientific publications published from 2009 onwards.

The EFSA GMO Panel performed a search of the scientific literature to identify new scientific publications specific to maize MON 810 that may report new information relevant for the risk assessment and/or management of maize MON 810. Subsequently, the EFSA GMO Panel evaluated whether the information reported in recent publications, identified by the literature search, would invalidate its previous risk assessment conclusions on maize MON 810, as well as its previous recommendations on risk mitigation measures and monitoring.

Following a search of the scientific literature published between 2009 and October 2012, the EFSA GMO Panel identified 165 peer-reviewed publications containing evidence specific to the risk assessment and/or management of maize MON 810, of which 68 publications were discussed and/or cited in previous EFSA GMO Panel scientific outputs. From the remaining 97 publications, eight were relevant for the molecular characterisation, 27 for food and feed safety assessment, 55 for the environmental risk assessment and/or risk management, two for the molecular characterisation and the environmental risk assessment and/or risk management and five for the food and feed safety assessment and the environmental risk assessment and/or risk management of maize MON 810.

The EFSA GMO Panel did not identify peer-reviewed scientific publications reporting new information that would invalidate its previous conclusions on the safety of maize MON 810. Therefore, the EFSA GMO Panel considers that its previous risk assessment conclusions on maize MON 810, as well as its previous recommendations for risk mitigation measures and monitoring, remain valid and applicable.

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BACKGROUND AS PROVIDED BY EFSA

The marketing of maize MON 810 (notification C/F/95/12-02) was authorised under Directive 90/220/EEC in the European Union (EU) for all, other than food, uses by the Commission Decision 98/294/EC (EC, 1998). A consent was granted to the applicant (Monsanto Europe S.A.) by France on 3 August 1998. Food uses of maize derivatives were notified according to Article 5 of the Novel Food Regulation (EC) No 258/97 on 6 February 1998.

On 15 June 2009, the EFSA GMO Panel issued a Scientific Opinion on the renewal of the authorisation for the continued marketing of: (1) existing food and food ingredients produced from maize MON 810; (2) feed consisting of and/or containing maize MON 810, including the use of seed for cultivation; and (3) food and feed additives, and feed materials produced from maize MON 810. The EFSA GMO Panel concluded that: "*maize MON 810 is as safe as its conventional counterpart with respect to potential effects on human and animal health*", and that: "*maize 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 (NT) Lepidoptera*". The EFSA GMO Panel recommended that: "*especially in areas of abundance of non-target Lepidoptera populations, the adoption of the cultivation of maize MON 810 be accompanied by management measures in order to mitigate the possible exposure of these species to maize MON 810 pollen*". In addition, the EFSA GMO Panel advised that: "*resistance management strategies continue to be employed and that the evolution of resistance in lepidopteran target pests continues to be monitored, in order to detect potential changes in resistance levels in pest populations*" (EFSA, 2009a).

On 30 November 2011, the EFSA GMO Panel adopted a Statement supplementing the environmental risk assessment conclusions and risk management recommendations on maize Bt11 cultivation. In its Statement, the EFSA GMO Panel concluded that: "*subject to appropriate management measures, maize Bt11 cultivation is unlikely to raise additional safety concerns for the environment compared to conventional maize*" (EFSA, 2011e). The EFSA GMO Panel considered that the environmental risk assessment conclusions and risk management recommendations on non-target Lepidoptera for maize Bt11 apply equally to maize MON 810 due to the similarities between both *Bt*-maize events (i.e., identity of amino acid sequence of the core of the Cry1Ab protein, similar biological activity against susceptible Lepidoptera, similar Cry1Ab protein expression level in pollen).

Recently, the EFSA GMO Panel further supplemented its previous risk management recommendations on maize Bt11 and MON 810 cultivation by reapplying the mathematical model developed by Perry et al. (2010, 2011, 2012), in order to consider additional hypothetical agricultural conditions, and to provide additional information on the factors affecting the insect resistance management (IRM) strategy (EFSA, 2012d).

Following requests of the European Commission to assess the annual post-market environmental monitoring (PMEM) reports submitted by the applicant on maize MON 810 cultivation in 2009 and 2010, the EFSA GMO Panel issued Scientific Opinions on the 2009 and 2010 PMEM reports on maize MON 810 on 7 September 2011 (EFSA, 2011b) and 7 March 2012 (EFSA, 2012a), respectively. The EFSA GMO Panel noted shortcomings in the methodology for case-specific monitoring (CSM) and general surveillance (GS), and made recommendations to strengthen PMEM plans for GM plants in general (EFSA, 2011a) and for maize MON 810 in particular (EFSA, 2011b, 2012a).

Several EU Member States invoked safeguard clause or emergency measures to provisionally restrict or prohibit the marketing of maize MON 810 on their territory. For all cases for which the EFSA GMO Panel has been asked by the European Commission to evaluate whether the invocation was justifiable on the basis of the scientific information submitted in support of a safeguard clause, the EFSA GMO Panel concluded that, in terms of risk to human and animal health and the environment,

no new scientific evidence had been presented that would invalidate its previous risk assessment conclusions on maize MON 810 (EFSA, 2004, 2005, 2006a,b, 2008a,b,c,d, 2012b,c).

On 20 June 2012, the EFSA GMO Panel was requested by the European Commission to deliver a Scientific Opinion updating the risk assessment and/or management of maize MON 810 in the light of recent scientific publications.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requested EFSA: "*to adopt an opinion gathering its previously adopted conclusions on maize MON 810 for each area of risk and taking into account recent relevant scientific publications, in accordance with Article 29 of Regulation (EC) No 178/2002*".

ASSESSMENT

1. INTRODUCTION

Maize MON 810 has been developed to provide protection against certain lepidopteran target pests, such as the European corn borer (ECB, *Ostrinia nubilalis*), and species belonging to the genus *Sesamia* (in particular the Mediterranean corn borer (MCB, *Sesamia nonagrioides*)), by the introduction of a part of a *Bacillus thuringiensis* (*Bt*) gene encoding the insecticidal Cry1Ab protein. The mode of action of the Cry1Ab protein and other Cry proteins is to bind selectively to specific receptors on the epithelial surface of the midgut of larvae of susceptible insect species, leading to death of larvae through pore formation, cell burst and subsequently septicaemia. Maize MON 810 is currently cultivated in the EU in countries such as the Czech Republic, Portugal, Romania, Slovakia and Spain. On an annual basis, the applicant reports to the European Commission and EU Member States the results of its monitoring activities on the cultivation of maize MON 810 in the EU.

This EFSA GMO Panel Scientific Opinion addresses all intended uses of maize MON 810, covering import and processing for food and feed uses, as well as cultivation.

In accordance with the terms of reference laid down by the European Commission, this Scientific Opinion is based on existing scientific outputs on maize MON 810 by the EFSA GMO Panel (i.e., EFSA, 2004, 2005, 2006a,b, 2008a,b,c,d, 2009a), focusing in particular on the most recent ones (e.g., EFSA, 2011b,d, 2012a,b,c,d). To comply with the current mandate of the European Commission, the EFSA GMO Panel performed a search of the scientific literature to identify new scientific publications specific to maize MON 810 that may report new information relevant for the risk assessment and/or management of maize MON 810. The EFSA GMO Panel scrutinised the new scientific publications identified during the literature search, and subsequently assessed whether the information reported in these publications would invalidate its previous conclusions on the safety of maize MON 810.

2. LITERATURE SEARCH

In response to the present request of the European Commission and in addition to the continuous screening of relevant scientific literature by the EFSA GMO Panel, an additional search of the scientific literature was performed. The aim of this search was to identify new scientific publications specific to maize MON 810 that may report new information relevant to the risk assessment and/or management of maize MON 810.

The scientific literature database ISI Web of Knowledge[4] (Thompson Reuters, New York, USA) was used for the literature search. Literature was searched and filtered in a stepwise manner. As a first step, the following combination of generic keywords, being both event- and trait-specific was used to retrieve all references for further consideration: "*TOPIC FIELD* = *MON*810* OR *Yieldgard* OR *Cry*1Ab* AND *maize*". The search by keywords using the 'topic' field enabled the retrieval of publications that contain these keywords, either in the publication's title, list of keywords, or abstract. The asterisk (wildcards) was used to cover all the possible written forms of the keywords MON 810 and Cry1Ab (e.g., MON810, MON 810, Cry1Ab, Cry 1Ab, Cry_1Ab). In the second step, search results were sorted by the area of scientific discipline (e.g., molecular characterisation, comparative analysis, food and feed safety assessment, environmental risk assessment (ERA) and PMEM) and subsequently considered by the EFSA GMO Panel (see sections below). The search for scientific publications targeted publications published between 2009 – the year during which the EFSA GMO Panel issued its Scientific Opinion on the renewal of the authorisation for the continued marketing of: (1) existing food and food ingredients produced from maize MON 810; (2) feed consisting of and/or containing maize MON 810, including the use of seed for cultivation; and (3) food and feed additives, and feed materials produced from maize MON 810 (EFSA, 2009a) – and October 2012. The EFSA GMO Panel also performed targeted searches of relevant peer-reviewed journals, in order to identify the most recent publications appearing ahead of print, and which may not have been included in the

[4] This database includes: Web of Science, CABI, FSTA, MedLine and Current Contents Connect databases

ISI Web of Knowledge yet. Publications on the coexistence of maize cropping systems, the detection, quantification, labelling and traceability of GMOs, socio-economics and public perception were excluded, as these topics are not in the remit of the EFSA GMO Panel. After having accounted for the scientific literature previously discussed and/or cited in the numerous EFSA GMO Panel scientific outputs (i.e., EFSA, 2009a, 2011b,c,d, 2012a,b,c,d,e), the EFSA GMO Panel found 97 relevant peer-reviewed publications written in English that it had not previously discussed (see sections below; Appendix A – rows highlighted in grey).

The EFSA GMO Panel identified a total number of 165 peer-reviewed publications containing evidence specific to the risk assessment and/or management of maize MON 810, of which 68 publications were discussed and/or cited in previous EFSA GMO Panel scientific outputs. From the remaining 97 publications, eight were relevant for the molecular characterisation, 27 for food and feed safety assessment, 55 for the environmental risk assessment and/or risk management, two for the molecular characterisation and the environmental risk assessment and/or risk management and five for the food and feed safety assessment and the environmental risk assessment and/or risk management of maize MON 810.

Even though no systematic review of the literature is carried out in this Scientific Opinion, the EFSA GMO Panel adhered to some fundamental principles of systematic review, which can be summarised as follows: methodological rigour and coherence in the retrieval and selection of publications; transparency; and reproducibility of the performed literature search (EFSA, 2010b).

3. MOLECULAR CHARACTERISATION

3.1. Introduction

The summary of the previous assessments of maize MON 810, presented below, covers the following key areas of molecular characterisation: (1) description of the methods used for the genetic modification; (2) source and characterisation of nucleic acid used for transformation; (3) description of the traits and characteristics which have been introduced; (4) information on the sequences actually inserted; (5) information on the expression of the inserted sequence; and (6) genetic stability of the inserted sequence and phenotypic stability of the GM plant.

3.1.1. Summary of previous conclusions by the EFSA GMO Panel

Maize MON 810 was developed through particle bombardment using a mixture of two plasmids: PV-ZMGT10 (which was not integrated in the plant), and PV-ZMBK07 which contains a *cry1Ab* expression cassette driven by the CaMV 35S promoter. Molecular characterisation data established that maize MON 810 contains one truncated copy of PV-ZMBK07 and as a result expresses the *cry1Ab* gene. Maize MON 810 contains the *cry1Ab* cassette at a single locus. The insert lacks the *nos* terminator, and no vector backbone sequences are present (EFSA, 2009a). Bioinformatic analyses revealed that the flanking regions of the insert in maize MON 810 show significant identity to maize genomic DNA sequences and indicated that the pre-insertion locus was preserved except for the addition of 400 bp of maize DNA at the 3' flank and 1000 bp of maize DNA at the 5' flank. Analysis also revealed that the 3' genomic region corresponds to a gene putatively coding for the HECT-ubiquitin ligase protein, suggesting that the insert may have interrupted this gene. However, phenotypic and compositional equivalence data for maize MON 810 and its conventional counterparts did not indicate any safety concerns arising from the interruption of this gene. 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 amino acids in different variants, all derived from the adjacent host genomic sequences and linked to the truncated Cry1Ab protein. These putative recombinant proteins showed no homology with any known protein and do not raise any new safety concerns. Bioinformatic analyses did not reveal biologically relevant similarities to allergens or toxins for any of the putative translation products of open reading frames spanning the 5' and 3' junction regions of the insert (EFSA, 2009a).

In field trials conducted in 1994 and 1995 in the USA, France and Italy, the levels of Cry1Ab protein in young leaf tissue ranged from 7.59 to 10.34 µg/g fresh weight (fw), in forage from 3.65 to 9.23 µg/g fw, and in grain from 0.19 to 0.69 µg/g fw (EFSA, 2009a). Levels of Cry1Ab in pollen ranged from undetectable to 0.097 µg/g fw (US EPA, 2001; Nguyen and Jehle, 2007). Southern analysis of maize MON 810 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations.

All previous assessments of maize MON 810 by the EFSA GMO Panel (EFSA, 2009a,b, 2011d) led to the conclusion that the molecular characterisation of maize MON 810 does not raise a safety issue.

3.1.2. Results from the literature search

From the literature search, eight new scientific publications containing evidence specific to maize MON 810 were identified and scrutinised for their possible relevance for the molecular characterisation of maize MON 810. The search also resulted in two publications (Brants et al., 2010 and Tahar et al., 2010) which commented on the publication by Aguilera et al. (2009).

a) Structure and stability of the insert in maize MON 810

- Aguilera et al. (2009) assessed the applicability of the 5' junction event-specific method for detecting and quantifying MON 810. This method has been validated by the Community Reference Laboratory for GM Food and Feed. Out of 26 varieties assessed, 2 (Aristis Bt, CGS4540) did not contain the 5' junction which was interpreted as an indication of instability. Two subsequent commentaries (Brants et al., 2010; Tahar et al., 2010) provided clarifications of these findings based on a further examination of the case. Variety CGS4540 was shown to be a maize hybrid derived from event Bt176 and not MON 810, whilst the Aristis Bt sourced by Aguilera et al. (2009) was considered to actually be Aristis, the non-GM counterpart part of Aristis Bt. However, the discrepancy was not actually resolved. The EFSA GMO Panel concludes that the publication of Aguilera et al. (2009), in combination with the clarifying commentaries, did not prove instability of the event MON 810.

- La Paz et al. (2010b) and Neumann et al. (2011) provided data indicating no mutation or rearrangement of the insert in maize MON 810. Neumann et al. (2011) did not identify any changes in the 5'or 3' junction sequences. The authors concluded that the results provided evidence that the MON 810 insert is stable. La Paz et al. (2010a) used a DNA mismatch endonuclease assay to show the lack of polymorphisms in the insert. Six SNPs were observed in the 5' flanking region, corresponding to a Zeon1 retrotransposon long terminal repeat. All six SNPs were more than 500 bp upstream of the point of insertion of the transgene and do not affect the reliability of the established PCR-based transgene detection and quantification methods. The authors concluded that the breeding of maize, subsequent to the introduction of the initial transformation event, has not resulted in any DNA sequence changes.

b) Expression of the Cry1Ab protein in maize MON 810

- La Paz et al. (2010b) demonstrated that the cry1Ab transgene gives rise to polyadenylated transcripts of different sizes that extend to around 1 kb downstream of the truncation site. A stop codon at position +7 downstream of the truncation site indicated the production of a recombinant protein with two additional amino acids. Several main 3' transcription termination regions were detected close to the truncation site and in the transgene 3' flanking sequence. Next to these main termination sites, the authors identified some sequence motifs that could potentially act as 3'-end-processing elements and drive termination of the transgene transcripts. Results also indicated that there were no significant differences in the levels of transgene mRNA accumulation, in major transcript sizes and in 3' termini profiles between five varieties grown under similar environmental conditions. One of the varieties tested was Aristis Bt. The data indicated that commercial varieties of maize MON 810 were stable in terms of transgene expression.

- The levels of Cry1Ab protein levels in maize MON 810 tissues have been reported in publications by several authors. Badea et al. (2010) focused on differences between plants grown in different soil types in Romania whilst Kamath et al. (2010) quantified the protein in tissues of maize MON 810 hybrids grown in several locations in India with particular attention to levels expressed within the oviposition window of the stem borers. Székács et al. (2010) provided data on variation in expression levels in a range of tissues at several stages during plant development. An examination of the protein levels in various tissues of maize MON 810-published by all of these authors indicates that the levels fell within the ranges previously reported in EFSA opinions (EFSA, 2009a,b, 2011d).

- The paper of Székács et al. (2012) emphasises the importance of using a standardised protocol when comparing Cry1Ab protein levels showing that, even when a standardised protocol was used, significant differences still occurred between laboratories in the values obtained.

3.1.3. Conclusion

Results reported by Aguilera et al. (2009), Badea et al. (2010), Brants et al. (2010), Kamath et al. (2010), La Paz et al. (2010a,b), Székács et al. (2010, 2012), Tahar et al. (2010) and Neumann et al. (2011) do not contain new information that would invalidate the previous conclusions on the molecular characterisation of maize MON 810 made by the EFSA GMO Panel. Therefore, the EFSA GMO Panel considers that its previous conclusions on maize MON 810 remain valid and applicable.

4. COMPARATIVE ANALYSIS

4.1. Introduction

The summary of the previous assessments of maize MON 810, presented below, covers the following key areas of the comparative analysis: (1) choice of comparator and production of material for the compositional assessment; (2) compositional analysis; and (3) agronomic traits and GM phenotype.

4.1.1. Summary of previous conclusions by the EFSA GMO Panel

The agronomic and phenotypic characteristics of maize MON 810 in relation to appropriate non-GM maize control materials having a comparable genetic background to the test material have been assessed by the EFSA GMO Panel in connection with the renewal applications for food and feed use and cultivation of maize MON810 as well as in connection with the assessment of several stacked GM maize events. It was concluded that: "*maize MON810 is agronomically and phenotypically equivalent to currently grown non-GM maize varieties, with the exception of the insect resistance conferred by the Cry1Ab protein*" (EFSA, 2009a).

In its 2009 Scientific Opinion for the continued marketing of maize MON 810, the EFSA GMO Panel concluded 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*" (EFSA, 2009a).

4.1.2. Results from the literature search

A number of publications related to the chemical composition of maize MON 810 have been published since the EFSA GMO Panel gave its latest opinion on this GM crop.

Two of these publications focused on specific plant constituents in grains, namely on proximates (Zhou et al., 2011) and on fatty acids (Jiménez et al., 2009). Zhou et al. (2011) studied the stability in proximate composition in maize MON 810 as compared to its conventional counterpart over multiple seasons (2001-2009), multiple locations (North, Central and South America, Europe), and multiple breeding germplasms (74 unique MON 810 hybrids). A meta-analysis of the compositional data of maize MON 810 and its comparators confirmed compositional equivalence of these materials over time. Jiménez et al. (2009) observed no differences in the level of major fatty acids between maize

MON 810 varieties and its non-GM comparators in two of the three genetic backgrounds studied, but a few small differences in the third genetic background. Several differences were noted in minor fatty acids but the fatty acid affected differed from one genetic background to the other. No consistent differences were noted. Kamota et al. (2011) analysed the composition of silage of maize MON 810 during a 42-day ensiling process. The findings suggest that Cry1Ab did not essentially influence the compositional quality of silage. Various other studies in which maize MON 810 was compared to non-GM maize have been published, using non-targeted techniques such as transcriptomics, proteomics and metabolomics (Manetti et al., 2006; Albo et al., 2007; Coll et al., 2008, 2009, 2010; Leon et al., 2009; Piccioni et al., 2009; Barros et al., 2010; Batista and Oliveira, 2010; Sissener et al., 2010; Balsamo et al., 2011; Frank et al., 2012). While these studies showed, in general, that most of the differences observed are accounted for by natural variability and environmental factors, these techniques are not routinely used in risk assessment of GMOs (EFSA, 2011f)

4.1.3. Conclusion

Results reported in the scientific literature described above do not provide new information that would invalidate the previous conclusions on the comparative assessment of maize MON 810 made by the EFSA GMO Panel. Therefore, the EFSA GMO Panel considers that its previous conclusions on maize MON 810 remain valid and applicable.

5. FOOD AND FEED SAFETY ASSESSMENT

5.1. Introduction

The summary of the previous assessments of maize MON 810, presented below, covers the following key areas of the food and feed safety assessment: (1) product description and intended use; (2) effect of processing; (3) toxicology; (4) allergenicity; (5) nutritional assessment of GM food and feed; and (6) post-market monitoring of GM food and feed.

5.1.1. Summary of previous conclusions by the EFSA GMO Panel

Cry1Ab protein produced in maize MON 810 is converted to the trypsin-resistant core protein by proteases in the digestive tract. The core protein (HD-1t), obtained through trypsinolysis of an *Escherichia coli*-produced Cry1Ab protein, was used for the safety studies after it had been demonstrated that it was functionally equivalent to the trypsin-resistant core protein present in maize MON 810. The Cry1Ab protein is quickly degraded in simulated gastric fluid. Bioinformatics studies showed that the amino acid sequence of the Cry1Ab protein expressed in maize MON 810 has no relevant similarity to proteins known to be toxic or allergenic to humans and other mammals. No toxicity of the Cry1Ab protein was observed in an acute oral toxicity study in mice or in a 28-day study where a Cry1Ab protein purified from *B. thuringiensis* var. *kurstaki* (strain HD-1) was administered by gavage to rats during the second and fourth week of the study. No oral toxicity of maize MON 810 was observed in a 90-day rat study where the experimental animals were fed *ad libitum* a diet containing up to 33% maize MON 810. Feeding studies with whole maize grains in the diet of broilers, dairy cows, and Atlantic salmon indicate that maize MON 810 is nutritionally equivalent to commercial non-GM maize cultivars. The Cry1Ab protein expressed in maize MON 810 was found unlikely to be allergenic and the potential for alteration in the allergenicity of the whole crop does not appear relevant to the EFSA GMO Panel since maize is not considered a common allergenic food. The EFSA GMO Panel was 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*" (EFSA, 2009a).

With regard to the safety of pollen, the EFSA GMO Panel refers to the conclusion of its statement on the safety of maize MON 810 pollen occurring in or as food: "*while the EFSA GMO Panel is not in a position to conclude on the safety of maize pollen in or as food in general, it concludes that the genetic modification in maize MON810 does not constitute an additional health risk if maize MON810 pollen were to replace that of non-GM maize in or as food*" (EFSA, 2011d; see also EFSA, 2013).

5.1.2. Results from the literature search

a) Toxicological and nutritional assessment

A number of publications on animal feeding studies with maize MON 810 have become available since the last opinion on this maize by the EFSA GMO Panel. These feeding studies included maize MON 810 grain and grain of the appropriate comparator in the diets of broilers (Řehout et al., 2009; Swiatkiewicz et al., 2010a), Japanese quails (Sartowska et al., 2012), lactating cows (Guertler et al., 2009, 2010; Paul et al., 2010; Steinke et al., 2010), pigs (Delgado and Wolt, 2010; Rossi et al., 2011; Stadnik et al., 2011; Swiatkiewicz et al., 2011; Buzoianu et al., 2012a,c; Walsh et al., 2012a), and Atlantic salmon (Frøystad-Saugen et al., 2009; Sissener et al., 2011). Rossi et al. (2011) noted that piglets supplied maize MON 810 performed better than piglets supplied the control maize and suggested that this difference was due to the lower level of fumonisin B1 in the diet. These additional feeding studies confirmed that maize MON 810 can be used as any other maize in animal feed.

Sissener et al. (2011) reported further details on a previously performed feeding study in Atlantic salmon supplied diets containing 30% maize MON 810 and a near-isogenic parental maize line (non-GM). It was reported that the level of deoxynivalenol (DON) differed between the materials from MON 810 and the comparator used for feed preparation, which, according to the same investigators, might account for the previously observed differences in growth, relative organ sizes, cellular stress and intestinal cell gene expression (e.g., Frøystad-Saugen et al., 2009) as well as in immunological parameters in Atlantic salmon exposed to maize MON 810 as compared to the conventional counterpart.

Guertler et al. (2012) slaughtered ten cows fed maize MON 810 and seven cows fed control maize and studied the gene expression in tissues of the gastrointestinal tract and the liver. The gene expression studied included major genes of the inflammation, cell cycle and apoptosis pathways. No significant difference in gene expression profile was found in cows fed GM and cows fed control maize. The investigators concluded that maize MON 810 does not have any effect on major genes involved in apoptosis, inflammation and cell cycle in the gastrointestinal tract and in the liver of dairy cows.

b) Allergenicity assessment

Several investigators have studied the allergenic or immunologic potential of the newly expressed Cry1Ab protein and/or the whole maize MON 810 using various experimental systems.

For the assessment of the newly expressed protein, De Luis et al. (2010) performed *in vitro* digestibility studies in the presence of pepsin at pH 2.0 with either the Cry1Ab protein extracted from maize MON 810 or produced by *B. thuringiensis*. They monitored the kinetics and rate of hydrolysis of the protein which varied upon the different conditions. The authors concluded that Cry1Ab was rapidly and extensively degraded by pepsin. In another study, Guimaraes et al. (2010) observed a limited degradation of the recombinant protein Cry1Ab when the hydrolysis was performed at pH 2.0 and above, in particular at conditions where the pepsin:Cry1Ab ratio was 1:20. The EFSA GMO Panel has considered the outcomes of these *in vitro* assays taking into account that different procedures were followed, and that the origin of the protein, the pH value and the pepsin:Cry1Ab ratio have an impact on the kinetics and rate of degradation. In line with its guidance document for the risk assessment of food and feed from GM plants (EFSA, 2011f), the EFSA GMO Panel assessed this information on *in vitro* hydrolysis together with all the available information on Cry1Ab (e.g., from other studies of different nature and other properties of the Cry1Ab protein) and did not identify any issue that would invalidate the previous Panel conclusions on the allergenicity of the Cry1Ab protein.

Kim et al. (2009) studied the reactivity of Cry1Ab as expressed in maize MON 810 and Bt11 against sera from maize-sensitised Korean children. No specific IgE binding was observed between the purified Cry1Ab protein (from *E. coli*) and the human sera used.

In a study comparing the adjuvanticity of the Cry1Ab protein and cholera toxin (CT) on allergenic sensitisation and elicitation to peanut in a mouse model, Guimaraes et al. (2008) observed that Cry1Ab protein had no adjuvant effects similar to those of CT. While a possible impact of Cry1Ab on the elicitation of the allergic reaction in the Balb/c mouse model under the conditions used was reported, the authors concluded that further studies are needed to clarify the mechanisms of action.

For the assessment of the whole maize MON 810, in a short-term feeding study, weanling pigs were fed diets containing either maize MON 810 or a non-GM comparator. The investigators observed no adverse effect of these diets on the gut microbiota, intestinal morphology and cytokine production in weanling pigs (Walsh et al., 2010). In an additional publication, these investigators concluded that feeding of maize MON 810 to pigs did not induce an allergic immune response as mainly evidenced by the lack of specific immunoglobulin (Ig) production against the Cry1Ab protein (Walsh et al., 2011). Walsh et al. (2012b) also investigated the effects of feeding maize MON 810 to pigs for 110 days on the immune response and found no indications for an allergic or immflammatory-type peripheral immune response. These conclusions were based on the absence of specific Ig production against the Cry1Ab protein, and the absence of alterations in T cell populations and inflammatory cytokine production. In a more recent study, Buzoianu et al. (2012b) studied the effects of feeding maize MON 810 to sows during gestation and lactation on maternal and offspring immunity. Following an assessment of the immune function of groups fed a diet containing maize MON 810 or a non-GM comparator, some differences were observed between the groups in a limited number of parameters. According to the authors, these differences did not indicate inflammation or allergy since neither specific Ig against Cry1Ab nor significant differences in cytokine production between groups was detected.

In experiments on mice, Adel-Patient et al. (2011) observed an immunogenic capacity of purified Cry1Ab without evidence of allergenic potential. These investigators also noted no difference in natural maize allergen profiles between maize MON 810 and its non-GM comparator. Similarly, Fonseca et al. (2012) compared the endogenous expression of five maize allergens in maize MON 810 and in its conventional counterpart and found no statistically significant differences in expression levels between the materials tested throughout seed development. In agreement with this observation, sera from two maize allergic individuals reacted very similarly against extracts of maize MON 810 and its non-GM comparator.

5.1.3. Conclusion

Results reported in the scientific literature described above do not provide new information that would invalidate the previous conclusions on the food and feed safety assessment of maize MON 810 made by the EFSA GMO Panel. Therefore, the EFSA GMO Panel considers that its previous conclusions on maize MON 810 remain valid and applicable.

6. ENVIRONMENTAL RISK ASSESSMENT AND RISK MANAGEMENT STRATEGIES

6.1. Environmental risk assessment

The outline of this EFSA GMO Panel Scientific Opinion follows the key areas of environmental risk as defined in Directive 2001/18/EC and EFSA (2010b): (1) changes in plant fitness due to the genetic modification; (2) potential for gene transfer and its environmental consequences; (3) interactions between the GM plant and target organisms; (4) interactions between the GM plant and non-target organisms; (5) effects on animal and human health; (6) interactions with biogeochemical processes and the abiotic environment; (7) impacts of the specific cultivation, management and harvesting techniques; and (8) risk management strategies (including PMEM).

The EFSA GMO Panel previously concluded 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*" (EFSA, 2009a).

6.1.1. Changes in plant fitness due to the genetic modification

In its 2009 Scientific Opinion for the continued marketing of maize MON 810, the EFSA GMO Panel evaluated the altered potential of maize MON 810 in terms of fitness, persistence and invasiveness .

6.1.1.1. Summary of previous conclusions by the EFSA GMO Panel

In its 2009 Scientific Opinion for the continued marketing of maize MON 810, the EFSA GMO Panel concluded that: "*the likelihood of unintended environmental effects due to the establishment and survival of maize MON 810 will be no different to that of conventional maize varieties*" (EFSA, 2009a).

6.1.1.2. Results from the literature search

From the literature search, no new scientific publications containing evidence specific to maize MON 810 for this specific area of risk were identified.

6.1.1.3. Conclusion

In the absence of new scientific evidence specific to maize MON 810 for this specific area of risk, the EFSA GMO Panel considers that its previous conclusions on changes in plant fitness due to the genetic modification remain valid and applicable.

6.1.2. Potential for gene transfer

In its 2009 Scientific Opinion for the continued marketing of maize MON 810, the EFSA GMO Panel evaluated the potential for horizontal and vertical gene flow of maize MON 810, as well as the potential environmental consequences of such gene transfer.

6.1.2.1. Summary of previous conclusions by the EFSA GMO Panel

Concerning the potential for horizontal gene transfer, the EFSA GMO Panel concluded that: "*it is very unlikely that the cry1Ab gene from maize MON 810 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*" (EFSA, 2009a).

Regarding a possible plant-to-plant gene transfer, the EFSA GMO Panel concluded that: "*maize MON 810 has no altered survival, multiplication or dissemination characteristics*", and that: "*the likelihood of unintended environmental effects due to the establishment and spread of maize MON 810 will be no different from that of conventional maize varieties*" (EFSA, 2009a).

6.1.2.2. Results from the literature search

From the literature search, the following ten new scientific publications containing evidence specific to maize MON 810 for this specific area of risk were identified and scrutinised for their possible relevance for the ERA of maize MON 810:

- Pirondini and Marmiroli (2008) reviewed available data on vertical and horizontal gene flow, but did not report new data that had not been previously considered by the EFSA GMO Panel.

- Some of the studies performed with maize MON 810 mixed into the diet of target animals have investigated the fate of the transgenic DNA and protein in the animal, including studies on the potential transfer of the whole or parts of the *cry1Ab* DNA and Cry1Ab protein to animal tissues or excreted fluids. In broiler chickens feed diets with maize MON 810 for six weeks, transgenic DNA was detected in the crop and gizzard content of birds fed diets with maize MON810 (Świątkiewicz et al., 2010b). Świątkiewicz et al. (2011) investigated the fate of DNA in the digestive tract, excreta and tissues of laying hens fed meal of maize MON 810 or its conventional counterpart, using PCR

analyses. Two specific pairs of primers targeting the transgenic promoter (35S) sequences of 170 bp and 123 bp, and a generic primer pair targeting a 226 bp region of an endogenous maize gene (invertase) were used to analyse samples taken. During the experiment, hens were fed *Bt*- or non-*Bt*-maize for 29 weeks from the 25th week of their age until the 54th week. Eggs were collected at 48 weeks. The authors detected DNA fragments from *Bt*-maize in all samples of crop digesta and gizzard of hens fed *Bt*-maize, and in 33% of the samples of the duodenum. However, DNA fragments of transgene fragments were not detectable in jejunum, ileum, caeca digesta, excreta, tissues (blood, liver, spleen, lungs) and eggs of hens fed *Bt*-maize meal. Whereas Świątkiewicz et al. (2010b) found no transgenic DNA further down in the gastrointestinal tract and in blood, liver, spleen and breast muscle of broiler chickens, Hanusova et al. (2011) detected transgenic DNA in 16% of blood samples but not in the liver or the kidneys. In pigs fed a diet containing maize MON 810, transgenic DNA was detectable in the stomach, duodenum and proximal ileum content, but not in the distal intestinal digesta, blood and examined organs (Świątkiewicz et al., 2011; Walsh et al., 2011; Buzoianu et al., 2012a). Three of the reports were from a long-term (25 months) feeding study on lactating dairy cows. Eighteen cows per group were fed diets with slightly more than 70% maize (MON 810 or a near-isogenic non-GM variety) in the form of kernels, maize stem pellets and silage (Guertler et al., 2010; Paul et al., 2010; Steinke et al., 2010). Feed samples were collected weekly, whereas samples of faeces, blood and milk were collected monthly, and urine samples bimonthly. Guertler et al. (2010) analysed samples for cry1Ab DNA by two PCR-based methods and the Cry1Ab protein by a sensitive and specific ELISA method. All samples of blood, milk and urine were free of recombinant DNA and protein. Whereas faeces contained fragments of the Cry1Ab protein, no transgenic DNA could be detected. At the end of the feeding trial digesta contents of rumen, abomasums, small intestine, large intestine and cecum were collected after slaughtering six cows of each feeding group (Paul et al., 2010). The authors estimated an extensive degradation of the Cry1Ab protein from the initial feed intake of 108 mg/day to 2.25-4.5 mg/day in faeces. In a previous study, Guertler et al. (2009) monitored the presence or absence of *cry1Ab* gene and the Cry1Ab protein in milk of multiparous cows fed maize MON 810 or non-*Bt*-maize during six months, using quantitative real-time PCR and ELISA. The quantitative real-time PCR and sandwich ELISA were optimised for *cry1Ab* and Cry1Ab determination at low levels of *cry1Ab* (100 copies) and Cry1Ab protein (0.4 ng/mL) in bovine milk, respectively. Neither *cry1Ab* nor Cry1Ab protein were detected in the 90 milk samples collected from cows fed either maize MON 810 or non-*Bt*-maize. Steinke et al. (2010) found that the milk yield during the two lactation periods, the milk composition and the body conditions was not consistently affected by the dietary treatment. The long-term feeding of dairy cows with maize demonstrated nutritional and indicated the compositional equivalence of maize MON 810 to its conventional counterpart. The results confirm that plant DNA in maize meal is substantially degraded to small fragments (Rizzi et al., 2012), and that DNA present in feed becomes substantially further degraded through digestion in the animal gastrointestinal tract by host and microbial factors. Therefore, the likelihood that a full length gene sequence persists in the lower intestinal tract is very low. These results are consistent with data previously considered by the EFSA GMO Panel (i.e., EFSA, 2009b).

- Galeano et al. (2011) investigated the level of cross-pollination between *Bt*-maize (Cry1Ab-expressing maize events MON 810 and Bt11) and conventional maize in Uruguay. The authors followed five pairs of fields in their study, each of which consisted of a GM maize field located nearby a field cropped to non-GM maize. Field size and the distance between the fields cropped to GM and non-GM maize varied between each pair of fields. The presence of GM material in the offspring of non-GM maize was observed in three of the five pairs of fields. The percentages of transgenic seedlings in the offspring of non-GM maize were 0.56% (1/180), 0.83% (1/120) and 0.13% (1/764) for three sampling sites located 40, 100 and 330 m away from the nearest *Bt*-maize source, respectively. The data confirm that concentrations of viable maize pollen and cross-pollination levels considerably decrease with increasing distance from the pollen source. Evidence indicates that approximately 95-99% of the released pollen is deposited within about 50 m from the pollen source, though vertical wind movements or gusts during pollen shedding can lift pollen up high in the atmosphere and distribute it over significant distances up to kilometres (reviewed by Eastham and Sweet, 2002; Devos et al., 2005, 2009; Sanvido et al., 2008; Riesgo et al., 2010). In

addition, the EFSA GMO Panel reiterates it does not consider cross-pollination in maize an environmental risk, but an agricultural management and coexistence issue (Devos et al., 2009) that is not in its remit.

6.1.2.3. Conclusion

Results reported by Pirondini and Marmiroli (2008), Guertler et al. (2009, 2010), Paul et al. (2010), Steinke et al. (2010), Galeano et al. (2011), Hanusova et al. (2011), Świątkiewicz et al. (2011), Walsh et al. (2011) and Buzoianu et al. (2012a) do not provide new information that would invalidate the previous conclusions on potential gene transfer from maize MON 810 and its potential environmental consequences made by the EFSA GMO Panel. Therefore, the EFSA GMO Panel considers that its previous conclusions on maize MON 810 remain valid and applicable.

6.1.3. Interactions of the GM plant with target organisms

The potential of maize MON 810 to cause adverse effects through direct or indirect interactions between the GM plant and target organisms was evaluated by the EFSA GMO Panel (EFSA, 2009a).

6.1.3.1. Summary of previous conclusions by the EFSA GMO Panel

In its 2009 Scientific Opinion for the continued marketing of maize MON 810, the EFSA GMO Panel identified: "*the possible evolution of resistance in target species, as a potential risk linked to the cultivation of maize MON 810*" (EFSA, 2009a, 2011d).

6.1.3.2. Results from the literature search

From the literature search, the following 13 new scientific publications containing evidence specific to maize MON 810 for this specific area of risk were identified and scrutinised for their possible relevance for the ERA of maize MON 810:

- Pirondini and Marmiroli (2008) reviewed available data on resistance evolution, but did not report new data that had not been previously considered by the EFSA GMO Panel.

- Bel et al. (2009) identified several major mutations in a cadherin gene in a laboratory-selected strain of European corn borer (ECB, *Ostrinia nubilalis*) with Cry1Ab resistance. The contribution of these major mutations to the resistance was analysed in resistant individuals that survived exposure to a high concentration of Cry1Ab protoxin. The results indicated that the presence of major mutations was drastically reduced in individuals that survived exposure. The authors concluded that their work supports a polygenic inheritance of resistance in the laboratory-selected ECB strain with Cry1Ab resistance, and that the mutations in a cadherin gene would contribute to resistance by means of an additive effect.

- Tiwari et al. (2009) determined the impact of ECB injury on whole-plant yield of non-*Bt*-maize (Pioneer 34B23) grown for silage by evaluating different levels of hand-infested third instar ECB per plant at three plant growth stages under field conditions (2004 and 2005; USA), and compared that with that of maize MON 810 (Pioneer 34B24). In 2004 and 2005, ECB had a significant negative impact on whole-plant yield with increasing infestation; however, whole-plant yield was not significantly affected by plant growth stage in either year. In 2004, the six larvae per plant treatment caused the greatest percentage of reduction (23.4%) in mean whole-plant yield compared with *Bt*-maize. In 2005, the five larvae per plant treatment caused the greatest percentage of reduction (8.3%) in mean whole-plant yield compared with *Bt*-maize. An exponential decay model fitted the relationship between mean whole-plant yield and ECB larvae infestation level from the pooled data of both years.

- Berés (2010) investigated the efficacy of maize MON 810 against ECB at five locations in Poland during the 2006-2007 growing seasons. A randomised complete block design with four replications was used at all locations. The authors observed that *Bt*-maize had a significantly reduced average

percentage of damaged plants (in particular cobs), and averaged more yield per hectare than non-*Bt*-maize across locations and seasons. The average plant damage of *Bt*-maize caused by ECB varied between 0.5-0.7% and significantly less than that observed on non-*Bt*-maize plants (40.0-44.0%). For *Bt*-maize, only slight symptoms of plant injuries, such as small number of holes in stalks and gnawing of cobs, were observed. The authors concluded that maize MON 810 offers an effective means to control ECB in Poland, assuming that appropriate insect resistance management (IRM) measures are implemented to delay the potential for resistance to evolve.

- Buntin (2010) investigated the efficacy of maize MON 810 for silage production against fall armyworm (FAW, *Spodoptera frugiperda*) and corn earworm (CEW_z, *Helicoverpa zea*) in central Georgia (southeastern USA) during 2006 and 2007. Hybrids with a temperate or semitropical background were planted at the recommended time in mid-April and in late June to simulate a double-crop corn planting. Whorl infestation and damage by FAW was significantly reduced in maize MON 810 when infestations were large. FAW infestation levels and damage were similar in both temperate and semitropical types. Maize MON 810 also had a small reduction in ear infestation and less kernel damage in ears infested by CEW_z than non-*Bt*-maize in most trials. CEW_z infestation level were less in the semitropical than the temperate hybrids in 2006 but were not different in 2007. Silage yield was not significantly different among maize MON 810 and non-*Bt*-maize in the first planting in both years. The *Bt*-trait prevented significant yield loss of 17.1% during the second planting in 2006 when FAW whorl infestations exceeded 39% in non-*Bt*-maize, but did not significantly affect silage yield in the late planting in 2007 when FAW infestations were low. Maize silage quality as measured by neutral detergent fiber, acid detergent fiber and crude protein content did not differ among maize MON 810 and non-*Bt*-maize indicating silage dry matter yield was the main silage component affected by the *Bt*-trait and insect damage

- Gómez et al. (2010) reviewed current data and models on the mode of action of the Cry1Ab protein (see also Bravo et al., 2012; Vachon et al., 2012), but did not report new data that had not been previously considered by the EFSA GMO Panel. The mode of action of Cry proteins is to bind selectively to specific receptors on the epithelial surface of the midgut of larvae of susceptible insect species, leading to death of larvae through pore formation, cell burst and subsequently septicemia. In their literature review, Vachon et al. (2012) concluded that available data still support the notion that Cry proteins act by forming pores, but that most events leading to pore formation, following binding of the activated toxins to their receptors, remain relatively poorly understood.

- Kamath et al. (2010) assessed the efficacy of several maize MON 810 hybrids against the spotted stem borer (SSB, *Chilo partellus*) and pink stem borer (PSB, *Sesamia inferens*), and quantified Cry1Ab protein expression levels in whorl leaf and stem tissues in India. Both species are target lepidopteran maize pests in India, and neonates initially feed on maize by scraping the leaf lamina before migrating to bore into the stem. *Bt*- and non-*Bt*-maize were planted at different locations during the two season field trials (four locations during the dry season (October 2005 to March 2006) and ten locations during the wet season (May to October) of 2006). At each location, *Bt*- and non-*Bt*-maize were planted in two replicated plots. In all maize MON 810 hybrids and over both seasons, Cry1Ab protein levels were the highest in the leaf tissues on which pest larvae start feeding before migrating towards the stem. The mean tissue Cry1Ab concentrations during the oviposition window of the borers, ranged from 50.05 to 21.01 ppm in whorl leaf, and between 9.26 and 3.47 ppm in stem tissue during the same period in the dry season of 2005/06. Similarly, Cry1Ab concentrations in whorl leaf and stem during the wet season of 2006 ranged between 19.30 to 11.08 ppm and 14.28 to 4.69 ppm, respectively. These results mirror the variability in Cry1Ab expression across *Bt*-maize hybrids and genetic backgrounds. Dose-response bioassays indicated that multiple populations of SSB and PSB are susceptible to Cry1Ab. For SSB, LC_{50} values were 0.008-0.068 µg Cry1Ab/mL; LC_{90} values were 0.020-0.354 µg Cry1Ab/mL, and MIC_{90} values were 0.005-0.078 µg Cry1Ab/mL. For PSB, LC_{50} values were 0.046-0.056 µg Cry1Ab/mL, LC_{90} values were 0.260-0.740 µg Cry1Ab/mL, and MIC_{90} values were 0.134-0.221 µg Cry1Ab/mL (see also

Jalali et al., 2010, below). Based on the results, the authors concluded that the maize MON 810 hybrids tested may be suitable to control SSB and PSB larvae in India.

- Tende et al. (2010) evaluated the susceptibility of SSB and African stem borer (ASB, *Busseola fusca*) to Cry1Ab and Cry1Ba under greenhouse conditions. Both Cry proteins expressed in *Bt*-maize leaves controlled SSB consistently, but did not provide complete control of ASB. No changes in susceptibility of SSB and ASB to Cry1Ab and Cry1Ba were observed after five generations of selection. The authors concluded that ASB is inherently less susceptible to Cry1Ab and Cry1Ba than SSB.

- Darvas et al. (2011) investigated whether reduced damage to *Bt*-maize plants caused by ECB and corn earworm (CEW_a, *Helicoverpa armigera*) would decrease *Fusarium verticillioides* infestation of maize plants and ears in Hungary. ECB and CEW_a larvae feeding on *F. verticillioides* mycelia can distribute its conidia via their faecal pellets. Laboratory experiments using third and fourth instars of ECB and CEW_a confirmed that: Cry1Ab protein levels vary across maize MON 810 plant parts; and that ECB larvae prefer feeding in stems, while CEW_a larvae feed on ears only. Field experiments with natural infestation indicated that: CEW_a larvae tend to change feeding place in case of *F. verticillioides* infection, when they move towards kernels via husk leaves; larval damage was followed by a *F. verticillioides* infection in only 20-30% of the cases; and that maize MON 810 effectively controlled maize ear infection by ECB and CEW_a, but that some larvae may be exposed to sublethal Cry1Ab levels and survive exposure.

- Mugo et al. (2011) investigated the performance of two maize MON 810 hybrids against ASB and SSB, which are two major lepidopteran maize pests in Kenya, under greenhouse conditions. The experimental design was a 4 × 4 alpha-lattice with ten replications, and with each plant being a replicate. Plants were infested artificially with ASB and SSB neonates. Maize MON 810 had less leaf damage, and fewer surviving larvae were recovered from the whole plant, compared to non-*Bt*-maize plants. The authors concluded that maize MON 810 may be used to control ASB and SSB, but that its efficacy also needs to be evaluated under field conditions.

- Guo et al. (2012) analysed gene regulation patterns in laboratory-selected Cry1Ab-resistant (Cry1Ab-R) and Cry1Ab-susceptible (Cry1Ab-S) sugarcane borer (SCB, *Diatraea saccharalis*) strains. Microarray analyses of 7145 cDNAs revealed 384 differentially expressed genes between larvae of the Cry1Ab-R and Cry1Ab-S strains. A total of 273 genes were significantly upregulated (2 to 51.6-fold), and 111 genes were significantly downregulated (2 to 22.6-fold) in the Cry1Ab-R strain compared with the Cry1Ab-S strain. The upregulation of three potential resistance-related genes, coding for a glutathione S-transferase (GST), a chymotrypsin-like protease (CHY) and a lipase (LP) was confirmed. In addition, ontology analysis revealed that more than twice the number of metabolic-related genes was upregulated compared with downregulated genes with the same biological function. Up to 35.2% of the upregulated genes in the resistant strain were associated with catalytic activity, while only 9.5% of the downregulated genes were related to the same catalytic molecular function. The results indicate that Cry1Ab resistance increases metabolic or catalytic activities with significant upregulations of metabolic- or catalytic-related genes. Therefore, the authors concluded that the underlying resistance mechanisms in SCB may be more complex than the cadherin-linked resistance observed in several *Bt*-resistant target insect species.

- George et al. (2012) conducted laboratory bioassays to investigate the impact in terms of survival and development of diets containing maize MON 810 leaves on ASB, which is a target lepidopteran maize pest in South Africa. Weight, development time and mortality of larvae exposed to diets containing either *Bt*-maize or non-*Bt*-maize were measured. Further, the authors assessed the binding of Cry1Ab to the midgut epithelial cells following ingestion. Subsequent effects on cell integrity, at the electron microscope (EM) level, were also investigated. Cry1Ab reduced larval weight by 60%, while larval weight in the control group increased by 25%; no effects on mortality were observed. Insect survival, developmental rate and pupal and adult weight were significantly reduced on *Bt*-maize compared with non-*Bt*-maize. These differences were more

pronounced with second instars than with third instars who are inherently less susceptible. Leaf area consumed by ASB larvae fed *Bt*-maize was significantly lower compared with the area consumed by larvae fed non-*Bt*-maize. EM studies revealed that consumption of *Bt*-maize deleteriously affected gut integrity. Effects were observed in columnar cells of the midgut epithelium, with the cytoplasm becoming highly vacuolated; the microvilli were disorganised, the mitochondria were abnormal, and there was an increase in the number of lysosomal bodies. The rough endoplasmic reticulum had also become dilated. The authors concluded that ASB larvae are susceptible to Cry1Ab and advocated the potential for maize MON 810 to control ASB when used as part of an integrated pest management (IPM) approach, though they recommended field studies to assess the efficacy of maize MON 810 against ASB under field conditions.

- Székács and Darvas (2012) reviewed recent publications to assess the potential impact that maize MON 810 may have on the environment if it was to be grown in the Pannonian Biogeographical Region (EU). In their publication, the authors questioned the compatibility of *Bt*-crops with IPM practices. The EFSA GMO Panel notes that the basic goal of IPM is to achieve effective crop protection in a manner that provides sustainable economic benefits to farmers and society, and minimal impact on the environment. IPM prescribes the use of multiple tactics to suppress target organisms, and to prevent or at least delay resistance evolution. Meissle et al. (2011) and others indicated that the incorporation of *Bt*-crops with current integrated approaches to pest management will help to ensure their long-term sustainability. Overall, the EFSA GMO Panel considers that the authors choose to cite a limited selection of publications in their opinion paper, and that they did not report new data specific to target organisms that had not previously been considered by the Panel (EFSA, 2008b).

6.1.3.3. Conclusion

The EFSA GMO Panel recommends caution when predicting future responses of ECB and MCB in relevant EU regions based on experiences elsewhere or with other target insect pest species, as resistance evolution is dependent upon many factors. Furthermore, caution must be exercised when extrapolating laboratory and greenhouse results with artificially-selected resistant strains to field conditions.

Results reported by Pirondini and Marmiroli (2008), Bel et al. (2009), Tiwari et al. (2009), Berés (2010), Buntin (2010), Gómez et al. (2010), Kamath et al. (2010), Tende et al. (2010), Darvas et al. (2011), Mugo et al. (2011), Guo et al. (2012), George et al. (2012) and Székács and Darvas (2012) do not provide new information that would invalidate the previous conclusions on interactions of maize MON 810 with target organisms made by the EFSA GMO Panel. Therefore, the EFSA GMO Panel considers that its previous conclusions on maize MON 810 remain valid and applicable.

6.1.4. Interactions of the GM plant with non-target organisms

The potential of maize MON 810 to have direct or indirect adverse effects on non-target organisms and the ecosystem services they provide in agro-ecosystems was previously evaluated by the EFSA GMO Panel (EFSA, 2009a) and the outcome of these evaluations has been recently updated in the light of new relevant scientific publications (EFSA, 2011a,b,e, 2012a,d).

6.1.4.1. Summary of previous conclusions by the EFSA GMO Panel

In its 2009 Scientific Opinion for the continued marketing of maize MON 810, the EFSA GMO Panel concluded that: "*there was no evidence to indicate that the placing of maize MON 810 and derived products on the market is likely to cause adverse effects on NTOs in the context of its proposed uses*" (EFSA, 2009a).

Concerning non-target Lepidoptera, the EFSA GMO Panel concluded that: "*on the basis of a modelling exercise (Perry et al., 2010), the amounts of maize MON 810 pollen grains found in and around maize fields are unlikely to adversely affect a significant proportion of non-target lepidopteran larvae*" (EFSA, 2009a). In its recent Statement on maize Bt11 (whose conclusions on non-target

Lepidoptera are equally applicable to maize MON 810), the EFSA GMO Panel indicated that: "*locally exposed non-target Lepidoptera that are 'extremely sensitive' to the Cry1Ab protein may be at risk if exposed to harmful amounts of maize Bt11/MON 810 pollen*" (EFSA, 2011e).

6.1.4.2. Results from the literature search

From the literature search, the following 23 new scientific publications containing evidence specific to maize MON 810 for this specific area of risk were identified and scrutinised for their possible relevance for the ERA of maize MON 810:

- Pirondini and Marmiroli (2008) reviewed available data on interactions between GM plants and non-target organisms, but did not report new data that had not been previously considered by the EFSA GMO Panel.

- Peterson et al. (2009) quantified the uptake of plant-produced Cry proteins, including the specific detection of Cry1Ab, from three different *Bt*-maize events (including maize MON 810 (4842S), a Cry3Bb1-expressing maize event and a maize event expressing both Cry1Ab and Cry3Bb1) by non-target carabids. The gut content of adult ground beetles collected from a field planted to the *Bt*-maize events and non-*Bt*-maize (4847) in Kentucky (USA) was screened for Cry1Ab via ELISA. Significant numbers of carabids tested positive for Cry1Ab from the lepidopteran-specific *Bt*-maize field: *Harpalus pensylvanicus* (39%, 25 of 64), *Stenolophus comma* (4%, 6 of 136), *Cratacanthus dubius* (50%, 1 of 2), *Clivina bipustulata* (50%, 1 of 2), and *Cyclotrachelus sodalis* (20%, 1 of 5). The highest proportion of *Bt*-toxin uptake was 4-6 weeks after anthesis. The uptake of Cry1Ab varied between carabid species, depending on their feeding behaviour. The EFSA GMO Panel does not consider the uptake of Cry1Ab by carabids through the consumption of *Bt*-maize plant material such as leaves and shed pollen lying on the soil surface or prey that had previously been feeding on *Bt*-maize an environmental hazard in itself, but is primarily concerned with assessing the consequences of such uptake and exposure.

- Balog et al. (2010) evaluated the abundance, species richness, diversity and similarity of non-target rove beetles in maize MON 810 (DK440BTY) and its conventional counterpart (DK440) under field conditions, and estimated the effect of prey abundance (here: the aphid *Rhopalosiphum padi*) on rove beetle populations. A field experiment was conducted in Hungary during three years (2001-2003), with plots (30 m × 30 m each) planted to *Bt*-maize and non-*Bt*-maize, and arranged alternatively, with six replications each. In total, 1538 rove beetles and 21 species were sampled with pitfall traps. The following five rove beetle species were the most abundant: *Platystethus spinosus*, *Aleochara bilineata*, *Tachyporus hypnorum*, *A. bipustulata* and *Xantholinus linearis*. The authors reported that the overall community structure of rove beetles was similar between *Bt*-maize and non-*Bt*-maize, indicating that they were not adversely affected by the Cry1Ab protein. In addition, no significant differences for non-aphidophagous predators and parasitoids were found after grouping staphylinids into guilds. The abundance of aphidophagous predators was significantly and marginally significantly higher in 2002 and 2003 in non-*Bt*-maize, respectively. Aphid abundance showed high fluctuations between plots and years, and was numerically higher in non-*Bt*-maize plots in the second half of the maize-growing season. The abundance of aphidophagous predators did not correlate with the total annual number of *R. padi* in the same year, but there was a linear correlation in successive years. The authors attributed the higher abundance of aphidophagous predators in non-*Bt*-maize plots in the second half of the maize-growing season to the unequal distribution of aphids within a field.

- García et al. (2010) studied prey-mediated effects of maize MON 810 on the rove beetle *Atheta coriaria*, using the spider mite *Tetranychus urticae* as prey in a tritrophic lower-tier study. No difference in the performance of *A. coriaria* fed, either *T. urticae* reared on *Bt*-maize, *T. urticae* reared on non-*Bt*-maize, or an artificial food diet was found in terms of development time, sex ratio, survivorship, fecundity and egg fertility. Cry1Ab was detected in both *T. urticae* and *A. coriaria* adults and larvae, with concentrations of Cry1Ab decreasing through the trophic chain. Proteolytic activities of *A. coriaria* adults fed spider mites reared on *Bt*-maize did not show

differences with those fed prey reared on non-*Bt*-maize, indicating that the nutritional quality of the prey was not affected by exposure to Cry1Ab. Based on the results, the authors concluded that *A. coriaria* is exposed to Cry1Ab when fed spider mites reared on maize MON 810, but that this exposure had no adverse effects on the performance or digestive physiology of the rove beetle.

- Bakonyi et al. (2011) analysed whether the reproduction, faecal pellet production or food preference of the collembolan species *Folsomia candida* is affected when fed maize MON 810 plant material for several consecutive generations under laboratory conditions. *F. candida* was fed *Bt*-maize for 0, 6, 16 and 22 months and the number of eggs and faecal pellets were determined. The experiment was repeated seven months later with the same populations. Food preference tests were additionally performed. Significant differences were found in food consumption, egg production and food preference between populations in some cases, but no time-response effect was observed. The four different populations showed different performances, e.g., in individual fertility. Based on the results, the authors concluded that long-term feeding of *F. candida* with maize MON 810 material had no harmful effects to this collembolan species.

- Lumbierres et al. (2011) measured the potential effect of the Cry1Ab-expressing maize events MON 810 and Bt176 on aphid abundance, aphid-parasitoid species composition and parasitism rates in a two-year field study in Spain. This publication presents the first experimental investigation concerning the functionality of naturally occurring parasitoids on a major pest guild of maize in Europe. The field study consisted of a complete randomised block design with four replications. No consistent differences in aphid abundance were found between *Bt*- and non-*Bt*-maize. Differences in aphid abundance between varieties were only significant in one year, and the only significant contrast was detected between maize MON 810, which hosted more aphids, and its near-isogenic counterpart. These differences were attributed by the authors to plant variety effects and year conditions, instead of intended and unintended changes in *Bt*-maize. The prevalent parasitoids were: *Lysiphlebus testaceipes*, *Lipolexis gracilis* and *Aphelinus* sp.. *Bt*-maize did not alter the aphid-parasitoid associations and had no effect on the aphid parasitism and hyperparasitism rates. Overall, parasitism rates between *Bt*- and non-*Bt*-maize as a group, or between each *Bt*-maize event and its near isogenic counterpart were not significantly different. The results suggest that *Bt*-maize has no negative impact on second, third and fourth levels of the trophic relationships studied.

- Shu et al. (2011) measured Cry1Ab concentrations in Cry1Ab-expressing maize stover (for events MON 810 (5422CBCL) and Bt11), soil-stover mixtures (named hereafter as substances), casts and guts of the earthworm *Eisenia fetida*, and assessed the potential effects of *Bt*-maize on the survival rate, relative growth rate and reproduction of *E. fetida*. *E. fetida* preferentially feeds on organic material than mineral soil. ELISA measurements detected immunoreactive Cry1Ab in substances, the casts, and the guts of *E. fetida* exposed to *Bt*-maize. Earthworms, bred in a medium containing stover of *Bt*-maize, had significantly higher relative growth rate and more new offspring and cocoons than those bred in substances with stover of non-*Bt*-maize. This effect was not attributed to Cry1Ab, but to the differences in the chemical composition of the plant material, of which both *Bt*-maize events contained more nutrients, including soluble sugar, organic carbon, nitrogen, phosphorous and potassium. There was a correlation between the amounts of Cry1Ab detected in the gut of the earthworms and in the time of exposure, suggesting that the proteins were not efficiently degraded in the gut. A significant decline was found in Cry1Ab concentrations from substances and casts of *E. fetida*. Overall, the authors demonstrated that the presence of Cry1Ab had no deleterious effect on growth and reproduction of *E. fetida*.

- Tan et al. (2011) identified specific arbuscular mycorrhizal fungal (AMF) communities associated with roots of the Cry1Ab-expressing maize events MON 810 (5422CBCL) and Bt11 and two non-*Bt*-maize comparators. The authors studied the community structure by PCR with specific primers directed towards the *Glomus* genus, which is the largest genus of AMF, on DNA extracted from roots and rhizosphere soil. No consistent significant differences of mycorrhizal colonization were found between *Bt*- and non-*Bt*-maize over the entire duration of the experiment. Differences caused

by the genetic modification were in the range of those between non-GM varieties. Maize genotypes had a greater influence on the differences between the communities than other factors such as plant age. Colonisation of roots by mycorrhiza was not affected by the genetic modification, and they were indistinguishable between the maize genotypes. Thus, no adverse effect of the genetic modification on the mycorrhizal community was detected, but an effect owing to the variety was detected.

- Viktorov (2011) did not report new data, but reviewed pathways through which plant-produced Cry1Ab proteins from *Bt*-maize can enter soil and aquatic environments, and whether such exposure may adversely affect non-target organisms, based on available scientific literature. These routes of exposure, as well as the environmental consequences of such exposure, were previously considered by the EFSA GMO Panel.

- Yu et al. (2011) reviewed available studies assessing potential adverse effects of GM plants and the Cry proteins they express on non-target organisms and the ecosystem services they provide, pathways of exposure through which GM plants may affect valued non-target organisms, as well as approaches to assess such potential effects, but did not report new data that had not been previously considered by the EFSA GMO Panel.

- Alcantara (2012) reported on a four-year on-farm monitoring study conducted in the Philippines between 2006 and 2009. The objectives of the study were to monitor potential long-term effects of maize MON 810 on arthropod community composition and diversity, including natural enemies, on commercial farms and in adjacent riparian ecosystems. The experimental design consisted of paired-fields that were replicated three times in each of the five growing seasons over four years. The experiment was carried out as a randomised complete-block design by using the three pairs of commercial farms, with each pair of farms serving as replicate. The farms in each pair: were approximately one hectare each; were separated by no more than one kilometre from each other; and were located within less than 25 m of the adjacent riparian areas. Arthropod counts were gathered by visual inspection of maize plants in three pairs of commercial farms and by sweep sampling in riparian sites nearby. Sampling showed that species composition and diversity between *Bt*- and non-*Bt*-maize and between riparian areas adjacent to *Bt*- and non-*Bt*-maize were similar. Principal response curves and analysis of variance showed that there were no indications of adverse effects of *Bt*-maize on the abundance of natural enemies either in crops or adjacent riparian sites. Crop type accounted for only small proportion of the total variance for the five seasons, while changes during the season accounted for the largest proportion of variance in the communities. Crop type had no significant influence on the community diversity index. In the riparian areas, there were no interactions between sampling date and crop type for any parameter. Moreover, there were no significant differences in either the diversity index or in the abundance of any of the natural enemies between the riparian sites associated with *Bt*-maize and those adjacent to non-*Bt*-maize. Overall, the author concluded that *Bt*-maize does not have any long-term adverse effect on arthropod communities in maize fields, or in adjacent riparian ecosystems.

- In the October issue of the peer-reviewed journal Environmental Entomology, a letter to the Editor by Andow and Lövei (2012) and a reply to it by Shelton et al (2012) were published. These two letters are the continuation of a scientific debate initiated by the publication by Lövei et al. (2009) that triggered the publication of successive responses by Shelton et al (2009a,b) and Andow et al. (2009). The two major points debated by both groups of authors are: (1) statistical considerations of power; and (2) the nature of effects of *Bt*-crops on natural enemies, whether direct or indirect. The original paper by Lövei et al. (2009) pointed to some possible methodological shortcomings in meta-analyses assessing the potential impact of *Bt*-crops on non-target organisms. Lövei et al. (2009) suggested non-zero effects on natural enemies due to the expression of Cry proteins and proteinase inhibitors in *Bt*-plants, in cases where the original analyses of the single studies taken individually had failed to detect such effects. Therefore, the authors argued that for at least some of these studies (without specifying which studies) a type II error has occurred whereby effects were present but were not detected. Shelton et al (2009a,b) replied highlighting the major role of indirect

effects, due to poor prey quality in tritrophic experiments, in determining such results. Further, Shelton et al (2009a,b) pointed out that it is impossible to be certain about the outcome of a statistical hypothesis test, and that Lövei et al. (2009) wrongly exclude the possibility of the meta-analysis itself having suffered a type I error, i.e., of falsely detecting differences where none exist. The two latest publications (Andow and Lövei, 2012; Shelton et al., 2012) defend the soundness of the statistical approach followed by Lövei et al. (2009) calling for a different approach to the ERA for natural enemies (Andow and Lövei, 2012), or the necessity to separate direct from indirect effects (Shelton et al., 2012). The EFSA GMO Panel previously evaluated the publications by Andow et al. (2009), Lövei et al. (2009) and Shelton et al (2009a,b), and therefore focuses on the two latest publications by Andow and Lövei (2012) and Shelton et al. (2012). The EFSA GMO Panel considers that both sets of authors failed to address the main reciprocal concerns raised in this ongoing correspondence. The EFSA GMO Panel considers that its ERA approach to assess potential adverse effects of GM plants on non-target organisms remains unaffected by the letters by Andow and Lövei (2012) and Shelton et al. (2012). This is because, according to its guidelines for the ERA of GM plants (EFSA, 2010a), both direct and indirect effects are to be assessed, as well as altered interactions between the GM plant and its receiving environments resulting from unintended changes in the GM plants. In addition, the guidelines for the ERA of GM plants guard against the problems of low-powered experiments by demanding that applicants specify the size of the effect each experiment is designed to detect, and supply a prospective statistical power analysis to give reassurance that the experiment has a sufficient chance of detecting that size of effect (EFSA, 2010a).

- Barriuso et al. (2012) investigated the potentially cumulative effect of continuous maize MON 810 (DKC6451YG) cultivation on rhizobacterial communities over a four-year period. The authors amplified partial bacterial 16S rRNA genes (including the V6 region) from bacterial DNA directly extracted from rhizosphere samples of the field planted to maize MON 810. The PCR products, which had a length of approximately 80 bp and which covered only approximately 5% of the whole gene, were sequenced by 454 titanium pyrosequencing. Each sample was composed of approximately 2,000 to 3,000 sequences with no experimental replicates of maize MON 810 and the near-isogenic control, DKC6450. However, a total of eight samplings were conducted at several growth stages of the maize over a period of four subsequent years. The taxonomic composition of the bacterial communities was similar to those found on other studies. The short sequence length of 80 bp, however, did not allow a sensitive resolution of the bacterial diversity beyond a family-genus level. Significant differences of the bacterial diversity between *Bt*- and non-*Bt*-maize were not detected, in contrast to differences caused by growth stages or year of cultivation (see also Baumgarte and Tebbe, 2005; Griffiths et al., 2007). In summary, this study gave no indication for direct effects of Cry1Ab, or indirect effects of maize MON 810 due to potentially altered root exudation, on maize rhizobacterial communities when compared with those of the non-*Bt*-maize.

- Dutra et al. (2012) investigated whether the generalist predator *Harmonia axyridis* displays a preference between prey larvae of the pest species fall armyworm (FAW, *Spodoptera frugiperda*) fed maize MON 810 or non-*Bt*-maize. A laboratory choice-test feeding studies was conducted to determine if *H. axyridis* shows any preference between FAW larvae fed *Bt*- and non-*Bt*-maize leaves. The predators were third instar larvae and female adults of *H. axyridis*. Ten and fifteen larvae of each prey type were offered to third instar and adult predators, respectively. FAW larvae that fed on non-*Bt*-maize leaves were significantly larger than those fed *Bt*-maize leaves, indicating a sublethal effect of Cry1Ab on FAW larvae, as has been previously reported by other authors (e.g., Sanders et al., 2007; Mendes et al., 2011). In all combinations of predator stage and prey age, the number of each prey type consumed did not differ significantly. ELISA measurements confirmed the presence of Cry1Ab in leaf tissue (23-33 mg/g dry weight), FAW (2.1-2.2 mg/g) and *H. axyridis* (0.01-0.2 mg/g). The results indicate that the Cry1Ab protein did not accumulate but was strongly diluted when transferred through trophic interactions. Comparable dilution levels have been reported from other laboratory studies assessing tritrophic interactions between *Bt*-plants, lepidopteran larvae and coccinellid predators (e.g., Li and Romeis, 2010; Álvarez-Alfageme et al., 2011; Devos et al., 2012; Tian et al., 2012). Based on these results, the authors concluded that both

H. axyridis adults and larvae show no preference between prey types fed either *Bt*- or non-*Bt*-maize. The lack of preference between prey types could act in favour of IRM strategies relying on seed blends (Section 6.2.1), assuming that the behaviour of pest and predator in the laboratory tests accurately reflects that under field conditions.

- Habuštová et al. (2012) performed a field study with maize MON 810 for three consecutive years in the Czech Republic, and compared the abundance and diversity of plant-dwelling arthropods between maize MON 810 and non-*Bt*-maize. Whole plants were collected (ten plants from each of five replicated plots of 0.5 ha) and specimen identified in the laboratory. CANOCO analysis was performed. Diversity measures were also analysed and an assessment was made using statistical methods to confirm that the sampling effort was adequate for the analyses performed. The authors found no significant differences between the *Bt*- and non-*Bt*-maize treatment, and concluded that *Bt*-maize does not affect plant-dwelling insects. The EFSA GMO Panel assumes that the conclusion from the authors can only be supported for the most abundant species for which there was sufficient statistical power to detect differences in abundance.

- Hansen et al. (2012) investigated the effect of maize MON 810 on the stored product pest, *Sitophilus zeamais* and its parasitoid, *Lariophagus distinguendus*, under laboratory conditions. Weevils were not harmfully affected by the maize MON 810 diet in terms of emergence or development time, but females emerging from *Bt*-maize kernels were larger. In contrast, 40% fewer parasitoid females emerged from weevil larvae that had consumed *Bt*-maize kernels, but they were not different in size or body mass compared to parasitoids emerging from hosts fed non-*Bt*-maize. Hansen et al. (2012) concluded that these effects cannot be explained by the known lepidopteran-specific toxicity of Cry1Ab. Even though the experimental design enabled the identification of an effect, a cause-effect relationship could not be determined. It is also known that parasitoids are more sensitive than predators to host/prey-mediated effects, but no apparent effects on the quality of hosts reared on *Bt*-maize were observed by Hansen et al. (2012). In terms of ERA, the relevance of the results for biological control could not be evaluated by the EFSA GMO Panel; no data are available about the use of *L. distinguendus* or other parasitoids in the biological control of coleopteran pests in stored maize in the EU. Usually, more traditional pest management methods are used under storage conditions.

- Kim et al. (2012) studied the potential impact of Cry1A-containing maize grains ground to powder on the survival and growth of the mealworm beetle *Tenebrio molitor*, which is a stored-product pest. In addition, Cry1A concentrations were measured in *T. molitor*. *T. molitor* fed *Bt*-maize had a slightly increased survival rate and head capsule width compared with non-*Bt*-maize, suggesting that Cry1A was not toxic to this species. ELISA measurements indicated the presence of Cry1A in the gut of *T. molitor* larvae after being fed *Bt*-maize, confirming Cry1A uptake by larvae. No Cry1A was detected in the hemolymph of larvae, suggesting little or no exposure of Cry1A via *T. molitor* to higher endoparasitoids.

- Meissle et al. (2012b) investigated whether the cereal leaf beetle (CLB, *Oulema melanopus*) is susceptible to Cry1Ab and Cry3Bb1 expressed in *Bt*-maize events MON 810 and MON 88017 under laboratory conditions, respectively, and whether CLB ingests *Bt*-toxins when feeding on *Bt*-maize leaves. CLB is a common pest of small grain cereals and grasses, and is present in Europe (Meissle et al., 2012a). Feeding experiments with Cry1Ab-containing leaf material were performed with CLB larvae. No difference in larval survival was found between larvae fed leaf material of maize MON 810 or non-*Bt*-maize. In contrast, exposure to Cry3Bb1 affected neonate larvae of *O. melanopus*. Statistical differences for the number of larvae and prepupae were observed among conventional varieties. Overall, the amount of eaten leaf material, development time to prepupal stage, and prepupal weight did not differ between CLB fed *Bt*-maize and those fed non-*Bt*-maize. ELISA measurements indicated that the Cry1Ab concentration in larvae reared on *Bt*-maize was 30% higher than that in leaves. Faeces contained 17% of the Cry1Ab concentrations in larvae, most likely due to digestion and microbial degradation. The lowest Cry1Ab concentrations were found in prepupae, with values lower than 3% compared with the feeding larvae. This is most likely because

larvae empty their guts before pupation and consequently excrete most *Bt*-containing plant material. The observed results confirm that Cry1Ab does not affect *O. melanopus* under laboratory conditions. The observed effects on neonate larvae exposed to Cry3Bb1-containing plant material demonstrate that the experiment was able to detect potential effects. For future tritrophic studies *O. melanopus* larvae might be a suitable test organism due to the reported accumulation of Cry proteins in larvae.

- Pérez-Hedo et al. (2012) determined the fate of Cry1Ab ingested in sublethal concentrations by two non-target lepidopteran species, true armyworm (TAW, *Mythimna unipuncta*) and CEW_a. Both species can occasionally cause severe damage to maize, and therefore are considered pests in Europe (EFSA, 2009a, 2012d; Meissle et al., 2012a). Cry1Ab concentrations were measured in tissues of the larvae fed lyophilized leaves of maize MON 810, as well as the recovery of larvae after exposure to *Bt*-maize for different periods. In addition, morphological effects of Cry1Ab on the midgut of TAW were assessed by light and transmission EM. The authors reported that multiple mechanisms could be involved in the low susceptibility of TAW and CEW_a to Cry1Ab. The low content of the Cry1Ab within the peritrophic membrane 48 hours after ingestion indicates a high rate of Cry1Ab elimination in the peritrophic membrane, a fast Cry1Ab excretion, or both. TAW larvae fed *Bt*-maize displayed a similar growth gain index to those fed non-*Bt*-maize, and showed an increasing elimination rate during the experiment. Little Cry1Ab reached the midgut epithelium, indicating a low permeability of the peritrophic membrane, a low affinity at the binding sites, or high activity of the digestive proteases. TAW and CEW_a larvae fed *Bt*-maize showed rapid recovery in weight gain and in the midgut epithelium, and also showed overcompensation mechanisms. Even though the results do not unequivocally explain why TAW and CEW_a have a lower susceptibility to Cry1Ab than ECB and MCB, they suggest that either only low concentrations of Cry1Ab reach the midgut epithelium, or that there was low affinity of potential binding sites in the gut epithelium for the Cry1Ab protein.

- Romeis et al. (2012) is a response to Hilbeck et al. (2012). The two sets of authors have previously performed lower-tier studies with the predatory two spotted ladybird beetle *Adalia bipunctata* (Schmidt et al., 2004, 2009; Álvarez-Alfageme et al., 2011), and criticised the experimental design of each other's studies (Álvarez-Alfageme et al., 2011; Hilbeck et al., 2012; see Appendix B for further details). In their response to Hilbeck et al. (2012), Romeis et al. (2012) defend the experimental design of their direct feeding bioassay as being more realistic in terms of exposure to Cry1Ab compared with the approach used by Hilbeck et al (2012), and point to some weaknesses in the experimental design followed by Hilbeck et al (2012). In addition, Romeis et al. (2012) commented on the results of two additional publications reporting no adverse effects of Cry1Ab on the same predator in a direct feeding bioassay (Porcar et al., 2010) and a tritrophic feeding bioassay (Álvarez-Alfageme et al., 2011). The observations reported by Hilbeck et al. (2012) and other publications listed above were previously evaluated by the EFSA GMO Panel (EFSA, 2009a, 2012b). The EFSA GMO Panel concluded that the data reported by Schmidt et al. (2004, 2009) and Hilbeck et al. (2012) were not sufficient to clearly identify a hazard, or to indicate a new mode of action of Cry1Ab on *A. bipunctata*. Further, like Hilbeck et al. (2012) and Romeis et al. (2012), the EFSA GMO Panel considered it unlikely that coccinellid larvae will be exposed to biologically relevant amounts of Cry1Ab from maize MON 810. The Cry1Ab protein content in maize MON 810 pollen (which is likely to be the most common source for possible Cry1Ab ingestion for *A. bipunctata*) is very low and ranges between 1-97 ηg/g fresh weight (Nguyen and Jehle, 2007; EFSA, 2009a). In addition, Cry1Ab is normally absent in aphids feeding on *Bt*-maize (Head et al., 2001; Raps et al., 2001; Romeis and Meissle, 2011), which constitute the main diet of many coccinelid larvae and therefore this alternative route of exposure to Cry1Ab from maize MON 810 can be considered negligible. Moreover, higher-tier studies available in the literature reported no adverse effects of *Bt*-maize (different events) on a range of coccinellid species (Marvier et al., 2007; Naranjo, 2009). Therefore, the EFSA GMO Panel reiterates that the risk of maize MON 810 to ladybirds is considered to be negligible.

- Székács and Darvas (2012) reviewed recent publications to assess the potential impact that maize MON 810 may have on the environment if it was to be grown in the Pannonian Biogeographical Region (EU). The authors reported very few new data specific to non-target organisms that had been not previously considered by the EFSA GMO Panel (EFSA, 2008b). Further, the EFSA GMO Panel considers there is a limited selection of publications that the authors choose to cite in their opinion paper. The only new data presented by Székács and Darvas (2012) are mortality values of the non-target Lepidoptera *Nymphalis io* (syn. *Inarchis io*) larvae exposed to maize MON 810. The EFSA GMO Panel previously assumed the Cry1Ab content in maize MON 810 pollen to be 1-97 (90) ng/g (EFSA, 2009a), while Székács and Darvas (2012) reported values of 101 and 109 ng/g for four of their six mortality estimates. Székács and Darvas (2012) estimates of mortality were highly variable and different to the values of 10-35 ng/g reported in the data package submitted to EFSA previously (EFSA, 2008b). At the four replicates at concentration levels of 101 and 109 ng/g, they reported a geometric mean of about 20% mortality when geometric mean pollen concentrations were about 582 grains/cm². This value is considerably greater than EFSA's assumed mortality-dose relationship, which would give a mortality estimate of about 8%. Additional differences concerning the protein concentrations (fresh weight versus dry weight as a basis) and the age of the larvae (L1 versus L5 larval stage) were identified between the values used by EFSA (2010) and Székács and Darvas (2012). The discrepancy in Cry1Ab content in maize MON 810 pollen and mortality values cannot be resolved, as Székács and Darvas (2012) do not provide further details about the experimental design and methodology used. The EFSA GMO Panel based its modelling on expression data provided by the applicant and Nguyen and Jehle (2007). In addition, the usefulness of the Perry et al. (2010) model on the mortality-dose relationships regarding maize MON 810 was clarified by Perry et al. (2011), and therefore the EFSA GMO Panel did not identify any new information in the Székács and Darvas (2012) publication that would raise safety concerns.

- Verbruggen et al. (2012) analysed the diversity of the fungal mycorrhizal communities of maize MON 810 in two genetic backgrounds (Monumental and DKC3421YG) and their near-isogenic comparators in greenhouse pot experiments with three replicates for each cultivar. A cultivation independent approach based on the PCR amplification of fungal rRNA genes from directly extracted nucleic acids (total DNA, total RNA) of the rhizosphere was applied. As RNA is very short lived, data on RNA were related to the active part of the fungal community, while data on DNA were more related to the general occurrence. The study demonstrated that under the given conditions of maize cultivation a significant proportion (8-21%) of the fungal rRNA and their corresponding genes originated from mycorrhizal fungi, confirming their quantitative importance for the overall microbial community of maize. Temporal variation in the mycorrhizal community was bigger than differences between the maize MON 810 cultivars and non-*Bt*-maize. There were no consistent differences between the communities associated with maize MON 810 and non-*Bt*-maize. Further, the authors reported that natural variation of the communities across fifteen agricultural in the Netherlands, as well as within field variation, was much higher than the variation induced by maize MON 810. Despite the methodologically innovative approach, which was performed to increase the sensitivity of the analyses in comparison to previous studies, there was no indication that maize MON 810 would cause adverse effects on mycorrhiza.

6.1.4.3. Conclusion

The EFSA GMO Panel considers that conclusions about potential adverse effects of maize MON 810 and the Cry1Ab protein it expresses on non-target organisms and the ecosystem services they provide and/or routes of exposure to plant-produced *Bt*-toxins can be drawn from the studies by Peterson et al. (2009), García et al. (2010), Lumbierres et al. (2011), Shu et al. (2011), Tan et al. (2011), Barriuso et al. (2012), Dutra et al. (2012), Hansen et al. (2012), Meissle et al. (2012b), Pérez-Hedo et al. (2012) and Verbruggen et al. (2012), and that the conclusions reached by the authors are supported by the data.

The EFSA GMO Panel considers that the conclusions of Habuštová et al. (2012) are only partially supported by the data, so that conclusions can be drawn only for some of the species studied.

The EFSA GMO Panel considers that no conclusions about potential adverse effects of maize MON 810 and the Cry1Ab protein it expresses on non-target organisms and the ecosystem services they provide can be drawn from the studies by Balog et al. (2010), Bakonyi et al. (2011), Alcantara (2012) and Kim et al. (2012), owing to the limitations in these studies.

The EFSA GMO Panel reiterates that in its guidelines for the ERA of GM plants it does not recommend the use of diversity indices for general risk assessment in pre-commercialisation studies. It is most unlikely that studies will yield sufficient samples of individuals to characterise indices adequately or that a sufficient degree of ecological background information will exist to give confidence that biodiversity can be represented adequately as a single number (EFSA, 2010a). By contrast, multivariate approaches may be useful, especially for summarising data and for analysing principal response curves (Perry et al., 2009).

Results reported by Pirondini and Marmiroli (2008), Peterson et al. (2009), García et al. (2010), Lumbierres et al. (2011), Shu et al. (2011), Tan et al. (2011), Viktorov (2011), Yu et al. (2011), Andow and Lövei (2012), Shelton et al (2012), Barriuso et al. (2012), Dutra et al. (2012), Habuštová et al. (2012), Hansen et al. (2012), Meissle et al. (2012b), Pérez-Hedo et al. (2012), Romeis et al. (2012), Székács and Darvas (2012) and Verbruggen et al. (2012) do not provide new information that would invalidate the previous conclusions on interaction of maize MON 810 with non-target organisms made by the EFSA GMO Panel. Therefore, the EFSA GMO Panel considers that its previous conclusions on maize MON 810 remain valid and applicable.

6.1.5. Effects on human and animal health

The potential of maize MON 810 to have adverse effects on human and animal health was previously evaluated by the EFSA GMO Panel (EFSA, 2009a, 2011d).

6.1.5.1. Summary of previous conclusions by the EFSA GMO Panel

The molecular analysis, comparative analysis and the food and feed safety assessment of maize MON 810 did not raise safety concerns for human and animal health. Therefore, in its previous Scientific Opinions on maize MON 810 (EFSA, 2009a, 2011d), the EFSA GMO Panel concluded that: "*maize MON 810 is as safe as its conventional counterpart with respect to potential effects on human and animal health*" (EFSA, 2009a).

6.1.5.2. Results from the literature search

See sections 3, 4 and 5 for further details.

6.1.5.3. Conclusion

See sections 3, 4 and 5 for further details.

6.1.6. Interactions with biogeochemical processes and the abiotic environment

In its 2009 Scientific Opinion for the continued marketing of maize MON 810, the EFSA GMO Panel considered the possible environmental exposure to the Cry1Ab protein that can be introduced into the soil via physical damage to plant tissues, via decomposition of shed root cells during plant growth, via decomposing plant residues remaining in fields after harvest, which might be incorporated into the soil during tillage operations, and possibly via root exudates (EFSA, 2009a).

6.1.6.1. Summary of previous conclusions by the EFSA GMO Panel

In its 2009 Scientific Opinion for the continued marketing of maize MON 810, the EFSA GMO Panel considered that: "*potential effects on soil microorganisms and microbial communities due to maize MON 810 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*" (EFSA, 2009a).

6.1.6.2. Results from the literature search

From the literature search, the following seven new scientific publications containing evidence specific to maize MON 810 for this specific area of risk were identified and scrutinised for their possible relevance for the ERA of maize MON 810:

- Daudu et al. (2009) investigated the decomposition rate of leaf and stem residues of maize MON 810 and non-*Bt*-maize in litterbags under field conditions in the central region of the Eastern Cape (South Africa) over a period of 112 days, as well as potential changes of the C and N contents of these plant residues. The experiment consisted of a split plot in a randomised complete block design with maize MON 810 or its conventional counterpart as the main plot treatments, replicated three times. No differences between *Bt*- and non-*Bt*-maize were observed in decomposition of the plant residues in the litterbags. Percent ash-free dry mass, and N and C contents decreased over time and varied between leaves and stems (e.g., decomposition of leaf residues was significantly faster than that of stems), but no differences were observed between residues of *Bt*- and non-*Bt*-maize. The higher decomposition rates of leaf than stem residues were attributed by the authors to differences in composition, with stem residues having a higher C/N ratio than leaf residues. The Cry1Ab protein concentration was reported to be higher in leaves than in stems, but in both cases it declined to <0.09% of initial levels in maize MON 810 14 days after the placement of litterbags. The Cry1Ab protein increased by up to 25% of the initial levels for maize MON 810 between 14 and 56 days, after which it decreased to undetectable levels (2 ng/g) by the end of the experiment (112 days after the placement of litterbags). No transfer of Cry1Ab from decomposing plant material to soil was detected. The results indicated that residues of maize MON 810 degraded at rates comparable to those of non-*Bt*-maize residues, and that the persistence of the free Cry1Ab protein in soil is minimal (see below).

- Badea et al. (2010) studied the fate (time-dependent degradation) of Cry1Ab-containing plant material incorporated into the soil of pots under greenhouse conditions. The experiment contained eight replicated pots for each soil type (four for maize MON 810 (DKC5784YG) and four for non-*Bt*-maize (DKC5783); three different soil types, namely eutric aluvio soil, eutricambi soil and cernozem cambic soil). The organic matter content of the soils was 1.8, 2.5 and 3.4%, and the clay content was 19.7, 34.0, and 39.3%, respectively. The level of Cry1Ab protein was determined using ELISA assay, with a threshold detectable concentration of 0.01 ng/g soil. The greenhouse study showed only sporadic detection of low amounts of Cry1Ab in soil samples following incorporation of plant residues, and gave no indication for increasing concentrations (accumulation) during the growth of the plants. The authors found no difference in the degradation time of plant debris between *Bt*- and non-*Bt*-maize. The average Cry1Ab concentrations in soil increased after incorporation of *Bt*-maize plant material into the pots, with the Cry1Ab concentration peaking at about six to nine weeks after incorporation, but declining slowly towards the 12-15 week (3-4 months) sampling interval. Overall, the results by Badea et al. (2010), though limited to pot experiments, support the previous conclusion that Cry1Ab does not persist or accumulate in different soil types (Hopkins and Gregorich, 2003, 2005; Baumgarte and Tebbe, 2005; Dubelman et al., 2005; Andersen et al., 2007; Hönemann et al., 2008; Icoz and Stotzky, 2008; Icoz et al., 2008; Gruber et al., 2011).

- Tan et al. (2010) investigated whether the cultivation of Cry1Ab-expressing maize events (Pioneer 34B24 and Nongda 1246*1428) or exposure to plant residues (leaves and stalks) of the Cry1Ab-expressing maize events (Pioneer 34B24 and Nongda 61) adversely affected community structures of bacteria and fungi under greenhouse conditions. Neither the actively growing *Bt*-maize nor its straw had any consistent apparent effect on the soil bacteria and fungi community structure. The age of the growing plants, or the timing of plant straw decomposition had more effect on the microbial community than other factors (i.e., the presence of Cry1Ab, plant variety). The authors concluded that *Bt*-maize plants and residues had no apparent lasting effect on soil bacterial and fungal communities.

- Raubuch et al. (2010) studied the decomposition of plant material from two maize MON 810 cultivars and two corresponding non-*Bt*-maize comparators in laboratory soil incubations. Respiration of soils, microbial biomass C and contents of ATP, ADP and AMP were measured during three weeks of incubation. Due to this relatively short period of monitoring, it can be assumed that the study analysed the first stage of decomposition in which typically the easily available nutrients (simple carbohydrates, amino acids, etc.) are degraded. On the second and third sampling, i.e. after two and four days, the authors found significantly elevated respiration in samples amended with both maize MON 810 cultivars in comparison to non-*Bt*-maize. Respiration rates at the other six sampling occasions were not different. On one sampling occasion, after two days, a difference in the ATP contents of the soils was also found between those amended with one maize MON 810 cultivar (Valmont) and its control (Prelude), which was not detected between the other maize MON 810 cultivar (Novelis) and its control (Nobilis). On the other seven samplings, there were no differences between *Bt*- and non-*Bt*-maize, or between the different cultivars. Data on the chemical properties of the different plant residues used in this study to amend the soils indicate a 20 to 80% higher sugar contents for both maize MON 810 cultivars, which may explain the increased respiration rates and the observation that this increased activity was not translated to microbial biomass. The overall data of this study indicate that the overall decomposition of residues of *Bt*- and non-*Bt*-maize is similar, but that minor differences in the kinetics related to variation in the easily degradable substrates may occur during the initial decomposition stages.

- Yanni et al. (2010) reviewed available studies that assessed the C input from residues in maize agroecosystems and that compared chemical characteristics and decomposition rates of *Bt*-maize residues with those of non-*Bt*-maize residues, but did not report new data that had not been previously considered by the EFSA GMO Panel.

- Emmerling et al. (2011) studied the fate of the Cry1Ab protein in different gut compartments of the detritivorous earthworm species, *Lumbricus terrestris*, after ingestion of maize MON 810 litter in soil microcosm experiments. The litter loss, the earthworm biomass, and Cry1Ab concentrations and fragmentation were analysed after two weeks of exposure. Western blotting and ELISA demonstrated that Cry1Ab was degraded inside the gut generating fragmentation products of different lengths (i.e., to approximately 31 and 17.23 kDa in the foregut and midgut, with the initial molecular weight of maize material being ~65kDa). No Cry1Ab protein fragments were identified in the hindgut and cast material, and only very small amounts could be detected. Comparisons were made to microcosms without earthworms, and it was concluded that detritivorous earthworms accelerate the degradation of Cry1Ab from MON 810 maize litter.

- Viktorov (2011) did not report new data, but reviewed pathways via which plant-produced Cry1Ab proteins from *Bt*-maize can enter soil and aquatic environments, and whether this may adversely affect non-target organisms, based on available scientific literature. These routes of exposure, as well as the environmental consequences of such exposure, were previously considered by the EFSA GMO Panel.

6.1.6.3. Conclusion

Results reported by Daudu et al. (2009), Badea et al. (2010), Tan et al. (2010), Raubuch et al. (2010), Yanni et al. (2010), Emmerling et al. (2011) and Viktorov (2011) do not provide new information that would invalidate previous conclusions on interactions of maize MON 810 with biogeochemical processes and the abiotic environment made by the EFSA GMO Panel. Therefore, the EFSA GMO Panel considers that its previous conclusions on maize MON 810 remain valid and applicable.

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

The consequences of changes in crop management practices associated with maize MON 810 were previously evaluated by the EFSA GMO Panel (EFSA, 2009a).

6.1.7.1. Summary of previous conclusions by the EFSA GMO Panel

In its 2009 Scientific Opinion for the continued marketing of maize MON 810, the EFSA GMO Panel concluded that: "*no new specific cultivation practices, management or harvesting techniques are associated to the cultivation of maize MON 810. The only difference between maize MON 810 and its conventional counterpart is due to fewer insecticide treatments needed to control lepidopteran target pests such as* O. nubilalis *and* S. nonagrioides" (EFSA, 2009a).

6.1.7.2. Results from the literature search

From the literature search, no new scientific publications containing evidence specific to maize MON 810 for this specific area of risk were identified.

6.1.7.3. Conclusion

In the absence of new scientific evidence specific to maize MON 810 for this area of risk, the EFSA GMO Panel considers that its previous conclusions on impacts of the specific cultivation, management and harvesting techniques associated with the cultivation of maize MON 810 remain valid and applicable.

6.2. Risk management strategies (including post-market environmental monitoring)

6.2.1. Risk mitigation measures

The EFSA GMO Panel previously considered that the potential risk of resistance evolution in target insect pests and that the risk of reductions in populations of certain extremely highly sensitive non-target lepidopteran species require management, and recommended the implementation of risk mitigation measures under certain conditions (EFSA, 2009a, 2011e, 2012d).

6.2.1.1. Summary of previous conclusions by the EFSA GMO Panel

In its 2009 Scientific Opinion for the continued marketing of maize MON 810, the EFSA GMO Panel concluded that: "*resistance management strategies continue to be employed*", and advised 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*" (EFSA, 2009a).

For target insect pests, the EFSA GMO Panel indicated that: "*even though no resistance has been reported for maize MON 810 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*". Therefore, the EFSA GMO Panel advised 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*" (EFSA, 2009a).

For non-target Lepidoptera, the EFSA GMO Panel considered 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*" (EFSA, 2009a).

In its Statement on maize Bt11 (whose conclusions on non-target Lepidoptera are equally applicable to maize MON 810), the EFSA GMO Panel indicated that: "*subject to the implementation of appropriate risk mitigation measures, the identified risks of maize Bt11 cultivation on non-target Lepidoptera can*

be reduced to a level of no concern. Special attention should be paid to the degree of large-scale exposure as risk mitigation measures are only needed when the proportion and uptake of maize Bt11 (and/or other Lepidoptera-resistant maize events such as maize MON 810 currently grown in the EU) are sufficiently high, regardless of the other parameters. If maize Bt11 (and/or maize MON 810) cultivation remains below 7.5% of the regional Utilized Agricultural Area[5,6] *(see www.oecd.org/), the global mortality is predicted to remain below 1%, even for 'extremely sensitive' species, and then risk mitigation measures using non-Bt-maize border rows are not required*" (EFSA, 2011e).

Recently, the EFSA GMO Panel further supplemented its previous recommendations for risk mitigation measures by reapplying the mathematical model developed by Perry et al. (2010, 2011, 2012), in order to consider additional hypothetical agricultural conditions and to provide additional information on the factors affecting the IRM strategy. The EFSA GMO Panel reiterated that: "*risk mitigation measures can appropriately delay resistance evolution in target Lepidoptera, and reduce the identified risks of maize MON 810 cultivation to a level of no concern for non-target Lepidoptera*" (EFSA, 2012d).

6.2.1.2. Results from the literature search

From the literature search, the following nine new scientific publications containing evidence specific to maize MON 810 for this specific area were identified and scrutinised for their possible relevance for the mitigation of maize MON 810:

- Prasifka et al. (2009) studied the larval behaviour of Cry1Ab-S and Cry1Ab-R ECB strains and their hybrid (F_1) progeny (Cry1Ab-H) after exposure to diets containing maize MON 810 plant material (Pioneer 34N44) under laboratory conditions. In no-choice tests, Cry1Ab-R (and usually Cry1Ab-H) larvae were less likely to be irritated after exposure to Cry1Ab-containing diet and hence to move away than Cry1Ab-S larvae. Likewise, choice tests indicated that Cry1Ab-R (and sometimes Cry1Ab-H) larvae were more likely to be found on diet with Cry1Ab than Cry1Ab-S neonates. The authors concluded that the differences in behaviour are due to reduced physiological susceptibility to Cry1Ab instead of a behavioural component to resistance. The findings reported by Prasifka et al. (2009) contribute to a better understanding of the behaviour of ECB, which is considered an important factor to consider when defining appropriate IRM strategies to delay resistance evolution.

- Wu et al. (2009) assessed whether fitness costs are associated with Cry1Ab resistance in different laboratory-selected SCB strains. Larval growth and development of Cry1Ab-S, Cry1Ab-R, Cry1Ab-H (two strains), and a back-crossed and reselected resistant strain (Cry1Ab-R') fed an artificial diet containing Cry1Ab or not under laboratory conditions or reared on non-*Bt*-maize plants under greenhouse conditions were analysed. Larvae of Cry1Ab-S and Cry1Ab-R' grew normally on a diet without Cry1Ab and on non-*Bt*-maize plants, and no significant differences were observed between the two strains in the remaining measured endpoints. Except for the development time on the non-Cry1Ab-containing diet, all other parameters on both the non-Cry1Ab-containing diet and non-*Bt*-maize plants were similar among the five genotypes. Larval development of Cry1Ab-S was significantly affected when fed a Cry1Ab-containing diet; in contrast, larval development of Cry1Ab-R and Cry1Ab-R' was not significantly affected when fed a Cry1Ab-containing diet. Pupal weight and sex ratio reared on Cry1Ab-diet were similar and there were no significant differences among the five genotypes. Neonate-to-pupation rate decreased as Cry1Ab concentrations increased but the decrease was more significant for Cry1Ab-S than for the other four genotypes. Results of this study show that no major fitness costs in larval growth and development are associated with Cry1Ab resistance in SCB. Larval growth, development, and survival, pupation rate, larval and pupal weight, and sex ratio of SCB fed a non-Cry1Ab-containing diet or reared on non-*Bt*-maize plants were similar among the five genotypes with few exceptions.

[5] For example, a maximum uptake of 25% of maize Bt11 (and/or maize MON 810) in a region where maize represents 30% or less of the arable land

[6] I.e., zv = 0.075, and with conservative assumptions for the other parameters y=a=x=0.5, yielding R = 0.009375

Because the above data indicate that fitness costs are not necessarily associated with resistance to Cry1Ab in SCB, the EFSA GMO Panel considers it prudent to infer that fitness costs may not help to substantially delay SCB resistance.

- Crespo et al. (2010) assessed fitness costs associated with Cry1Ab resistance in two laboratory-selected ECB strains with Cry1Ab resistance. Cry1Ab-R larvae exhibited reduced pupal weight and increased developmental time compared with Cry1Ab-S and Cry1Ab-H larvae derived from reciprocal crosses of resistant and susceptible parents. Resistant adult individuals exhibited a higher proportion of unsuccessful matings and lower fertility than the susceptible ones. These results suggest that significant fitness costs are associated with resistance to Cry1Ab in ECB in both resistant strains tested, which could delay resistance evolution. However, results did not indicate strong evidence of fitness costs in the Cry1Ab-H larvae, though a slight but not significant reduction in some demographic parameters was observed. How the selection for Cry1Ab resistance in ECB under artificial conditions may affect the performance of the hybrid progeny in the field remains unclear, as other aspects of the insect biology, including diapause, pheromone response, flight capacity, mating competition, and first-male paternity, were not assessed in this study.

- Lopez et al. (2010) examined the extent with which the entomopathogenic microsporidium *Nosema pyrausta* alone or in combination with Cry1Ab exposure decreases the survival and delays larval development of Cry1Ab-R and Cry1Ab-S ECB. Entomopathogens can serve as biological control agents and affect fitness costs associated with resistance. Theoretically, treating refuges with entomopathogens for the target insect pest could magnify fitness costs and be useful to delay resistance evolution (Gassmann et al., 2009). However, a concern is the non-synchronous emergence of ECB from refuge and *Bt*-maize fields caused by feeding on *Bt*-maize or infection with *N. pyrausta*, as this could result in non-random (assortative) mating and cause an increase in the rate of resistance evolution. Greater larval delays of Cry1Ab-R ECB feeding on *Bt*-maize could lead to temporal isolation from adults emerging from refuge maize. Feeding on Cry1Ab-containing diet significantly increased number of days from hatch to pupation and decreased survival in Cry1Ab-R ECB under laboratory conditions. Infection with *N. pyrausta* increased mortality and lengthened development in both the resistant and susceptible populations. The combination of Cry1Ab-containing diet and infection with *N. pyrausta* in Cry1Ab-R ECB lengthened development and increased mortality to a greater extent than either factor alone. Compared with uninfected Cry1Ab-R ECB feeding on Cry1Ab-containing diet, survival of Cry1Ab-R ECB infected with *N. pyrausta* on Cry1Ab-containing diet was decreased dramatically (approximately 4%). If similar patterns to those observed under laboratory conditions occurred under field conditions, then the authors considered that infection with *N. pyrausta* would compromise the ability of Cry1Ab-R larvae to survive to adulthood on *Bt*-maize and to confer resistance to the next generation, which would slow down the rate of resistance evolution.

- Tamez-Guerra (2010) reviewed achievements of a cooperation between the USA and Mexico to develop IRM measures to delay resistance evolution in target pests in Mexico, but did not report new data that had not been previously considered by the EFSA GMO Panel.

- Prasifka et al. (2010) studied the behaviour and survival of ECB larvae with different genotypes (Cry1Ab-R, Cry1Ab-S and Cry1Ab-H) after exposure to maize MON 810 plant material (Pioneer 34N44) in feeding bioassays. On diet containing maize MON 810, all genotypes moved greater total distances, spent more time moving, more time away from the maize MON 810-containing diet and displayed less turning per unit moved (meander) relative to the control diet. Compared with Cry1Ab-S or Cry1Ab-H larvae, Cry1Ab-R larvae moved a lower total distance and displayed more meandering. However, when placed onto maize MON 810 plants, a greater percentage of Cry1Ab-R larvae moved onto adjacent non-*Bt*-maize plants compared with Cry1Ab-S ones. Resistant larvae were also more likely to survive the 48-72 h period between hatching and the dissection of plants. The difference in on-plant dispersal seems to reflect greater survival after toxin exposure for resistant larvae rather than increased activity. Based on the results, the authors concluded that simplified Petri dish tests may not be predictive of larval movement between *Bt*- and non-*Bt*-maize

plants. The results also indicate that even though Cry1Ab-R larvae moved more slowly, on average they survived long enough to disperse onto adjacent non-*Bt*-maize plants. The potential for larval movement between *Bt*-maize and refuge plants, and the exposure of later instars to sublethal doses of *Bt*-toxins may reduce the efficacy of IRM strategies relying on seed blends (also termed seed mixtures or refuge in a bag), as these factors may lower the selective differential between susceptible and resistant genotypes, and increase the effective dominance of resistance by producing more heterozygote individuals (Mallet and Porter, 1992; Goldstein et al., 2010; Onstad et al., 2011). The findings reported by Prasifka et al. (2010) will be useful in evaluating the efficacy of seed blends for *Bt*-maize and ECB as refuge strategy for managing ECB resistance in *Bt*-maize (see also Razze et al., 2011; Razze and Mason, 2012) . The EFSA GMO Panel previously indicated that seed blends would not be an appropriate strategy for managing resistance evolution when *Bt*-maize events express a single *Bt*-toxin and are truly high dose, and/or when larval movement of the target insect pests is substantial (EFSA, 2012a; Siegfried and Hellmich, 2012).

- Pérez-Hedo et al. (2011) determined the effect of the ingestion of sublethal amounts of the Cry1Ab protein on the development and hormonal balance in larvae of the MCB. To simulate a seed blend scenario where larvae may be exposed to sublethal Cry1Ab concentrations under field conditions, diapausing and non-diapausing larvae were either fed maize MON 810 leaves, or exposed to different Cry1Ab concentrations in their diet. The levels of juvenile hormone (JH) and ecdysteroids, and a number of parameters of the development of the larvae were compared. MCB larvae surviving sublethal exposure to Cry1Ab had higher levels of JH, whereas their level of ecdysteroids did not increase sufficiently to allow pupation. In non-diapausing larvae, the higher levels of JH led to a longer larval development and more larval moults. The authors considered this response a defence mechanism that allows some larvae to survive *Bt*-toxin ingestion. In diapausing larvae, the number of moults increased due to feeding on *Bt*-maize, but the duration of development did not increase. Further, changes in the hormone levels in diapausing larvae were undetectable, most likely due to the higher level of JH in the haemolymph of diapausing larvae, which may have masked these changes, and due to a lack of ecdysteroid titer increase. The higher JH concentration together with the lower ecdysteroid concentration may favour longer larval development and a higher number of larval moults, and may allow some developed larvae to recover from ingestion of the *Bt*-toxin, and to pupate and produce viable adults. The findings reported by Pérez-Hedo et al. (2011) will be useful in evaluating the efficacy of seed blends for *Bt*-maize and MCB as refuge strategy for managing MCB resistance in *Bt*-maize (see also Prasifka et al., 2010, above).

- Razze et al. (2011) assessed the feeding behaviour of neonate ECB on maize MON 810 and a conventional (near-isogenic) counterpart (Pioneer 33D31) during the first 48 hours after egg eclosion. Feeding experiments revealed that there was significantly less feeding on *Bt*-maize compared with non-*Bt*-maize. A higher quantity of plant material was found in the gut of larvae recovered from leaves of non-*Bt*-maize compared with *Bt*-maize. At the end of 48 hours among the larvae that had left the plant, a greater proportion from *Bt*-maize had plant material in the gut than did those from non-*Bt*-maize. The authors also found that >50% of the larvae initially dispersed from their natal plant, whether it was a *Bt*- or non-*Bt*-maize plant, with no evidence of feeding prior to movement; they left the plant before there was evidence in the gut of feeding. These results indicate that ECB neonates on *Bt*-maize are more likely to disperse than those on non-*Bt*-maize after initial feeding on the plant, but that >50% of the larvae are likely to disperse off their natal plant prior to feeding, regardless of its suitability as a host (Goldstein et al., 2010). The authors recommended further research to explore the relationship between larval feeding and dispersal on *Bt*-maize to understand movement between *Bt*- and non-*Bt*-maize plants under field conditions and the likelihood of survival after ingesting *Bt*-toxins, as this will allow determination of the efficacy of the efficacy of IRM strategies relying on seed blends.

- Kruger et al. (2012) compared life-history characteristics, as well as fecundity and longevity of field-collected Cry1Ab-R and Cry1Ab-S ASB. Instances of field-evolved resistance to Cry1Ab-expressing maize were previously reported for ASB in South Africa (van Rensburg 2007; Kruger et

al. 2009, 2011); larvae were able to survive on maize MON 810. Reasons for these instances of field-selected resistance ranged from the insufficient planting of refuges of non-*Bt*-maize to the assortative mating between resistant and susceptible insects. South African farmers declared non-irrigated conventional maize as refuges for irrigated *Bt*-maize, which most likely decreased random mating and egg laying, as ASB prefer high humidity (van Rensburg 2007; Kruger et al. 2011). Kruger et al. (2012) compared sex ratio, pupal mass, fecundity and longevity of field-collected Cry1Ab-R and Cry1Ab-S ASB populations under laboratory conditions. Field-collected Cry1Ab-R individuals were fed *Bt*-maize, while Cry1Ab-S ASB populations were fed non-*Bt*-maize. The authors observed slight adverse effects of *Bt*-maize on the fitness of Cry1Ab-R ASB. The sex ratio was biased towards males in some resistant populations and towards females in Cry1Ab-S populations. The Cry1Ab-R ASB population had a lower mean pupal mass, shorter longevity of moths and reduced fecundity. These results indicate a decrease of the general fitness of Cry1Ab-R ASB populations on *Bt*-maize compared to Cry1Ab-S populations on non-*Bt*-maize. However, further studies are required to assess whether fitness costs are associated with Cry1Ab resistance in ASB. Fitness costs associated with resistance occur when fitness on the non-*Bt*-crop is lower for resistant insects than the susceptible ones (Gassmann et al., 2009). As the most likely cause of instability of resistance to a *Bt*-toxin is the fitness cost associated with resistance (Tabashnik, 1994), such costs could cause declines in resistance when the selection exerted by *Bt*-maize ceases. In refuges where resistant insects are not exposed to the *Bt*-toxin, fitness costs would exert control over the frequency of resistance alleles, and delay or reverse resistance by selecting against resistant genotypes, thereby increasing the effectiveness of refuges for delaying resistance (Gould, 1998; Carrière and Tabashnik, 2001; Crowder and Carrière, 2009). Refuges would delay resistance evolution not only by providing susceptible individuals to mate with resistant individuals, but also by selecting against resistance.

6.2.1.3. Conclusion

The EFSA GMO Panel recommends caution when predicting future responses of ECB and MCB in relevant EU regions based on experiences elsewhere or with other target insect pest species, as resistance evolution is dependent upon many factors. Furthermore, caution must be exercised when extrapolating laboratory and greenhouse results with artificially-selected resistant strains to field conditions.

Results reported by Prasifka et al. (2009, 2010), Wu et al. (2009), Crespo et al. (2010), Lopez et al. (2010), Tamez-Guerra (2010), Pérez-Hedo et al. (2011), Razze et al. (2011) and Kruger et al. (2012) do not provide new information that would invalidate the previous recommendations on risk mitigation made by the EFSA GMO Panel. Therefore, the EFSA GMO Panel considers that its previous conclusions on maize MON 810 remain valid and applicable.

6.2.2. Post-market environmental monitoring

Upon request of the European Commission, the EFSA GMO Panel recently updated its previous evaluation of the initial PMEM plan for maize MON 810 (EFSA, 2009a) and made several recommendations to strengthen the PMEM plan proposed by the applicant (EFSA, 2011a,b, 2012a,d).

6.2.2.1. Summary of previous conclusions by the EFSA GMO Panel

In its 2009 Scientific Opinion for the continued marketing of maize MON 810, the EFSA GMO Panel recommended that: "*resistance management strategies continue to be employed and case-specific monitoring is conducted by the applicant under Directive 2001/18/EC*" (EFSA, 2009a). For non-target Lepidoptera, the EFSA GMO Panel considered 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*", and therefore: "*no case-specific monitoring plan for non-target Lepidoptera is deemed necessary*". Overall, the EFSA GMO Panel agreed with the general methods and approaches of the general surveillance plan, but advised 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" (EFSA, 2009a).

Upon request from the European Commission to assess the annual PMEM reports of maize MON 810 for the 2010 and 2011 growing seasons (EFSA, 2011b, 2012a) according to the updated EFSA GMO Panel Scientific Opinion on the PMEM of GM plants (EFSA, 2011a), the EFSA GMO Panel provided detailed recommendations to the applicant for the improvement of the IRM/CSM and GS of maize MON 810 (for further details, see EFSA, 2011b, 2012a). In addition, the EFSA GMO Panel confirmed from its evaluation of the PMEM results on maize MON 810 that: "*no adverse effects on the environment, human and animal health due to maize MON 810 cultivation were identified during the 2009 and 2010 growing seasons*" (EFSA, 2011b, 2012a).

6.2.2.2. Results from the literature search

From the literature search, the following six new scientific publications containing evidence specific to maize MON 810 for this specific area were identified and scrutinised for their possible relevance for the monitoring of maize MON 810:

- Huang et al. (2009) estimated the frequency of resistance alleles to Cry1Ab in SCB populations collected from the Gulf Coast area (Texas, USA). SCB is a stalk-boring pest species of sugarcane, maize and other crops, which only occurs in South, Central and North America, but not in Europe (Meissle et al., 2012a). SCB is a major target of *Bt*-maize in South America and the mid-Southern region of USA. In total, 473 two-parent field-collected family-lines of four populations were examined for Cry1Ab resistance, using F_1- and F_2-screens (see also Huang et al., 2007a, 2008). No major resistance alleles were detected in these family-lines. The estimated frequency for major Cry1Ab resistance alleles in SCB was <0.0016. Six family-lines were identified to possess minor resistance alleles. The overall frequency for minor resistance alleles in the data combined across the four populations was estimated to be 0.0037. Success of IRM relying on the high dose/refuge (HDR) strategy is aided if the initial resistance alleles are rare in the target insect pest population. The frequency should be typically <0.001, which has been taken as a default value when modelling the evolution of resistance to *Bt*-toxins (Roush 1994), so that nearly all resistance alleles are in heterozygote genotypes that are eliminated by the *Bt*-crop (Andow 2008). The results reported by Huang et al. (2009) indicate that the major Cry1Ab resistance allele frequency in SCB is low even after nine years of maize MON 810 cultivation in the Gulf Coast area of Texas, that the rare initial resistance condition of the HDR strategy is still met in this area, but that minor resistance alleles in SCB appear to occur at a higher frequency compared to other corn stalk borer species. To delay resistance evolution in SCB, it is important maize MON 810 expresses a sufficiently high dose of Cry1Ab that can kill heterozygous SCB with major resistance and homozygotes with minor resistance.

- Using dose-response bioassays, Jalali et al. (2010) measured the susceptibility of field-collected SSB, PSB and CEW_a populations from representative maize growing areas in India to Cry1Ab, and established the baseline susceptibility data for these three maize pest species to Cry1Ab. The EFSA GMO Panel considers that the development of baseline susceptibility data represents the first step toward the development of a monitoring program designed to detect changes in susceptibility that may result from repeated and prolonged exposure to *Bt*-toxins (Siegfried et al., 2000). In this study, mortality and growth inhibition were followed as endpoints. Median LC_{50} values ranged between 0.008 and 0.068 µg Cry1Ab/mL diet for 18 populations of SSB (across two seasons), between 0.12 and 1.99 µg Cry1Ab/mL for seven populations of CEW, and between 0.46 and 0.56 µg Cry1Ab/mL for two populations of PSB (for a complete overview of LC_{90}, MIC_{50} and MIC_{90} values, see full text publication). Results indicated that multiple populations of SSB, PSB and CEW_a are susceptible to Cry1Ab. Based on the baseline susceptibility data generated by Jalali et al. (2010), future variation in susceptibility of SSB, PSB and CEW_a populations to Cry1Ab and resistance evolution can be documented. Several authors (Siegfried et al., 2007; Siegfried and Hellmich, 2012) indicated that baseline susceptibility data will serve as a benchmark against which future changes in susceptibility can be measured when monitoring for the evolution of resistance.

- Tamez-Guerra (2010) reviewed achievements of a cooperation between the USA and Mexico to develop monitoring methods to survey *Bt*-crops and pest dynamics in Mexico, but did not report new data that had not been previously considered by the EFSA GMO Panel.

- Alcantara et al. (2011) quantified the baseline susceptibility of Asian corn borer (ACB, *Ostrinia furnacalis*) to Cry1Ab in field-collected populations in the Philippines, developed a diagnostic concentration for monitoring of ACB resistance to Cry1Ab-expressing maize, and used the diagnostic concentration to monitor field populations for changes in susceptibility to Cry1Ab. Results from the bioassays indicated that ACB populations are highly susceptible to Cry1Ab; the median LC_{50} for the different collections ranged from 0.42 to 2.37 ng/cm^2. Monitoring of field populations during 2009 in areas where *Bt*-maize had been grown for three years revealed some enhanced survival of neonates at the diagnostic concentration but progeny of the diagnostic concentration survivors did not survive on *Bt*-maize. Therefore, the authors concluded that ACB populations in the Philippines remain susceptible to Cry1Ab-expressing maize.

- Huang et al. (2012a) reported data from a six-year resistance monitoring program, during which the resistance allele frequency and susceptibility of SCB to Cry1Ab was investigated in Louisiana and Mississippi (USA) during 2004-2009 (see also Huang et al., 2007b, 2008). Huang et al. (2012a) collected a total of 986 SCB individuals from maize fields in six locations of Louisiana and Mississippi during 2007-2009, and examined whether Cry1Ab resistance evolved, using F_1- and/or F_2-screens (Huang et al., 2007a, 2008). Major resistance alleles to maize MON 810 in the SCB populations sampled from non-*Bt*-maize plants during 2007 and 2008 in Louisiana and 2009 in Mississippi were rare. From a total of 487 SCB individuals collected from three locations in Louisiana in 2007 and 2008, only one was identified with major resistance alleles. In addition, no major resistance alleles were detected in 242 SCB individuals collected from three locations in Mississippi in 2009. The frequency of major resistance alleles was estimated to be 0.002 for the Louisiana populations and <0.0061 for the Mississippi populations. The resistance frequency estimated for the Louisiana populations in 2007 and 2008 was not significantly different from those reported previously for SCB populations sampled in 2004-2006 (Huang et al., 2012a and references therein). However, among 200 SCB individuals sampled from non-*Bt*-maize plants in 2009 in Louisiana, six were identified to possess major resistance alleles. The estimated major resistance allele frequency for the populations sampled from non-*Bt*-maize plants in 2009 in Louisiana was 0.0176, which was significantly greater than those estimated for the populations collected in 2004-2008. Similarly, the frequency of minor resistance alleles to maize MON 810 for the Louisiana populations collected in 2009 was also significantly greater than those estimated for the populations sampled before. In addition, two out of 57 SCB individuals collected from maize MON 810 plants in Louisiana in 2009 were identified to carry major resistance alleles to Cry1Ab. The overall results from the six-year resistance monitoring program indicate that resistance allele frequency to Cry1Ab in field populations of SCB in Louisiana was low (averaged 0.0011) during 2004-2008, but that there was a significant increase in 2009. This increase in resistance allele frequency was not observed for the Mississippi populations, in spite of eleven years of cultivation of maize MON 810.

  The authors concluded that the timely switching from maize MON 810 to the pyramided *Bt*-maize will prevent further increases in Cry1Ab resistance allele frequency and thus contribute to the sustainable use of *Bt*-maize for managing SCB in the region. The pyramiding in the same plant of two or multiple *Bt*-toxins[7], acting independently on target insect pest midgut receptors, is expected to delay the evolution of resistance to either *Bt*-toxin effectively when most individuals that are resistant to one *Bt*-toxin are killed by the other, and when selection for resistance to one of the *Bt*-toxins does not cause cross-resistance to the other (Storer et al., 2012a). However, Ghimire et al. (2011) demonstrated that larvae of a laboratory-selected Cry1Ab-resistant SCB colony are also resistant to Cry1F in leaf tissue bioassays and intact plant tests conducted under greenhouse conditions, pointing to the potential for cross-resistance between maize MON 810 and 1507.

[7] A pyramided *Bt*-crop combines related traits such as insect resistance against target insect pest species of the same Order

Results from this study suggest that the mode of action of Cry1Ab and Cry1F (i.e., the binding sites for these proteins in the insect midgut) could overlap. Even though other studies suggested only very low levels or lack of cross-resistance between Cry1Ab and Cry1F (Siqueira et al., 2004; Pereira et al., 2008; Xu et al., 2010; Crespo et al., 2011), it is prudent to infer the potential for cross-resistance in deploying pyramided *Bt*-crops that express both Cry1Ab and Cry1F proteins, as their efficacy will be diminished or offset, if cross-resistance occurs (see Storer et al., 2012b). In their study, Huang et al. (2012b) could not completely exclude the possibility of differences in Cry1F susceptibility between two field-collected SCB populations, though both populations were susceptible to the three purified Cry1Aa, Cry1Ab and Cry1Ac proteins.

6.2.2.3. Conclusion

The EFSA GMO Panel recommends caution when predicting future responses of ECB and MCB in relevant EU regions based on experiences elsewhere or with other target insect pest species, as resistance evolution is dependent upon many factors.

Results reported by Huang et al. (2009, 2012a,b), Jalali et al. (2010), Tamez-Guerra (2010) and Alcantara et al. (2011) do not provide new information that would invalidate the previous recommendations on monitoring made by the EFSA GMO Panel. Therefore, the EFSA GMO Panel considers that its previous conclusions on maize MON 810 remain valid and applicable.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Following a search of the scientific literature published between 2009 and October 2012, the EFSA GMO Panel identified 165 peer-reviewed publications containing evidence specific to the risk assessment and/or management of maize MON 810, of which 68 publications were discussed and/or cited in previous EFSA GMO Panel scientific outputs. From the remaining 97 publications, eight were relevant for the molecular characterisation, 27 for food and feed safety assessment, 55 for the environmental risk assessment and/or risk management, two for the molecular characterisation and the environmental risk assessment and/or risk management and five for the food and feed safety assessment and the environmental risk assessment and/or risk management of maize MON 810.

The EFSA GMO Panel did not identify any peer-reviewed scientific publications reporting new information that would invalidate its previous conclusions on the safety of maize MON 810. Therefore, the EFSA GMO Panel considers that its previous risk assessment conclusions on maize MON 810, as well as its previous recommendations for risk mitigation measures and monitoring, remain valid and applicable.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Deputy Director General for the Health and Consumers of the European Commission, dated 20 June 2012, to the EFSA executive Director requesting an EFSA opinion gathering all available information related to the environmental risk assessment of maize MON 810 for cultivation.

2. Acknowledgement letter, dated 11 July 2012, from the EFSA executive Director to the Director General for the Health and Consumers of the European Commission.

3. Letter, dated 27 September 2012, from the EFSA executive Director to the Director General for the Health and Consumers of the European Commission prioritising the Commission mandates in the area of GMOs currently pending with EFSA and requesting to provide additional evidence to support previous EFSA Opinions on maize 1507, Bt11 and MON 810.

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Verbruggen E, Kuramae EE, Hillekens R, de Hollander M, Kiers ET, Röling WFM, Kowalchuk GA, van der Heijden MGA, 2012. Testing potential effects of Bt maize on mycorrhizal fungal communities via DNA- and RNA-based pyrosequencing and molecular fingerprinting. Applied and Environmental Microbiology 78, 7384-7392.

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Walsh MC, Buzoianu SG, Gardiner GE, Rea MC, Hart OM, Ross P, Lawlor PG, 2010. Effect of short-term feeding of genetically modified Bt maize (MON810) on gut microbiota, intestinal morphology and immune status of weanling pigs. Advances in Animal Biosciences 1, 180.

Walsh MC, Buzoianu SG, Gardiner GE, Rea MC, Gelencsér E, Janosi A, Epstein MM, Ross RP, Lawlor PG, 2011. Fate of transgenic DNA from orally administered *Bt* MON810 maize and effects on immune response and growth in pigs. PLoS ONE 6(11), e27177.

Walsh MC, Buzoianu SG, Gardiner GE, Rea MC, Ross RP, Cassidy JP, Lawlor PG, 2012a. Effects of short-term feeding of *Bt* MON810 maize on growth performance, organ morphology and function in pigs. British Journal of Nutrition 107, 364-371.

Walsh MC, Buzoianu SG, Rea MC, O'Donovan O, Gelencsér E, Ujhelyi G, Ross RP, Gardiner GE, Lawlor PG, 2012b. Effects of feeding Bt MON810 maize to pigs for 110 days on peripheral immune response and digestive fate of the *cry1Ab* gene and truncated Bt toxin.PLoS ONE 7(5), e36141.

Wu X, Huang F, Leonard BR, Ghimire M, 2009. Growth and development of *Bacillus thuringiensis* Cry1Ab-susceptible and Cry1Ab-resistant sugarcane borer on diet and conventional maize plants. Entomologia Experimentalis et Applicata 133, 199-207.

Xu L, Wang Z, Zhang J, He K, Ferry N, Gatehouse AMR, 2010. Cross-resistance of Cry1Ab-selected Asian corn borer to other Cry toxins. Journal of Applied Entomology 134, 429-438.

Yanni SF, Whalen JK, Ma BL, 2010. Crop residue chemistry, decomposition rates, and CO2 evolution in Bt and non-Bt corn agroecosystems in North America: a review. Nutrient Cycling in Agroecosystems 87, 277-293.

Yu HL, Li YH, Wu KM, 2011. Risk assessment and ecological effects of transgenic *Bacillus thuringiensis* crops on non-target organisms. Journal of Integrative Plant Biology 53, 520-538.

Zhang W, Shi F, 2011. Do genetically modified crops affect animal reproduction? A review of the ongoing debate. Animal 5, 1048-1059.

Zhou J, Harrigan GG, Berman KH, Webb EG, Klusmeyer TH, Nemeth MA, 2011. Stability in the composition equivalence of grain from insect-protected maize and seed from glyphosate-tolerant soybean to conventional counterparts over multiple seasons, locations, and breeding germplasms. Journal of Agricultural and Food Chemistry 59, 8822-8828.

APPENDICES

A. PUBLICATIONS OBTAINED FROM ISI WEB OF KNOWLEDGE USING KEYWORD SEARCHES, AND FROM TARGETED SEARCHES OF PEER-REVIEWED JOURNALS

Authors of publication	Title of publication	Journal	Publication year	Issue in the remit of the EFSA GMO Panel and relevant to this EC mandate	Peer-reviewed publication	Publication in English	Publication previously discussed and/or cited in scientific outputs of the EFSA GMO Panel
Adel-Patient K, Guimaraes VD, Paris A, Drumare MF, Ah-Leung S, Lamourette P, Nevers MC, Canlet C, Molina J, Bernard H, Creminon C, Wal JM	Immunological and metabolomic impacts of administration of Cry1Ab protein and MON 810 maize in mouse	PLoS ONE	2011	YES	YES	YES	NO
Aguilera M, Querci M, Pastor S, Bellocchi G, Milcamps A, van den Eede G	Assessing copy number of MON 810 integrations in commercial seed maize varieties by 5' event-specific real-time PCR validated method coupled to 2 (Delta Delta CT) analysis	Food Analytical Methods	2009	YES	YES	YES	NO
Albert H	GM authorization for Mon 810 pollen	Biofutur	2012	NO	-	-	-
Alcantara EP	Postcommercialization monitoring of the long-term impact of Bt corn on non-target arthropod communities in commercial farms and adjacent riparian areas in the Philippines	Environmental Entomology	2012	YES	YES	YES	NO

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Alcantara E, Estrada A, Alpuerto V, Head G	Monitoring Cry1Ab susceptibility in Asian corn borer (Lepidoptera: Crambidae) on Bt corn in the Philippines	Crop Protection	2011	YES	YES	YES	NO
Al-Hmoud N, Al-Rousan H, Hayek BO, Ibrahim MA	Detection of genetically modified maize and soybean food products in the Jordanian market	Biotechnology	2010	NO	-	-	-
Alvarez-Alfageme F, Bigler F, Romeis J	Laboratory toxicity studies demonstrate no adverse effects of Cry1Ab and Cry3Bb1 to larvae of *Adalia bipunctata* (Coleoptera: Coccinellidae): the importance of study design	Transgenic Research	2011	YES	YES	YES	YES
Alvarez-Alfageme F, Ortego F, Castanera P	Bt maize fed-prey mediated effect on fitness and digestive physiology of the ground predator *Poecilus cupreus* L. (Coleoptera: Carabidae)	Journal of Insect Physiology	2009	YES	YES	YES	YES
Andow DA, Farrell SL, Hu Y	Planting patterns of in-field refuges observed for Bt maize in Minnesota	Journal of Economic Entomology	2010	YES	YES	YES	YES
Antofie MM, Sand C	Insights into the biotech policy and Europeans tendency	Research Journal of Agricultural Science	2009	NO	-	-	-
Aris A	Response to comments from Monsanto scientists on our study showing detection of glyphosate and Cry1Ab in blood of women with and without pregnancy	Reproductive Toxicology	2012	YES	YES	YES	YES[8]

[8] For more details about the assessment of the original publication by the EFSA GMO Panel see letter from EFSA to the European Commission (DG Sanco) dated 19 August 2011 with reference PB/HF/AFD/mt (2011) 5863329. This letter is available upon request to EFSA. The EFSA GMO Panel notes that this commentary does not add to the risk assessment of maize MON 810

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Arvinth S, Arun S, Selvakesavan RK, Srikanth J, Mukunthan N, Kumar PA, Premachandran MN, Subramonian N	Genetic transformation and pyramiding of aprotinin-expressing sugarcane with cry1Ab for shoot borer (*Chilo infuscatellus*) resistance	Plant Cell Reports	2010	NO	-	-	-
Ascher J, Ceccherini MT, Guerri G, Nannipieri P, Pietramellara G	"e-motion" of extracellular DNA (e-DNA) in soil	Fresenius Environmental Bulletin	2009	NO	-	-	-
Aviron S, Sanvido O, Romeis J, Herzog F, Bigler F	Case-specific monitoring of butterflies to determine potential effects of transgenic Bt-maize in Switzerland	Agriculture Ecosystems & Environment	2009	YES	YES	YES	YES
Badea EM, Chelu F, Lacatusu A	Results regarding the levels of Cry1Ab protein in transgenic corn tissue (MON810) and the fate of Bt protein in three soil types	Romanian Biotechnological Letters	2010	YES	YES	YES	NO
Badea EM, Pamfil D	The status of agricultural biotechnology and biosafety in Romania	Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Animal Science and Biotechnologies	2009	NO	-	-	-
Bai YY, Yan RH, Ye GY, Huang FN, Cheng JA	Effects of transgenic rice expressing *Bacillus thuringiensis* Cry1Ab protein on ground-dwelling collembolan community in postharvest seasons	Environmental Entomology	2010	NO	-	-	-

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Bakonyi G, Dolezsai A, Matrai N, Szekacs A	Effects of consumption of Bt-maize (MON 810) on the collembolan *Folsomia candida*, over multiple generations: a laboratory study	Insects	2011	YES	YES	YES	NO
Balog A, Kiss J, Szekeres D, Szenasi A, Marko V	Rove beetle (Coleoptera: Staphylinidae) communities in transgenic Bt (MON810) and near isogenic maize	Crop Protection	2010	YES	YES	YES	NO
Balog A, Szenasi A, Szekeres D, Kiss J	Staphylinids (Coleoptera: Staphylinidae) in genetically modified maize ecosystems: species densities and trophic interactions	IOBC/wprs Bulletin	2010	YES	NO	-	-
Balsamo GM, Cangahuala-Inocente GC, Bertoldo J B, Terenzi H, Arisi ACM	Proteomic analysis of four Brazilian MON810 maize varieties and their four non-genetically-modified isogenic varieties	Journal of Agricultural and Food Chemistry	2011	YES	YES	YES	NO
Baniulis D, Sikorskaite S, Bendokas V, Staniene G, Gelvonauskiene D, Stanys V	Application of proteolytic digestion test to assess allegenicity risk of genetically modified plants	Sodininkyste ir Darzininkyste	2011	YES	YES	NO	-
Barriuso J, Valverde JR, Mellado RP	Effect of Cry1Ab protein on rhizobacterial communities of Bt-maize over a four-year cultivation period	PLoS ONE	2012	YES	YES	YES	NO
Barros E, Lezar S, Anttonen MJ, van Dijk JP, Rohlig RM, Kok EJ, Engel KH	Comparison of two GM maize varieties with a near-isogenic non-GM variety using transcriptomics, proteomics and metabolomics	Plant Biotechnology Journal	2010	YES	YES	YES	NO

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Batista R, Oliveira M	Plant natural variability may affect safety assessment data	Regulatory Toxicology and Pharmacology	2010	YES	YES	YES	NO
Bel Y, Siqueira HAA, Siegfried BD, Ferre J, Escriche B	Variability in the cadherin gene in an *Ostrinia nubilalis* strain selected for Cry1Ab resistance	Insect Biochemistry and Molecular Biology	2009	YES	YES	YES	NO
Beres PK	Harmfulness of *Ostrinia nublialis Hbn.* on some non-Bt versus genetically modified Bt maize (*Zea mays* L.) cultivars in Poland in 2006-2007	Journal of Plant Protection Research	2010	YES	YES	YES	NO
Beres PK	Reduction of damage caused by *Ostrinia nubilalis* Hbn in south-eastern Poland in 2007 through the cultivation of transgenic maize varieties	IOBC/wprs Bulletin	2010	YES	NO	-	-
Bergerova E, Godalova Z, Siekel P	Combined effects of temperature, pressure and low pH on the amplification of DNA of plant derived foods	Czech Journal of Food Sciences	2011	NO	-	-	-
Bohn T, Traavik T, Primicerio R	Demographic responses of *Daphnia magna* fed transgenic Bt-maize	Ecotoxicology	2010	YES	YES	YES	YES
Branquinho MR, Ferreira RTB, Cardarelli-Leite P	Survey of compliance with labelling legislation in food containing GMOs in Brazil	Journal of Food Composition and Analysis	2010	NO	-	-	-
Brants I, Ben Tahar S, Salva I	Commentary to publications in food analytics methods journal as related to genetic stability of maize event MON810	Food Analytical Methods	2010	YES	YES	YES	NO

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Buntin GD	Corn expressing Cry1Ab endotoxin for management of fall armyworm and corn earworm (Lepidoptera: Noctuidae) in silage production	Journal of Entomological Science	2010	YES	YES	YES	NO
Burkness EC, Hutchison WD	Bt pollen dispersal and Bt kernel mosaics: integrity of non-Bt refugia for lepidopteran resistance management in maize	Journal of Economic Entomology	2012	YES	YES	YES	YES
Burkness EC, O'Rourke PK, Hutchison WD	Cross-pollination of nontransgenic corn ears with transgenic Bt corn: efficacy against lepidopteran pests and implications for resistance management	Journal of Economic Entomology	2011	YES	YES	YES	YES
Burkness EC, Dively G, Patton T, Morey AC, Hutchison WD	Novel Vip3A *Bacillus thuringiensis* (Bt) maize approaches high-dose efficacy against *Helicoverpa zea* (Lepidoptera: Noctuidae) under field conditions: Implications for resistance management	GM Crops	2010	NO	-	-	-
Buzoianu SG, Walsh MC, Rea MC, O'Sullivan O, Cotter PD, Ross RP, Gardiner GE, Lawlor PG	High-throughput sequence-based analysis of the intestinal microbiota of weanling pigs fed genetically modified MON810 maize expressing *Bacillus thuringiensis* Cry1Ab (Bt maize) for 31 days	Applied and Environmental Microbiology	2012	YES	YES	YES	YES
Buzoianu SG, Walsh MC, Rea MC, O'Sullivan O, Crispie F, Cotter PD, Ross RP, Gardiner GE, Lawlor PG	The effect of feeding Bt MON810 maize to pigs for 110 days on intestinal microbiota	PLoS ONE	2012	YES	YES	YES	NO

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Buzoianu SG, Walsh MC, Rea MC, O'Donovan O, Gelencsér E, Ujhelyi G, Szabo E, Nagy A, Ross RP, Gardiner GE, Lawlor PG	Effects of feeding Bt maize to sows during gestation and lactation on maternal and offspring immunity and fate of transgenic material	PLoS ONE	2012	YES	YES	YES	NO
Cao Y, Wu G, Wu Y, Nie S, Zhang L, Lu C	Characterization of the transgenic rice event TT51-1 and construction of a reference plasmid	Journal of Agricultural and Food Chemistry	2011	NO	-	-	-
Caprioara-Buda M, Meyer W, Jeynov B, Corbisier P, Trapmann S, Emons H	Evaluation of plasmid and genomic DNA calibrants used for the quantification of genetically modified organisms	Analytical and Bioanalytical Chemistry	2012	NO	-	-	-
Chambers CP, Whiles MR, Rosi-Marshall EJ, Tank JL, Royer TV, Griffiths NA, Evans-White MA, Stojak AR	Responses of stream macroinvertebrates to Bt maize leaf detritus	Ecological Applications	2010	YES	YES	YES	YES
Cheeke TE, Pace BA, Rosenstiel TN, Cruzan MB	The influence of fertilizer level and spore density on arbuscular mycorrhizal colonization of transgenic Bt11 maize (*Zea mays*) in experimental microcosms	FEMS Microbiology Ecology	2011	NO	-	-	-
Cheeke TE, Rosenstiel TN, Cruzan MB	Evidence of reduced arbuscular mycorrhizal fungal colonization in multiple lines of Bt maize	American Journal of Botany	2012	YES	YES	YES	YES
Chege PG, Clark TL, Hibbard BE	Initial larval feeding on an alternate host enhances western corn rootworm (Coleoptera: Chrysomelidae) beetle emergence on Cry3Bb1-expressing maize	Journal of the Kansas Entomological Society	2009	NO	-	-	-

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Chen M, Ye G, Liu Z, Fang Q, Hu C, Peng Y, Shelton AM	Analysis of Cry1Ab toxin bioaccumulation in a food chain of Bt rice, an herbivore and a predator	Ecotoxicology	2009	NO	-	-	-
Chunjiao Z, Wentao X, Zhifang Z, Yunbo L, Xinghua Y, Nan Z, Kunlun H	Universal primer-multiplex-polymerase chain reaction (UP-M-PCR) and capillary electrophoresis-laser-induced fluorescence analysis for the simultaneous detection of six genetically modified maize lines	Journal of Agricultural and Food Chemistry	2011	NO	-	-	-
Coll A, Nadal A, Collado R, Capellades G, Messeguer J, Mele E, Palaudelmas M, Pla M	Gene expression profiles of MON810 and comparable non-GM maize varieties cultured in the field are more similar than are those of conventional lines	Transgenic Research	2009	YES	YES	YES	NO
Coll A, Nadal A, Collado R, Capellades G, Kubista M, Messeguer J, Pla M	Natural variation explains most transcriptomic changes among maize plants of MON810 and comparable non-GM varieties subjected to two N-fertilization farming practices	Plant Molecular Biology	2010	YES	YES	YES	NO
Coll A, Nadal A, Rossignol M, Puigdomenech P, Pla M	Proteomic analysis of MON810 and comparable non-GM maize varieties grown in agricultural fields	Transgenic Research	2011	YES	YES	YES	NO
Consmueller N, Beckmann V, Petrick M	An econometric analysis of regional adoption patterns of Bt maize in Germany	Agricultural Economics	2010	NO	-	-	-
Corbisier P, Bhat S, Partis L, Xie Vicki Rui D, Emslie KR	Absolute quantification of genetically modified MON810 maize (*Zea mays* L.) by digital polymerase chain reaction	Analytical and Bioanalytical Chemistry	2010	NO	-	-	-

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Crava CM, Bel Y, Ferre J, Escriche B	Absence of Cry1Ab resistance in a Spanish *Ostrinia nubilalis* population from an infested greenhouse	IOBC/wprs Bulletin	2010	YES	NO	-	-
Crava MC, Bel Y, Escriche B	*Bacillus thuringiensis* susceptibility variation among *Ostrinia nubilalis* populations	IOBC/wprs Bulletin	2009	YES	NO	-	-
Crespo ALB, Rodrigo-Simon A, Siqueira HAA, Pereira EJG, Ferre J, Siegfried BD	Cross-resistance and mechanism of resistance to Cry1Ab toxin from *Bacillus thuringiensis* in a field-derived strain of European corn borer, *Ostrinia nubilalis*	Journal of Invertebrate Pathology	2011	YES	YES	YES	YES
Crespo ALB, Spencer TA, Alves AP, Hellmich RL, Blankenship EE, Magalhaes LC, Siegfried BD	On-plant survival and inheritance of resistance to Cry1Ab toxin from *Bacillus thuringiensis* in a field-derived strain of European corn borer, *Ostrinia nubilalis*	Pest Management Science	2009	YES	YES	YES	YES
Crespo ALB, Spencer TA, Tan SY, Siegfried BD	Fitness costs of Cry1Ab resistance in a field-derived strain of *Ostrinia nubilalis* (Lepidoptera: Crambidae)	Journal of Economic Entomology	2010	YES	YES	YES	NO
Czapla A, Kurczak P, Kiekiewicz M	The rose-grain aphid (*Metopolophium dirhodum* Walker) bionomy parameters on chosen maize cultivars	Progress in Plant Protection	2011	YES	YES	NO	-
Dabrowski ZT, Klukowski Z, Hurej M	Comparison of evaluation methods on the GMO plants' effect on the trophic relations under field conditions.	Progress in Plant Protection	2009	YES	YES	NO	-
Darvas B, Banati H, Takacs E, Lauber E, Szecsi A, Szekacs A	Relationships of *Helicoverpa armigera*, *Ostrinia nubilalis* and *Fusarium verticillioides* on MON 810 maize	Insects	2011	YES	YES	YES	NO

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Daudu CK, Muchaonyerwa P, Mnkeni PNS	Litterbag decomposition of genetically modified maize residues and their constituent *Bacillus thuringiensis* protein (Cry1Ab) under field conditions in the central region of the Eastern Cape, South Africa	Agriculture, Ecosystems & Environment	2009	YES	YES	YES	NO
De Maria G	EFSA assesses 2010 Post-Market Environmental Monitoring report for MON810 maize	Agro Food Industry Hi-Tech	2012	NO	-	-	-
de Polania IZ, Arevalo Maldonado HA, Mejia Cruz RJL	*Spodoptera frugiperda*: response of different populations to the Cry1Ab toxin	Revista Colombiana de Entomologia	2009	YES	YES	NO	-
de Vendomois JS, Roullier F, Cellier D, Seralini GE	A comparison of the effects of three GM corn on mammalian health	International Journal of Biological Sciences	2009	YES	YES	YES	YES[9]
de Vendomois JS, Cellier D, Velot C, Clair E, Mesnage R, Seralini GE	Debate on GMOs health risks after statistical findings in regulatory tests	International Journal of Biological Sciences	2010	YES	YES	YES	YES[10]
Debode F, Marien A, Janssen E, Berben G	Design of multiplex calibrant plasmids, their use in GMO detection and the limit of their applicability for quantitative purposes owing to competition effects	Analytical and bioanalytical chemistry	2010	NO	-	-	-
Deroin P	OGM New episode in the Mon 810 scandal	Biofutur	2012	NO	-	-	-

[9] See Minutes of the 55th plenary meeting of the EFSA GMO Panel of 27-28 January 2010 (http://www.efsa.europa.eu/en/events/event/gmo100127.htm)

[10] See Minutes of the 55th plenary meeting of the EFSA GMO Panel of 27-28 January 2010 (http://www.efsa.europa.eu/en/events/event/gmo100127.htm). The EFSA GMO Panel considers that the commentary by the authors does not add to the risk assessemnt of maize MON 810

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Digilio MC, Sasso R, Di Leo MG, Iodice L, Monti MM, Santeramo R, Arpaia S, Guerrieri E	Interactions between Bt-expressing tomato and non-target insects: the aphid *Macrosiphum euphorbiae* and its natural enemies	Journal of Plant Interactions	2012	NO	-	-	-
Dinon AZ, Bosco KT, Arisi ACM	Monitoring of Bt11 and Bt176 genetically modified maize in food sold commercially in Brazil from 2005 to 2007	Journal of the Science of Food and Agriculture	2010	NO	-	-	-
Dorhout DL, Rice ME	Intraguild competition and enhanced survival of western bean cutworm (Lepidoptera: Noctuidae) on transgenic Cry1Ab (MON810) *Bacillus thuringiensis* corn	Journal of Economic Entomology	2010	YES	YES	YES	YES
Douville M, Gagne F, Andre C, Blaise C	Occurrence of the transgenic corn cry1Ab gene in freshwater mussels (*Elliptio complanata*) near corn fields: Evidence of exposure by bacterial ingestion	Ecotoxicology and Environmental Safety	2009	YES	YES	YES	YES
Dutra CC, Koch LK, Burkness EC, Meissle M, Romeis J, Hutchison WD, Fernandes MG	*Harmonia axyridis* (Coleoptera: Coccinellidae) exhibits no preference between Bt and non-Bt maize fed *Spodoptera frugiperda* (Lepidoptera: Noctuidae)	PLoS ONE	2012	YES	YES	YES	NO
Dyer GA, Serratos-Hernandez JA, Perales HR, Gepts P, Pineyro-Nelson A, Chavez A, Salinas-Arreortua N, Yunez-Naude A, Taylor JE, Alvarez-Buylla ER	Dispersal of transgenes through maize seed systems in Mexico	PLoS ONE	2009	NO	-	-	-

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Edgerton MD, Fridgen J, Anderson JR, Ahlgrim J, Criswell M, Dhungana P, Gocken T, Li Z, Mariappan S, Pilcher CD, Rosielle A, Stark SB	Transgenic insect resistance traits increase corn yield and yield stability	Nature America	2012	NO	-	-	-
Eizaguirre M, Madeira F, Lopez C	Effects of Bt maize on non-target lepidopteran pests	IOBC/wprs Bulletin	2010	YES	NO	-	-
Emmerling C, Strunk H, Schoebinger U, Schrader S	Fragmentation of Cry1Ab protein from Bt-maize (MON810) through the gut of the earthworm species *Lumbricus terrestris* L.	European Journal of Soil Biology	2011	YES	YES	YES	NO
Engels H, Bourguet D, Cagan L, Manachini B, Schuphan I, Stodola TJ, Micoud A, Brazier C, Mottet C, Andow DA	Evaluating resistance to Bt toxin Cry1Ab by F2 screen in European populations of *Ostrinia nubilalis* (Lepidoptera: Crambidae)	Journal of Economic Entomology	2010	YES	YES	YES	YES
Erasmus A, van Rensburg JBJ, van den Berg J	Effects of Bt maize on *Agrotis segetum* (Lepidoptera: Noctuidae): a pest of maize seedlings	Environmental Entomology	2010	YES	YES	YES	YES
Farinos GP, de la Poza M, Ortego F, Castanera P	Susceptibility to the Cry1F toxin of field populations of *Sesamia nonagrioides* (Lepidoptera: Noctuidae) in Mediterranean maize cultivation regions	Journal of Economic Entomology	2012	NO	-	-	-
Farinos GR, Andreadis SS, de la Poza M, Mironidis GK, Ortego F, Savopoulou-Soultani M, Castanera P	Comparative assessment of the field-susceptibility of *Sesamia nonagrioides* to the Cry1Ab toxin in areas with different adoption rates of Bt maize and in Bt-free areas	Crop Protection	2011	YES	YES	YES	YES

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Farrar RR, Shepard BM, Shapiro M, Hassell RL, Schaffer ML, Smith CM	Supplemental control of lepidopterous pests on Bt transgenic sweet corn with biologically-based spray treatments	Journal of Insect Science	2009	NO	-	-	-
Feng Y, Jin Q, Wang J	Systemic induced effects of mechanical wounding on the chemical defence of Bt corn (*Zea mays*)	Chinese Journal of Plant Ecology	2010	NO	-	-	-
Fliessbach A, Messmer M, Nietlispach B, Infante V, Maeder P	Effects of conventionally bred and *Bacillus thuringiensis* (Bt) maize varieties on soil microbial biomass and activity	Biology and Fertility of Soils	2012	NO	-	-	-
Folcher L, Delos M, Marengue E, Jarry M, Weissenberger A, Eychenne N, Regnault-Roger C	Lower mycotoxin levels in Bt maize grain	Agronomy for Sustainable Development	2010	NO	-	-	-
Folcher L, Jarry M, Weissenberger A, Eychenne N, Delos M, Regnault-Roger C	Biocontrol of *Ostrinia nubilalis* and *Sesamia nonagrioides* by Bt maize in South Western France: search of biological indicators by a model-based approach for managing mycotoxin risks	IOBC/wprs Bulletin	2009	NO	-	-	-
Folloni S, Bellocchi G, Prospero A, Querci M, Moens W, Ermolli M, van den Eede G	Statistical evaluation of real-time PCR protocols applied to quantify genetically modified maize	Food Analytical Methods	2010	NO	-	-	-
Folloni S, Bellocchi G, Kagkli DM, Pastor-Benito S, Aguilera M, Mazzeo A, Querci M, van den Eede G, Ermolli M	Development of an ELISA reverse-based Assay to assess the presence of mycotoxins in cereal flour	Food Analytical Methods	2011	NO			
Fonseca C, Planchon S, Renaut J, Oliveira MM, Batista R	Characterization of maize allergens-MON810 vs. its non-transgenic counterpart	Journal of Proteomics	2012	YES	YES	YES	NO

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Frank T, Roehlig RM, Davies HV, Barros E, Engel K-H	Metabolite profiling of maize kernels - genetic modification versus environmental influence	Journal of Agricultural and Food Chemistry	2012	YES	YES	YES	NO
Froystad-Saugen MK, Lilleeng E, Bakke-McKellep AM, Vekterud K, Valen EC, Hemre GI, Krogdahl A	Distal intestinal gene expression in Atlantic salmon (*Salmo salar* L.) fed genetically modified maize	Aquaculture Nutrition	2009	YES	YES	YES	NO
Fu Q, Zhang Y, Huang W, Hu H, Chen D, Yang C	Remaining dynamics of Cry1Ab proteins from transgenic Bt corn in soil	Journal of Food Agriculture & Environment	2012	NO	-	-	-
Gabriela Macias-de la Cerda C, Cantu-Iris M, Cruz-Requena M, Rodriguez-Herrera R, Manuel Gonzalez-Vazquez V, Noe Aguilar-Gonzalez C, Carlos Loyola-Licea J, Carlos Contreras-Esquivel J	Transgenic sequences detected in corn, soybean and cotton grains imported to Mexico	Indian Journal of Genetics and Plant Breeding	2012	NO	-	-	-
Galeano P, Debat CM, Ruibal F, Fraguas LF, Galvan GA	Cross-fertilization between genetically modified and non-genetically modified maize crops in Uruguay	Environmental Biosafety Research	2010	YES	YES	YES	NO
Garcia M, Ortego F, Castanera P, Farinos GP	Effects of exposure to the toxin Cry1Ab through Bt maize fed-prey on the performance and digestive physiology of the predatory rove beetle *Atheta coriaria*	Biological Control	2010	YES	YES	YES	NO
Garcia-Canas V, Mondello M, Cifuentes A	Simultaneous detection of genetically modified organisms by multiplex ligation-dependent genome amplification and capillary gel electrophoresis with laser-induced fluorescence	Electrophoresis	2010	NO	-	-	-

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
George DM, Rind FC, Bendall MW, Taylor MA, Gatehouse AMR	Developmental studies of transgenic maize expressing Cry1Ab on the African stem borer, *Busseola fusca*; effects on midgut cellular structure	Pest Management Science	2012	YES	YES	YES	NO
Hagh ZH, Rahnama H, Panahandeh J, Baghban Kohneh Rouz B, Jafari KMA, Mahna N	Green-tissue-specific, C(4)-PEPC-promoter-driven expression of Cry1Ab makes transgenic potato plants resistant to tuber moth (*Phthorimaea operculella*, Zeller)	Plant Cell Reports	2009	NO	-	-	-
Ghimire MN, Huang F, Leonard R, Head GP, Yang Y	Susceptibility of Cry1Ab-susceptible and -resistant sugarcane borer to transgenic corn plants containing single or pyramided *Bacillus thuringiensis* genes	Crop Protection	2011	YES	YES	YES	YES
Gomez I, Arenas I, Pacheco S, Bravo A, Soberon M	New insights into the mode of action of Cry1Ab toxin used in transgenic insect-resistant crops	Southwestern Entomologist	2010	YES	YES	YES	NO
Gruber H, Paul V, Guertler P, Spiekers H, Tichopad A, Meyer HHD, Mueller M	Fate of Cry1Ab protein in agricultural systems under slurry management of cows fed genetically modified maize (*Zea mays* L.) MON810: A quantitative assessment	Journal of Agricultural and Food Chemistry	2011	YES	YES	YES	YES
Gruber H, Paul V, Meyer HHD, Mueller M	Determination of insecticidal Cry1Ab protein in soil collected in the final growing seasons of a nine-year field trial of Bt-maize MON810	Transgenic Research	2012	YES	YES	YES	YES
Guan Q, Wang X, Teng D, Yang Y, ian F, Yin Q, Wang J	Construction of a standard reference plasmid for detecting GM cottonseed meal	Applied Biochemistry and Biotechnology	2011	NO	-	-	-

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Guertler P, Paul V, Albrecht C, Meyer HHD	Sensitive and highly specific quantitative real-time PCR and ELISA for recording a potential transfer of novel DNA and Cry1Ab protein from feed into bovine milk	Analytical and Bioanalytical Chemistry	2009	YES	YES	YES	NO
Guertler P, Paul V, Steinke K, Wiedemann S, Preissinger W, Albrecht C, Spiekers H, Schwarz FJ, Meyer HHD	Long-term feeding of genetically modified corn (MON810) - Fate of cry1Ab DNA and recombinant protein during the metabolism of the dairy cow	Livestock Science	2010	YES	YES	YES	NO
Guertler P, Brandl C, Meyer HHD, Tichopad A	Feeding genetically modified maize (MON810) to dairy cows: comparison of gene expression pattern of markers for apoptosis, inflammation and cell cycle	Journal of Consumer Protection and Food Safety	2012	YES	YES	YES	NO
Guimaraes V, Drumare MF, Lereclus D, Gohar M, Lamourette P, Nevers MC, Vaisanen-Tunkelrott ML, Bernard H, Guillon B, Creminon C, Wal JM, Adel-Patient K	*In vitro* digestion of Cry1Ab proteins and analysis of the impact on their immunoreactivity	Journal of Agricultural and Food Chemistry	2010	YES	YES	YES	NO
Guo Z, Zhu YC, Huang F, Luttrell R, Leonard R	Microarray analysis of global gene regulation in the Cry1Ab-resistant and Cry1Ab-susceptible strains of *Diatraea saccharalis*	Pest Management Science	2012	YES	YES	YES	NO
Habustova O, Dolez al P, Spitzer1 L, Svobodova Z, Hussein H, Sehnal F	Impact of Cry1Ab toxin expression on the non-target insects dwelling on maize plants	Journal of Applied Entomology	2012	YES	YES	YES	NO

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Hansen LS, Lovei GL, Szekacs A	Survival and development of a stored-product pest, *Sitophilus zeamais* (Coleoptera: Curculionidae), and its natural enemy, the parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae), on transgenic *Bt* maize	Pest Management Science	2012	YES	YES	YES	NO
Hanusova L, Rehout V, Citek J	Transgene fragments in the blood and tissue of chicken fed with genetically modified soy and maize	Animal Nutrition and Feed Technology	2011	YES	YES	YES	NO
Hardke JT, Leonard BR, Huang F, Jackson RE	Damage and survivorship of fall armyworm (Lepidoptera: Noctuidae) on transgenic field corn expressing *Bacillus thuringiensis* Cry proteins	Crop Protection	2011	YES	YES	YES	YES
Hendriksma HP, Haertel S, Steffan-Dewenter I	Testing pollen of single and stacked insect-resistant Bt-maize on in vitro reared honey bee larvae	PLoS ONE	2011	YES	YES	YES	YES
Herman RA, Dunville CM, Juberg DR, Fletcher DW, Cromwell GL	Performance of broiler chickens fed event DAS-40278-9 maize containing the aryloxyalkanoate dioxygenase-1 protein	Regulatory Toxicology and Pharmacology	2011	NO	-	-	-
Hönemann L, Nentwig W	Are survival and reproduction of *Enchytraeus albidus* (Annelida: Enchytraeidae) at risk by feeding on Bt-maize litter?	European Journal of Soil Biology	2009	YES	YES	YES	YES
Höss S, Nguyen HT, Menzel R, Pagel-Wieder S, Miethling-Graf R, Tebbe CC, Jehle JA, Traunspurger W	Assessing the risk posed to free-living soil nematodes by a genetically modified maize expressing the insecticidal Cry3Bb1 protein	Science of the Total Environment	2011	NO	-	-	-

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Hofmann F, Epp R, Kruse L, Kalchschmied A, Maisch B, Muller E, Kuhn U, Kratz W, Ober S, Radtke J, Schlechtriemen U, Schmidt G, Schroder W, Ohe W, Vogel R, Wedl N, Wosniok W	Monitoring of Bt-maize pollen exposure in the vicinity of the nature reserve Ruhlsdorfer Bruch in northeast Germany 2007 to 2008	Umweltwissenschaften und Schadstoff-Forschung	2010	YES	YES	YES	YES
Holck AL, Dromtorp SM, Heir E	Quantitative, multiplex ligation-dependent probe amplification for the determination of eight genetically modified maize events	European Food Research and Technology	2009	NO	-	-	-
Huang F, Andow DA, Buschman LL	Success of the high-dose/refuge resistance management strategy after 15 years of Bt crop use in North America	Entomologia Experimentalis et Applicata	2011	YES	YES	YES	YES
Huang F, Ghimire MN, Leonard BR, Daves C, Levy R, Baldwin J	Extended monitoring of resistance to *Bacillus thuringiensis* Cry1Ab maize in *Diatraea saccharalis* (Lepidoptera: Crambidae)	GM Crops and Food: Biotechnology in Agriculture and the Food Chain	2012	YES	YES	YES	NO
Huang F, Ghimire MN, Leonard BR, Zhu YC, Head GP	Susceptibility of field populations of sugarcane borer from non-Bt and Bt maize plants to five individual Cry toxins	Insect Science	2012	YES	YES	YES	NO
Huang F, Ghimire MN, Leonard BR, Wang J, Daves C, Levy R, Cook D, Head GP, Yang Y, Temple J, Ferguson R	F2 screening for resistance to pyramided *Bacillus thuringiensis* maize in Louisiana and Mississippi populations of *Diatraea saccharalis* (Lepidoptera: Crambidae)	Pest Management Science	2011	NO	-	-	-

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Huang F, Parker R, Leonard R, Yong Y, Liu J	Frequency of resistance alleles to *Bacillus thuringiensis*-corn in Texas populations of the sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae)	Crop Protection	2009	YES	YES	YES	NO
Hutchison WD, Burkness EC, Mitchell PD, Moon RD, Leslie TW, Fleischer SJ, Abrahamson M, Hamilton KL, Steffey KL, Gray ME, Hellmich RL, Kaster LV, Hunt TE, Wright RJ, Pecinovsky K, Rabaey TL, Flood BR, Raun ES	Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers	Science	2010	YES	YES	YES	YES
Hutchison WD, Storer NP	Expanded use of pyramided transgenic maize hybrids expressing novel *Bacillus thuringiensis* toxins in the southern US potential for areawide suppression of *Helicoverpa zea* (Lepidoptera: Noctuidae) in the Mississippi Delta	Southwestern Entomologist	2010	NO	-	-	-
Jae-Hwan K, Su-Youn K, Hyungjae L, Young-Rok K, Hae-Yeong K	An event-specific DNA microarray to identify genetically modified organisms in processed foods	Journal of Agricultural and Food Chemistry	2010	NO	-	-	-
Jafari M, Norouzi P, Malboobi MA, Ghareyazie B, Valizadeh M, Mohammadi SA, Mousavi M	Enhanced resistance to a lepidopteran pest in transgenic sugar beet plants expressing synthetic cry1Ab gene	Euphytica	2009	NO	-	-	-

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Jalali SK, Lalitha Y, Kamath SP, Mohan KS, Head GP	Baseline-sensitivity of lepidopteran corn pests in India to the Cry1Ab insecticidal protein of *Bacillus thuringiensis*	Pest Management Science	2010	YES	YES	YES	NO
Jany KD	Honey genetically altered? The recommendations of the advocate general Bot to the ECJ in 'Pollen from genetically modified maize MON810 in honey' A one estimation	Deutsche Lebensmittel-Rundschau	2011	NO	-	-	-
Jasbeer K, Son R, Farinazleen MG, Kqueen CY	Real-time PCR-based detection and quantification of genetically modified maize in processed feeds commercialised in Malaysia	Food Control	2010	NO	-	-	-
Jensen PD, Dively GP, Swan CM, Lamp WO	Exposure and nontarget effects of transgenic Bt corn debris in streams	Environmental Entomology	2010	YES	YES	YES	YES
Kadlec J, Rehout V, Citek J, Hanusova L, Hosnedlova B	The influence of GM Bt maize MON 810 and RR soya in feed mixtures upon slaughter, haematological and biochemical indicators of broiler chickens	Journal of Agrobiology	2009	YES	YES	YES	NO
Kamath SP, Anuradha S, Vidya HS, Mohan KS, Dudin Y	Quantification of *Bacillus thuringiensis* Cry1Ab protein in tissues of YieldGard (R) (MON810) corn hybrids tested at multiple field locations in India	Crop Protection	2010	YES	YES	YES	NO
Kamle S, Kumar A, Bhatnagar RK	Development of multiplex and construct specific PCR assay for detection of cry2Ab transgene in genetically modified crops and product	GM Crops	2011	NO	-	-	-

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Kamota A, Muchaonyerwa P, Mnkeni PNS	Effects of ensiling of *Bacillus thuringiensis* (Bt) maize (MON810) on degradation of the crystal 1Ab (Cry1Ab) protein and compositional quality of silage	African Journal of Biotechnology	2011	YES	YES	YES	NO
Kee WP, Bumkyu L, Chang-Gi K, Do YK, Ji-Young P, Eun-Mi K, Soon-Chun J, Kyung-Hwa C, Won KY, Hwan MK	Monitoring the occurrence of genetically modified maize at a grain receiving port and along transportation routes in the Republic of Korea	Food Control	2010	YES	YES	YES	YES
Khan MS, Ali S, Iqbal J	Developmental and photosynthetic regulation of delta-endotoxin reveals that engineered sugarcane conferring resistance to 'dead heart' contains no toxins in cane juice	Molecular Biology Reports	2011	NO	-	-	-
Kim J, Seo Y, Kim J, Han YS, Lee KS, Kim S, Kim H, Ahn K, Lee S, Kim HY	Allergenicity assessment of Cry proteins in insect-resistant genetically modified maize Bt11, MON810, MON863	Food Science and Biotechnology	2009	YES	YES	YES	NO
Kim YH, Hwang CE, Kim T, Lee SH	Risk assessment system establishment for evaluating the potential impacts of imported *Bacillus thuringiensis* maize on a non-target insect, *Tenebrio molitor*	Journal of Asia-Pacific Entomology	2012	YES	YES	YES	NO
Kim YH, Hwang CE, Kim TS, Lee JH, Lee Si H	Assessment of potential impacts due to unintentionally released Bt maize plants on non-target aphid *Rhopalosiphum padi* (Hemiptera: Aphididae)	Journal of Asia-Pacific Entomology	2012	YES	YES	YES	YES

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Kleppin L, Schmidt G, Schroder W	Cultivation of GMO in Germany: support of monitoring and coexistence issues by WebGIS technology	Environmental Sciences Europe	2011	NO	-	-	-
Knecht S, Nentwig W	Effect of Bt maize on the reproduction and development of saprophagous Diptera over multiple generations	Basic and Applied Ecology	2010	YES	YES	YES	YES
Kramarz P, de Vaufleury A, Gimbert F, Cortet J, Tabone E, Andersen MN, Krogh PH	Effects of Bt-maize material on the life cycle of the land snail *Cantareus aspersus*	Applied Soil Ecology	2009	YES	YES	YES	YES
Krizova L, Pavlok S, Kocourek F, Nedelnik J, Vesely A	The effect of artificial inoculation with Fusarium strains on nutritive value of maize and ensiling process	Scientia Agriculturae Bohemica	2009	NO	-	-	-
Krizova L, Richter M, Kocourek F, Nedelnik J, Dolezal P	The effect of artificial inoculation with selected Fusarium strains on nutritional quality and ensiling process of Bt maize	Journal of Central European Agriculture	2010	NO	-	-	-
Kruger M, van Rensburg JBJ, van den Berg J	Transgenic Bt maize: farmers' perceptions, refuge compliance and reports of stem borer resistance in South Africa	Journal of Applied Entomology	2012	YES	YES	YES	YES
Kruger M, van Rensburg JBJ, van den Berg J	Reproductive biology of Bt-resistant and susceptible field-collected larvae of the maize stem borer, *Busseola fusca* (Lepidoptera: Noctuidae)	African Entomology	2012	YES	YES	YES	NO
Kruger M, van Rensburg JBJ, van den Berg J	Resistance to Bt maize in *Busseola fusca* (Lepidoptera: Noctuidae) from Vaalharts, South Africa	Environmental Entomology	2011	YES	YES	YES	YES

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Kumar R	A real-time immuno-PCR assay for the detection of transgenic Cry1Ab protein	European Food Research and Technology	2012	NO	-	-	-
Kyrova V, Ostry V, Laichmannova L, Ruprich J	An occurrence of genetically modified foodstuffs on the Czech food market	Acta Alimentaria	2010	NO	-	-	-
la Mura M, Allnutt TR, Greenland A, Mackay I, Lee D	Application of QUIZ for GM quantification in food	Food Chemistry	2011	NO	-	-	-
La Paz JL, Pla M, Papazova N, Puigdomenech P, Vicient CM	Stability of the MON 810 transgene in maize	Plant Molecular Biology	2010	YES	YES	YES	NO
La Paz JL, Vicient C, Puigdomenech P, Pla M	Characterization of polyadenylated cryIA(b) transcripts in maize MON810 commercial varieties	Analytical and Bioanalytical Chemistry	2010	YES	YES	YES	NO
Lang A, Otto M	A synthesis of laboratory and field studies on the effects of transgenic *Bacillus thuringiensis* (Bt) maize on non-target Lepidoptera	Entomologia Experimentalis et Applicata	2010	YES	YES	YES	YES
Langrell SRH, Allnutt TR, Laval V, Bertheau Y, Pla M, Papazova N, Lee D	Validation of a real-time PCR on-site quantification method for MON810 maize	Food Analytical Methods	2011	NO	-	-	-
Lawhorn CN, Neher DA, Dively GP	Impact of coleopteran targeting toxin (Cry3Bb1) of Bt corn on microbially mediated decomposition	Applied Soil Ecology	2009	NO	-	-	-
Lee B, Kim C, Park J, Park KW, Kim HJ, Hoonbok Y, Soon-Chun J, Won Kee Y, Kim HK	Monitoring the occurrence of genetically modified soybean and maize in cultivated fields and along the transportation routes of the Incheon port in South Korea	Food Control	2009	YES	YES	YES	YES

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Lee D, La Mura M, Allnutt T, Powell W, Greenland A	Isothermal amplification of genetically modified DNA sequences directly from plant tissues lowers the barriers to high-throughput and field-based genotyping	Journal of Agricultural and Food Chemistry	2009	NO	-	-	-
Lehman RM, Osborne SL, Prischmann-Voldseth DA, Rosentrater KA	Insect-damaged corn stalks decompose at rates similar to Bt-protected, non-damaged corn stalks	Plant and Soil	2010	YES	YES	YES	YES
Leon C, Rodriguez-Meizoso I, Lucio M, Garcia-Canas V, Ibanez E, Schmitt-Kopplin P, Cifuentes A	Metabolomics of transgenic maize combining Fourier transform-ion cyclotron resonance-mass spectrometry, capillary electrophoresis-mass spectrometry and pressurized liquid extraction	Journal of Chromatography A	2009	YES	YES	YES	NO
Leslie TW, Biddinger DJ, Mullin CA, Fleischer SJ	Carabidae population dynamics and temporal partitioning: response to coupled neonicotinoid-transgenic technologies in maize	Environmental Entomology	2009	NO	-	-	-
Leslie TW, Biddinger DJ, Rohr JR, Fleischer SJ	Conventional and seed-based insect management strategies similarly influence nontarget coleopteran communities in maize	Environmental Entomology	2010	NO	-	-	-
Li Y, Meissle M, Romeis J	Use of maize pollen by adult *Chrysoperla carnea* (Neuroptera: Chrysopidae) and fate of Cry proteins in Bt-transgenic varieties	Journal of Insect Physiology	2010	YES	YES	YES	YES

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Li Y, Romeis J	Bt maize expressing Cry3Bb1 does not harm the spider mite, *Tetranychus urticae*, or its ladybird beetle predator, *Stethorus punctillum*	Biological Control	2010	NO	-	-	-
Lili C, Jinchao G, Qidi W, Guoyin K, Litao Y	Development of the visual loop-mediated isothermal amplification assays for seven genetically modified maize events and their application in practical samples analysis	Journal of Agricultural and Food Chemistry	2011	NO	-	-	-
Lopez MD, Sumerford DV, Lewis LC	*Nosema pyrausta* and Cry1Ab-incorporated diet led to decreased survival and developmental delays in European corn borer	Entomologia Experimentalis et Applicata	2010	YES	YES	YES	NO
Lu X, Wu H, Wang M, Li B, Yang C, Sun H	Developing a method of oligonucleotide microarray for event specific detection of transgenic maize (*Zea mays*)	Acta Agronomica Sinica	2009	NO	-	-	-
Lu H, Wu W, Chen Y, Wang H, Devare M, Thies JE	Soil microbial community responses to Bt transgenic rice residue decomposition in a paddy field	Journal of Soils and Sediments	2010	NO	-	-	-
Lu H, Wu W, Chen Y, Zhang X, Devare M, Thies JE	Decomposition of Bt transgenic rice residues and response of soil microbial community in rapeseed-rice cropping system	Plant and Soil	2010	NO	-	-	-
Lumbierres B, Albajes R, Pons X	Positive effect of Cry1Ab-expressing Bt maize on the development and reproduction of the predator *Orius majusculus* under laboratory conditions	Biological Control	2012	YES	NO	-	-

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Lumbierres B, Stary P, Pons X	Effect of Bt maize on the plant-aphid-parasitoid tritrophic relationships	Biocontrol	2011	YES	YES	YES	NO
McNaughton J, Roberts M, Rice D, Smith B, Hinds M, Delaney B, Iiams C, Sauber T	Evaluation of broiler performance and carcass yields when fed diets containing corn grain from transgenic stacked-trait product DAS-Ø15Ø7-1xDAS-59122-7xMON-ØØ81Ø-6xMON-ØØ6Ø3-6	Journal of Applied Poultry Research	2011	NO	-	-	-
Meissle M, Knecht S, Waldburger M, Romeis J	Sensitivity of the cereal leaf beetle *Oulema melanopus* (Coleoptera: Chrysomelidae) to Bt maize-expressed Cry3Bb1 and Cry1Ab	Arthropod-Plant Interactions	2012	YES	YES	YES	NO
Meissle M, Romeis J, Bigler F	Bt maize and integrated pest management - a European perspective	Pest Management Science	2011	YES	YES	YES	YES
Meissle M, Romeis J	Insecticidal activity of Cry3Bb1 expressed in Bt maize on larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*	Entomologia Experimentalis et Applicata	2009	NO	-	-	-
Meissle M, Romeis J	The web-building spider *Theridion impressum* (Araneae: Theridiidae) is not adversely affected by Bt maize resistant to corn rootworms	Plant Biotechnology Journal	2009	NO	-	-	-
Mejia RAC, Zenner de Polania I	Expression of the Cry1Ab toxin in transgenic corn yieldgard (R) in the eastern plains of Colombia	Southwestern Entomologist	2012	YES	YES	NO	-
Michelotto MD, Finoto EL, Martins ALM, Duarte AP	Interaction between transgenics and insecticides in the control of key pests on off-season maize hybrids	Arquivos do Instituto Biologico (Sao Paulo)	2011	YES	YES	NO	-

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Milcamps A, Rabe S, Cade R, De Framond AJ, Henriksson P, Kramer V, Lisboa D, Pastor-Benito S, Willits MG, Lawrence D, van den Eede G	Validity assessment of the detection method of maize event Bt10 through investigation of its molecular structure	Journal of Agricultural and Food Chemistry	2009	NO	-	-	-
Mueller M, Freitag B, Koeder F	Plant biotechnology in German media: A linguistic analysis of the public image of genetically modified organisms	Biotechnology Journal	2010	NO	-	-	-
Mueting Sara, Lydy M	Environmental fate of the transgenic insecticidal protein Cry1Ab in water within a Bt maize agricultural ecosystem	Proceedings abstract	2011	YES	NO	-	-
Mugo S, Murenga MG, Karaya H, Tende R, Taracha C, Gichuki IJ, M'bijjewe K, Chavangi A	Control of *Busseola fusca* and *Chilo partellus* stem borers by *Bacillus thuringiensis* (Bt)-delta-endotoxins from Cry1Ab gene event MON810 in greenhouse containment trials	African Journal of Biotechnology	2011	YES	YES	YES	NO
Mugo SN, Mwimali M, Taracha CO, Songa JM, Gichuki ST, Tende R, Karaya H, Bergvinson DJ, Pellegrineschi A, Hoisington DA	Testing public Bt maize events for control of stem borers in the first confined field trials in Kenya	African Journal of Biotechnology	2011	NO	-	-	-
Muller A K, Schuppener M, Rauschen S	Assessing the impact of Cry1Ab expressing corn pollen on larvae of *Aglais urticae* in a laboratory bioassay	IOBC/wprs Bulletin	2012	YES	NO	-	-
Murenga M, Danson J, Mugo S, Githiri SM, Wanjala B	Quantification of Bt delta-endotoxins in leaf tissues of tropical Bt maize populations	African Journal of Biotechnology	2012	NO	-	-	-

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Murenga MG, Githiri SM, Mugo SN, Olubayo FM	Levels of control of *Chilo partellus* stem borer in segregating tropical Bt maize populations in Kenya	African Journal of Biotechnology	2011	NO	-	-	-
Nakayama T, Hiep HM, Furui S, Yonezawa Y, Saito M, Takamura Y, Tamiya E	An optimal design method for preventing air bubbles in high-temperature microfluidic devices	Analytical and Bioanalytical Chemistry	2010	NO	-	-	-
Natterer A	Case note: political issue of GMOs - ECJ on MON 810	European Food and Feed Law Review	2011	NO	-	-	-
Nedelnik J, Moravcova H, Vejrazka K	Mycotoxins, GMO and bulk feed	Proceedings abstract	2010	NO	-	-	-
Neumann G, Brandes C, Joachimsthaler A, Hochegger R	Assessment of the genetic stability of GMOs with a detailed examination of MON810 using scorpion probes	European Food Research and Technology	2011	YES	YES	YES	NO
Nguyen HT, Jehle JA	Stability of Cry1Ab protein during long-term storage for standardization of insect bioassays	Environmental Biosafety Research	2009	NO	-	-	-
Njontie C, Schiemann J, Husken A	Research projects to ensure the coexistence by maize (*Zea mays* L.)	Mitteilungen aus dem Julius Kuhn-Institut	2009	NO	-	-	-
Oguchi T, Onishi M, Mano J, Akiyama H, Teshima R, Futo S, Furui S, Kitta K	Development of multiplex PCR method for simultaneous detection of four events of genetically modified maize: DAS-59122-7, MIR604, MON863 and MON88017	Journal of the Food Hygienic Society of Japan	2010	NO	-	-	-
Ortego F, Pons X, Albajes R, Castanera P	European commercial genetically modified plantings and field trials	Book, entitled "Environmental impact of genetically modified crops"	2009	YES	NO	-	-

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Papazova N, Zhang D, Gruden K, Vojvoda J, Yang L, Gasparic Mb, Blejec A, Fouilloux S, De Loose M, Taverniers I	Evaluation of the reliability of maize reference assays for GMO quantification	Analytical and Bioanalytical Chemistry	2010	NO	-	-	-
Paul V, Guertler P, Wiedemann S, Meyer HHD	Degradation of Cry1Ab protein from genetically modified maize (MON810) in relation to total dietary feed proteins in dairy cow digestion	Transgenic Research	2010	YES	YES	YES	NO
Perez-Hedo M, Albajes R, Eizaguirre M	Modification of hormonal balance in larvae of the corn borer *Sesamia nonagrioides* (Lepidoptera: Noctuidae) due to sublethal *Bacillus thuringiensis* protein ingestion	Journal of Economic Entomology	2011	YES	YES	YES	NO
Perez-Hedo M, Lopez C, Albajes R, Eizaguirre M	Low susceptibility of non-target lepidopteran maize pests to the Bt protein Cry1Ab	Bulletin of Entomological Research	2012	YES	YES	YES	NO
Perez-Hedo M, Marques T, Lopez C, Albajes R, Eizaguirre M	Determination of the Cry1Ab toxin in *Helicoverpa armigera* larvae fed on a diet containing lyophilized Bt leaves	IOBC/wprs Bulletin	2012	YES	NO	-	-
Perry JN	The effect of Bt-maize on butterflies - reckoning the risk	Outlooks on Pest Management	2011	YES	YES	YES	YES
Perry JN, Devos Y, Arpaia S, Bartsch D, Gathmann A, Hails RS, Kiss J, Lheureux K, Manachini B, Mestdagh S, Neemann G, Ortego F, Schiemann J, Sweet JB	A mathematical model of exposure of non-target Lepidoptera to Bt-maize pollen expressing Cry1Ab within Europe	Proceedings of the Royal Society B-Biological Sciences	2010	YES	YES	YES	YES

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Perry JN, Devos Y, Arpaia S, Bartsch D, Gathmann A, Hails RS, Kiss J, Lheureux K, Manachini B, Mestdagh S, Neemann G, Ortego F, Schiemann J, Sweet JB	The usefulness of a mathematical model of exposure for environmental risk assessment	Proceedings of the Royal Society B-Biological Sciences	2011	YES	YES	YES	YES
Perry JN, Devos Y, Arpaia S, Bartsch D, Ehlert C, Gathmann A, Hails RS, Hendriksen NB, Kiss J, Messean A, Mestdagh S, Neemann G, Nuti M, Sweet JB, Tebbe CC	Estimating the effects of Cry1F Bt-maize pollen on non-target Lepidoptera using a mathematical model of exposure	Journal of Applied Ecology	2012	NO	-	-	-
Peterson JA, Obrycki JJ, Harwood JD	Quantification of Bt-endotoxin exposure pathways in carabid food webs across multiple transgenic events	Biocontrol Science and Technology	2009	YES	YES	YES	NO
Piccioni F, Capitani D, Zolla L, Mannina L	NMR metabolic profiling of transgenic maize with the Cry1A(b) gene	Journal of Agricultural and Food Chemistry	2009	YES	YES	YES	NO
Pirondini A, Marmiroli N	Environmental risk assessment in GMO analysis	Rivista di Biologia-Biology Forum	2010	YES	YES	YES	NO
Popescu CF, Visoiu E, Buciumeanu E, Teodorescu A, Gheorghe RN, Tanasescu C, Ciocirlan CN	From plant tissue culture to modern biotechnology at the National Research and Development Institute for Biotechnology in Horticulture Stefanesti: Achievements and Prospects	Romanian Biotechnological Letters	2010	NO	-	-	-
Prasifka JR, Hellmich RL, Sumerford DV, Siegfried BD	*Bacillus thuringiensis* resistance influences European corn borer (Lepidoptera: Crambidae) larval behavior after exposure to Cry1Ab	Journal of Economic Entomology	2009	YES	YES	YES	NO

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Prasifka JR, Hellmich RL, Crespo ALB, Siegfried BD, Onstad DW	Video-tracking and on-plant tests show Cry1Ab resistance influences behavior and survival of neonate *Ostrinia nubilalis* following exposure to Bt maize	Journal of Insect Behavior	2010	YES	YES	YES	NO
Priestley AL, Brownbridge M	Field trials to evaluate effects of Bt-transgenic silage corn expressing the Cry1Ab insecticidal toxin on non-target soil arthropods in northern New England, USA	Transgenic Research	2009	YES	YES	YES	YES
Qiu C, Sangha JS, Song F, Zhou Z, Yin A, Gu K, Tian D, Yang J, Yin Z	Production of marker-free transgenic rice expressing tissue-specific Bt gene	Plant Cell Reports	2010	NO	-	-	-
Randhawa GJ, Singh M, Sharma R	Validation of ST-LS1 as an endogenous reference gene for detection of AmA1 and cry1Ab genes in genetically modified potatoes using multiplex and real time PCR	American Journal of Potato Research	2009	NO	-	-	-
Randhawa GJ, Singh M, Chhabra R, Sharma R	Qualitative and quantitative molecular testing methodologies and traceability systems for commercialised Bt cotton events and other Bt crops under field trials in India	Food Analytical Methods	2010	NO	-	-	-
Rasco ET, Mangubat JR, Burgonio AB, Logrono ML, Villegas VN, Fernandez EC	Agronomic performance and Asiatic corn borer resistance of tropical converted transgenic corn hybrids containing the truncated Cry1A(b) gene (Bt-11) in Davao city, Philippines	Philippine Journal of Crop Science	2010	NO	-	-	-

Authors of publication	Title of publication	Journal	Publication year	Out of scope	Peer-reviewed publication	Publication in English	Previously discussed and/or cited
Raubuch M, Behr K, Roose K, Joergensen RG	Specific respiration rates, adenylates, and energy budgets of soil microorganisms after addition of transgenic Bt-maize straw	Pedobiologia	2010	YES	YES	YES	NO
Rauschen S, Schaarschmidt F, Gathmann A	Occurrence and field densities of Coccinellidae in the maize herb layer: implications for environmental risk assessment	IOBC/wprs bulletin	2010	YES	NO	-	-
Rauschen S, Schaarschmidt F, Gathmann A	Occurrence and field densities of Coleoptera in the maize herb layer: implications for Environmental Risk Assessment of genetically modified Bt-maize	Transgenic Research	2010	YES	YES	YES	YES
Rauschen S	A case of 'pseudo science'? A study claiming effects of the Cry1Ab protein on larvae of the two-spotted ladybird is reminiscent of the case of the green lacewing	Transgenic Research	2010	YES	YES	YES	YES
Rauschen S, Schultheis E, Pagel-Wieder S, Schuphan I, Eber S	Impact of Bt-corn MON88017 in comparison to three conventional lines on *Trigonotylus caelestialium* (Kirkaldy) (Heteroptera: Miridae) field densities	Transgenic Research	2009	NO	-	-	-
Raybould A, Graser G, Hill K, Ward K	Ecological risk assessments for transgenic crops with combined insect-resistance traits: the example of Bt11 x MIR604 maize	Journal of Applied Entomology	2012	NO	-	-	-

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Raybould A, Higgins LS, Horak MJ, Layton RJ, Storer NP, De La Fuente JM, Herman RA	Assessing the ecological risks from the persistence and spread of feral populations of insect-resistant transgenic maize	Transgenic Research	2012	YES	YES	YES	YES
Razze JM, Mason E	Dispersal behaviour of neonate European corn borer (Lepidoptera: Crambidae) on Bt corn	Ecology and Behavior	2012	YES	YES	YES	YES
Razze JM, Mason CE, Pizzolato TD	Feeding behavior of neonate *Ostrinia nubilalis* (Lepidoptera: Crambidae) on Cry1Ab Bt corn: Implications for resistance management	Journal of Economic Entomology	2011	YES	YES	YES	NO
Reavy-Jones FPF, Wiatrak P, Greene JK	Evaluating the performance of transgenic corn producing *Bacillus thuringiensis* toxins in South Carolina	Journal of Agricultural and Urban Entomology	2009	YES	YES	YES	YES
Reavy-Jones FPF, Wiatrak P	Evaluation of new transgenic corn hybrids producing multiple *Bacillus thuringiensis* toxins in South Carolina	Journal of Entomological Science	2011	YES	YES	YES	YES
Regnault-Roger C, Folcher L, Delos M, Jarry M, Weissenberger A, Eychenne N	Bt maize: a tool for improving food safety of grains at harvest	Julius-Kühn-Archives (proceedings abstract)	2010	YES	NO	-	-
Rehout V, Kadlec J, Citek J, Hradecka E, Hanusova L, Hosnedlova B, Lad F	The influence of genetically modified Bt maize MON 810 in feed mixtures on slaughter, haematological and biochemical indices of broiler chickens	Journal of Animal and Feed Sciences	2009	YES	YES	YES	NO
Ricroch A, Berge JB, Kuntz M	Is the German suspension of MON810 maize cultivation scientifically justified?	Transgenic Research	2010	NO	-	-	-

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Ridley WP, Harrigan GG, Breeze ML, Nemeth MA, Sidhu RS, Glenn KC	Evaluation of compositional equivalence for multitrait biotechnology crops	Journal of Agricultural and Food Chemistry	2011	NO	-	-	-
Rimachi Gamarra LF, Alcantara Delgado J, Aquino Villasante Y, Ortiz R	Detecting adventitious transgenic events in a maize center of diversity	EJB, Electronic Journal of Biotechnology	2011	NO	-	-	-
Romeis J, Alvarez-Alfageme F, Bigler F	Putative effects of Cry1Ab to larvae of *Adalia bipunctata* - reply to Hilbeck et al. (2012)	Environmental Sciences Europe	2012	YES	YES	YES	NO
Rossi F, Morlacchini M, Fusconi G, Pietri A, Piva G	Effect of insertion of Bt gene in corn and different fumonisin content on growth performance of weaned piglets	Italian Journal of Animal Science	2011	NO	-	-	-
Roth L, Zagon J, Ehlers A, Kroh LW, Broll H	A novel approach for the detection of DNA using immobilized peptide nucleic acid (PNA) probes and signal enhancement by real-time immuno-polymerase chain reaction (RT-iPCR)	Analytical and Bioanalytical Chemistry	2009	NO	-	-	-
Ryffel GU	Dismay with GM maize - a science-based solution to public resistance against genetically modified crops that could be compatible with organic farming	EMBO Reports	2011	NO	-	-	-
Sanvido O, Romeis J, Bigler F	An approach for post-market monitoring of potential environmental effects of Bt-maize expressing Cry1Ab on natural enemies	Journal of Applied Entomology	2009	YES	YES	YES	YES

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Sanvido O, Romeis J, Bigler F	Environmental change challenges decision-making during post-market environmental monitoring of transgenic crops	Transgenic Research	2011	YES	YES	YES	YES
Sartowska K, Korwin-Kossakowska A, Sender G, Jozwik A, Prokopiuk M	The impact of genetically modified plants in the diet of Japanese quails on performance traits and the nutritional value of meat and eggs - preliminary results	Archiv Für Geflügelkunde	2012	YES	YES	YES	NO
Schuppener M, Muehlhause J, Mueller AK, Rauschen S	Environmental risk assessment for the small tortoiseshell *Aglais urticae* and a stacked Bt-maize with combined resistances against Lepidoptera and Chrysomelidae in central European agrarian landscapes	Molecular Ecology	2012	NO	-	-	-
Selwet M	Maize plants infestation by *Fusarium* spp. and deoxynivalenol in genetically modified corn hybrid and traditional maize cultivars	Polish Journal of Microbiology	2011	NO	-	-	-
Sharma P, Nain V, Lakhanpaul S, Kumar PA	Synergistic activity between *Bacillus thuringiensis* Cry1Ab and Cry1Ac toxins against maize stem borer (*Chilo partellus* Swinhoe)	Letters in Applied Microbiology	2010	NO	-	-	-
Sharma P, Nain V, Lakhanpaul S, Kumar PA	Binding of *Bacillus thuringiensis* Cry1A toxins with brush border membrane vesicles of maize stem borer (*Chilo partellus* Swinhoe)	Journal of Invertebrate Pathology	2011	NO	-	-	-

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Shelton AM, Naranjo SE, Romeis J, Hellmich RL	Errors in logic and statistics plague a meta-analysis (response to Andow and Lovei 2012)	Environmental Entomology	2012	YES	YES	YES	NO
Shu Y, Ma H, Du Y, Li Z, Feng Y, Wang J	The presence of *Bacillus thuringiensis* (Bt) protein in earthworms *Eisenia fetida* has no deleterious effects on their growth and reproduction	Chemosphere	2011	YES	YES	YES	NO
Shuang W, Zhen C, Jun M, Wei-Bin B, Xi-Yang W	Multiplex tandem PCR assays for the detection of genetically modified organisms	Scientia Agricultura Sinica	2012	NO	-	-	-
Siegfried BD, Hellmich RL	Understanding successful resistance management. The European corn borer and Bt corn in the United States	GM Crops and Food: Biotechnology in Agriculture and the Food Chain	2012	YES	YES	YES	YES
Sieradzki Z, Kwiatek K	Validation of real-time PCR methods for the quantification of genetically-modified maize and soybean	Bulletin of the Veterinary Institute in Pulawy	2009	NO	-	-	-
Sieradzki Z, Mazur M, Kwiatek K	Occurrence of genetically modified crops in animal feeding stuffs in Poland	Krmiva	2010	NO	-	-	-
Sissener NH, Johannessen LE, Hevroy EM, Wiik-Nielsen CR, Berdal KG, Nordgreen A, Hemre G	Zebrafish (*Danio rerio*) as a model for investigating the safety of GM feed ingredients (soya and maize); performance, stress response and uptake of dietary DNA sequences	British Journal of Nutrition	2010	YES	YES	YES	NO
Sissener NH, Hemre GI, Lall SP, Sagstad A, Petersen KWJ, Rohloff J, Sanden M	Are apparent negative effects of feeding GM MON810 maize to Atlantic salmon, *Salmo salar*, caused by confounding factors?	British Journal of Nutrition	2011	YES	YES	YES	NO

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Soberon M, Gill SS, Bravo A	Signaling versus punching hole: How do *Bacillus thuringiensis* toxins kill insect midgut cells?	Cellular and Molecular Life Sciences	2009	YES	YES	YES	YES
Sobiech L, Skrzypczak W, Sulewska H, Michalski T	Occurrence of *Ostrinia nubilalis* Hbn. and *Ustilago maydis* (DC) corda on conventional and Bt maize cultivars	Progress in Plant Protection	2011	YES	YES	NO	-
Sorokina EY, Chernyshova ON	Detection of recombinant-DNA in foods from stacked genetically modified plants	Voprosy Pitaniia	2012	NO	-	-	-
Stadnik J, Karwowska M, Dolatowski ZJ, Swiatkiewicz M, Kwiatek K	Effect of genetically modified feeds on physico-chemical properties of pork	Annals of Animal Science	2011	YES	YES	YES	NO
Stadnik J, Karwowska M, Dolatowski ZJ, Swiatkiewicz S, Kwiatek K	Effect of genetically modified, insect resistant corn (MON 810) and glyphosate tolerant soybean meal (roundup ready) on physico-chemical properties of broilers' breast and thigh muscles	Bulletin of the Veterinary Institute in Pulawy	2011	YES	NO	-	-
Steinke K, Guertler P, Paul V, Wiedemann S, Ettle T, Albrecht C, Meyer H H D, Spiekers H, Schwarz F J	Effects of long-term feeding of genetically modified corn (event MON810) on the performance of lactating dairy cows	Journal of Animal Physiology and Animal Nutrition	2010	YES	YES	YES	NO
Steinke K, Spiekers H	Foreign genes in animal feeds. Feeding trials using genetically modified maize	Neue Landwirtschaft	2009	NO	-	-	-
Stephens EJ, Losey JE, Allee LL, DiTommaso A, Bodner C, Breyre A	The impact of Cry3Bb Bt-maize on two guilds of beneficial beetles	Agriculture Ecosystems & Environment	2012	NO	-	-	-
Storer NP, Thompson GD, Head GP	Application of pyramided traits against Lepidoptera in insect resistance management for Bt crops	GM Crops and Food: Biotechnology in Agriculture and the Food Chain	2012	YES	YES	YES	YES

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Storer NP, Babcock JM, Schlenz M, Meade T, Thompson GD, Bing JW, Huckaba RM	Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico	Journal of Economic Entomology	2010	NO	-	-	-
Sulan B, Jie Z, Shucheng L, Haodong C, Terzaghi W, Xin Z, Xiurong C, Jin T, Hongxia L, Wensheng H, Ying C, Yaochuan Z	Detection of six genetically modified maize lines using optical thin-film biosensor chips.	Journal of Agricultural and Food Chemistry	2010	NO	-	-	-
Su-Youn K, Jae-Hwan K, Hyungjae L, Hae-Yeong K	Detection system of stacked genetically modified maize using multiplex PCR	Food Science and Biotechnology	2010	NO	-	-	-
Swiatkiewicz S, Koreleski J, Arczewska A, Twardowska M, Kwiatek K, Tomczyk G, Kozaczynski W, Mazur M, Bednarek D	Safety of transgenic feed materials in poultry nutrition - results of Polish study	Zycie Weterynaryjne	2010	NO	-	-	-
Swiatkiewicz S, Swiatkiewicz M, Koreleski J, Kwiatek K	Nutritional efficiency of genetically-modified insect resistant corn (MON 810) and glyphosate-tolerant soybean meal (roundup ready) for broilers	Bulletin of the Veterinary Institute in Pulawy	2010	YES	NO	-	-
Swiatkiewicz S, Koreleski J, Arczewska-Wlosek A, Swiatkiewicz M, Twardowska M, Markowski J, Mazur M, Sieradzki Z, Kwiatek K	Detection of transgenic DNA from Bt maize and herbicide tolerant soybean meal in tissues, eggs and digestive tract content of laying hens fed diets containing genetically modified plants	Annals of Animal Science	2011	YES	YES	YES	NO

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Szekacs A, Darvas B	Environmental assessment of MON 810 maize in the pannonian biogeographical region	Acta Phytopathologica et Entomologica Hungarica	2012	YES	YES	YES	NO
Szekacs A, Lauber E, Juracsek J, Darvas B	CRY1AB toxin production of MON 810 transgenic maize	Environmental Toxicology and Chemistry	2010	YES	YES	YES	NO
Szekacs A, Lauber E, Takacs E, Darvas B	Detection of Cry1Ab toxin in the leaves of MON 810 transgenic maize	Analytical and Bioanalytical Chemistry	2010	YES	YES	YES	YES
Szekacs A, Weiss G, Quist D, Takacs E, Darvas B, Meier M, Swain T, Hilbeck A	Inter-laboratory comparison of Cry1Ab toxin quantification in MON 810 maize by enzyme-immunoassay	Food and Agricultural Immunology	2012	YES	YES	YES	NO
Tabashnik BE, Carriere Y	Insect resistance to genetically modified crops	Book chapter (review)	2009	YES	NO	-	-
Tabashnik BE, van Rensburg JBJ, Carriere Y	Field-evolved insect resistance to Bt crops: definition, theory, and data	Journal of Economic Entomology	2009	YES	YES	YES	YES
Tahar SB, Salva I, Brants IO	Genetic stability in two commercialized transgenic lines (MON810)	Nature Biotechnology	2010	YES	YES	YES	NO
Takacs E, Darvas B, Szekacs A	Analytical difficulties and certain biological aspects of Cry1Ab toxin determination in MON 810 genetically modified maize	Acta Phytopathologica et Entomologica Hungarica	2012	NO	-	-	-
Tamez-Guerra P	A review of US and Mexican cooperation to develop insect resistance management and monitoring methods for surveying transgenic crops expressing *Bacillus thuringiensis* proteins 2003 to 2010	Southwestern Entomologist	2010	YES	YES	YES	NO

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Tan F, Wang J, Chen Z, Feng Y, Chi G, Rehman SU	Assessment of the arbuscular mycorrhizal fungal community in roots and rhizosphere soils of Bt corn and their non-Bt isolines	Soil Biology & Biochemistry	2011	YES	YES	YES	NO
Tan F, Wang J, Feng Y, Chi G, Kong H, Qiu H, Wei S	Bt corn plants and their straw have no apparent impact on soil microbial communities	Plant and Soil	2010	YES	YES	YES	NO
Tan SY, Cayabyab BF, Alcantara EP, Ibrahim YB, Huang F, Blankenship EE, Siegfried BD	Comparative susceptibility of *Ostrinia furnacalis*, *Ostrinia nubilalis* and *Diatraea saccharalis* (Lepidoptera: Crambidae) to *Bacillus thuringiensis* Cry1 toxins	Crop Protection	2011	YES	YES	YES	YES
Tank JL, Rosi-Marshall EJ, Royer TV, Whiles MR, Griffiths NA, Frauendorf TC, Treering DJ	Occurrence of maize detritus and a transgenic insecticidal protein (Cry1Ab) within the stream network of an agricultural landscape	Proceedings of the National Academy of Sciences of the United States of America	2010	YES	YES	YES	YES
Taube F, Theobald W	Genetic engineering - An assessment model, part 2 (case study)	Umweltwissenschaften und Schadstoff-Forschung	2010	NO	-	-	-
Tende RM, Mugo SN, Nderitu JH, Olubayo FM, Songa JM, Bergvinson DJ	Evaluation of *Chilo partellus* and *Busseola fusca* susceptibility to delta-endotoxins in Bt maize	Crop Protection	2010	YES	YES	YES	NO
Tian JC, Liu ZC, Chen M, Chen Y, Chen XX, Peng YF, Hu C, Ye GY	Laboratory and field assessments of prey-mediated effects of transgenic Bt rice on *Ummeliata insecticeps* (Araneida: Linyphiidae)	Environmental Entomology	2010	NO	-	-	-

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Tiwari S, Youngman RR, Laub CA, Brewster CC, Jordan TA, Teutsch C	European corn borer (Lepidoptera: Crambidae) infestation level and plant growth stage on whole-plant corn yield grown for silage in Virginia	Journal of Economic Entomology	2009	YES	YES	YES	NO
Trapmann S, Corbisier P, Schimmel H, Emons H	Towards future reference systems for GM analysis	Analytical and Bioanalytical Chemistry	2010	NO	-	-	-
Twardowski JP, Beres P, Hurej M, Klukowski Z	Ground beetles (Col., Carabidae) in Bt-maize - preliminary results from the first large scale field experiment in Poland	IOBC/wprs Bulletin	2010	YES	NO	-	-
van de Wiel CCM, Groeneveld RMW, Dolstra O, Kok EJ, Scholtet IMJ, Thissen JTNM, Smulders MJM, Lotz LAP	Pollen-mediated gene flow in maize tested for coexistence of GM and non-GM crops in the Netherlands: effect of isolation distances between fields	NJAS - Wageningen Journal of Life Sciences	2009	YES	YES	YES	YES
van Kretschmar JB, Bailey WD, Arellano C, Thompson GD, Sutula CL, Roe RM	Feeding disruption tests for monitoring the frequency of larval lepidopteran resistance to Cry1Ac, Cry1F and Cry1Ab	Crop Protection	2011	YES	YES	YES	YES
van Wyk A, van den Berg J, van Rensburg JBJ	Comparative efficacy of Bt maize events MON810 and Bt11 against *Sesamia calamistis* (Lepidoptera: Noctuidae) in South Africa	Crop Protection	2009	YES	YES	YES	YES
Verbruggen E, Kuramae EE, Hillekens R, de Hollander M, Kiers ET, Röling WFM, Kowalchuk GA, van der Heijden MGA	Testing potential effects of Bt maize on mycorrhizal fungal communities via DNA- and RNA- based pyrosequencing and molecular fingerprinting	Applied and Environmental Microbiology	2012	YES	YES	YES	NO

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Vijayakumar KR, Martin A, Gowda LR, Prakash V	Detection of genetically modified soya and maize: Impact of heat processing	Food Chemistry	2009	NO	-	-	-
Viktorov AG	Transfer of Bt corn byproducts from terrestrial to stream ecosystems	Russian Journal of Plant Physiology	2011	YES	YES	YES	NO
Vyhnanek T, Hanacek P	Optimisation of qualitative and semi-quantitative detection of genetically modified crops by PCR	Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis	2009	NO	-	-	-
Walsh MC, Buzoianu SG, Gardiner GE, Rea MC, Ross RP, Lawlor PG	Short-term feeding of genetically modified Bt maize (MON810) to weanling pigs: Effects on gut microbiota, intestinal morphology and immune status	Journal of Dairy Science	2010	YES	YES	YES	NO
Walsh MC, Buzoianu SG, Gardiner GE, Rea MC, Gelencser E, Janosi A, Epstein MM, Ross RP, Lawlor PG	Fate of transgenic DNA from orally administered Bt MON810 maize and effects on immune response and growth in pigs	PLoS ONE	2011	YES	YES	YES	NO
Walsh MC, Buzoianu SG, Rea MC, O'Donovan O, Gelencser E, Ujhelyi G, Ross RP, Gardiner GE, Lawlor PG	Effects of feeding Bt MON810 maize to pigs for 110 days on peripheral immune response and digestive fate of the cry1Ab gene and truncated Bt toxin	PLoS ONE	2012	YES	YES	YES	NO
Walsh MC, Buzoianu SG, Gardiner GE, Rea MC, Ross RP, Cassidy JP, Lawlor PG	Effects of short-term feeding of Bt MON810 maize on growth performance, organ morphology and function in pigs	British Journal of Nutrition	2012	YES	YES	YES	NO
Wang YH, Chen XQ, Xue J, Yang FP, Li XL, Zhang XD	Expression of Cry1Ab/1Ac gene in transgenic maize	Molecular Plant Breeding	2010	YES	YES	NO	-

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Wang Y, Xu W, Zhao W, Hao J, Luo Y, Tang X, Zhang Y, Huang Kunlun	Comparative analysis of the proteomic and nutritional composition of transgenic rice seeds with Cry1ab/ac genes and their non-transgenic counterparts	Journal of Cereal Science	2012	NO	-	-	-
Wang Y, Li Y, Romeis J, Chen X, Zhang J, Chen H, Peng Y	Consumption of Bt rice pollen expressing Cry2Aa does not cause adverse effects on adult *Chrysoperla sinica* Tjeder (Neuroptera: Chrysopidae)	Biological Control	2012	NO	-	-	-
Wendt C, Freier B, Volkmar C, Schorling M, Wieacker K	Assessment of Bt maize effects on non-target arthropods in field studies using the evaluation approach of "good ecological state"	IOBC/wprs Bulletin	2010	YES	NO	-	-
Wiedemann S, Lutz B, Albrecht C, Kuehn R, Killermann B, Einspanier R, Meyer, HHD	Fate of genetically modified maize and conventional rapeseed, and endozoochory in wild boar (*Sus scrofa*)	Mammalian Biology	2009	NO	-	-	-
Wilhelm R, Sanvido O; Castanera P, Schmidt K, Schiemann J	Monitoring the commercial cultivation of Bt maize in Europe--conclusions and recommendations for future monitoring practice	Environmental Biosafety Research	2009	YES	YES	YES	YES
Williams WP, Windham GL, Krakowsky MD, Scully BT, Ni XZ	Aflatoxin accumulation in BT and non-BT maize testcrosses	Journal of Crop Improvement	2010	NO	-	-	-
Wu H, Sun H, Li B, Yang C, Lu X	Detection of genetically modified maize by multiplex PCR-gene chip	Journal of Agricultural Biotechnology	2009	NO	-	-	-

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Wu X, Huang F, Leonard BR, Ghimire M	Growth and development of *Bacillus thuringiensis* Cry1Ab-susceptible and Cry1Ab-resistant sugarcane borer on diet and conventional maize plants	Entomologia Experimentalis et Applicata	2009	YES	YES	YES	NO
Wu X, Leonard BR, Zhu YC, Abel CA, Head GP, Huang F	Susceptibility of Cry1Ab-resistant and -susceptible sugarcane borer (Lepidoptera: Crambidae) to four *Bacillus thuringiensis* toxins	Journal of Invertebrate Pathology	2009	NO	-	-	-
Xiaolei Z, Lili C, Ping S, Junwei J, Dabing Z, Litao Y	High sensitive detection of Cry1Ab protein using a quantum dot-based fluorescence-linked immunosorbent assay	Journal of Agricultural and Food Chemistry	2011	NO	-	-	-
Xing Z, Wang Z, He K, Bai S	Degradation dynamics of Cry1Ab insecticidal protein within transgenic *Bacillus thuringiensis* corn root debris and rhizosphere soil in field	Scientia Agricultura Sinica	2010	YES	YES	NO	-
Xu L, Wang Z, Zhang J, He K, Ferry N, Gatehouse AMR	Cross-resistance of Cry1Ab-selected Asian corn borer to other Cry toxins	Journal of Applied Entomology	2010	YES	YES	YES	YES
Xu W, Yuan Y, Luo Y, Bai W, Zhang C, Huang K	Event-specific detection of stacked genetically modified maize Bt11 x GA21 by UP-M-PCR and real-time PCR	Journal of Agricultural and Food Chemistry	2009	NO	-	-	-
Xu Y, Wang ZY, He KL, Bai SX	Histopathological changes in the midgut of larvae of the Asian corn borer, *Ostrinia furnacalis* (Lepidoptera: Crambidae), fed on Bt-transgenic corn expressing Cry1Ab protein	Acta Entomologica Sinica	2009	YES	YES	NO	-
Xu W, Cao S, He X, Luo YB, Guo X, Yuan Y, Huang K	Safety assessment of Cry1Ab/Ac fusion protein	Food and Chemical Toxicology	2009	NO	-	-	-

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Yanni SF, Whalen JK, Ma BL	Crop residue chemistry, decomposition rates, and CO2 evolution in Bt and non-Bt corn agroecosystems in North America: a review	Nutrient Cycling in Agroecosystems	2010	YES	YES	YES	NO
Yoke-Kqueen C, Yee-Tyan C, Siew-Ping K, Son R	Development of multiplex-PCR for Genetically Modified Organism (GMO) detection targeting EPSPS and Cry1Ab genes in soy and maize samples	International Food Research Journal	2011	NO	-	-	-
Yu HL, Li YH, Wu KM	Risk assessment and ecological effects of transgenic *Bacillus thuringiensis* crops on non-target organisms	Journal of Integrative Plant Biology	2011	YES	YES	YES	NO
Yuan Y, Ke X, Chen F, Krogh PH, Ge F	Decrease in catalase activity of *Folsomia candida* fed a Bt rice diet	Environmental Pollution	2011	NO	-	-	-
Zaulet M, Rusu L, Kevorkian S, Luca C, Mihacea S, Badea EM, Costache M	Detection and quantification of GMO and sequencing of the DNA amplified products	Romanian Biotechnological Letters	2009	NO	-	-	-
Zeilinger AR, Andow DA, Zwahlen C, Stotzky G	Earthworm populations in a northern US Cornbelt soil are not affected by long-term cultivation of Bt maize expressing Cry1Ab and Cry3Bb1 proteins	Soil Biology & Biochemistry	2010	YES	YES	YES	YES
Zhai Z, Xu W, Zhang N, Yan X, Wang Y, Luo Y, Huang K	Event-specific transgenic detection of genetically modified maize LY038	Journal of Agricultural Biotechnology	2011	NO	-	-	-
Zhang N, Xu W, Bai W, Zhai Z, Luo Y, Yan X, He J, Huang K	Event-specific qualitative and quantitative PCR detection of LY038 maize in mixed samples	Food Control	2011	NO	-	-	-

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Zhang M, Tang Q, Chen Z, Liu J, Cui H, Shu Q, Xia Y, Altosaar I	Genetic transformation of Bt gene into sorghum (*Sorghum bicolor* L.) mediated by *Agrobacterium tumefaciens*	Chinese Journal of Biotechnology	2009	NO	-	-	-
Zhang W, Shi F	Do genetically modified crops affect animal reproduction? A review of the ongoing debate	Animal	2011	NO	-	-	-
Zhang Y, Lai C, Su R, Zhang M, Xiong Y, Qing H, Deng Y	Quantification of Cry1Ab in genetically modified maize leaves by liquid chromatography multiple reaction monitoring tandem mass spectrometry using O-18 stable isotope dilution	Analyst	2012	NO	-	-	-
Zhou J, Harrigan GG, Berman KH, Webb EG, Klusmeyer TH, Nemeth MA	Stability in the composition equivalence of grain from insect-protected maize and seed from glyphosate-tolerant soybean to conventional counterparts over multiple seasons, locations, and breeding germplasms	Journal of Agricultural and Food Chemistry	2011	YES	YES	YES	NO
Zhu YY, Tian X, Zhao D	Construction and application of the vector containing the Bar::gus fusion gene and LoxP/FRT recognition site	Genomics and Applied Biology	2011	NO	-	-	-
Zurbrügg C, Hönemann L, Meissle M, Romeis J, Nentwig W	Decomposition dynamics and structural plant components of genetically modified Bt maize leaves do not differ from leaves of conventional hybrids	Transgenic Research	2010	YES	YES	YES	YES
Zurbrügg C, Nentwig W	Ingestion and excretion of two transgenic Bt corn varieties by slugs	Transgenic Research	2009	YES	YES	YES	YES

B. OVERVIEW OF LOWER-TIER STUDIES EXPOSING *ADALIA BIPUNCTATA* TO CRY1AB

	Schmidt et al. (2004, 2009)	**Porcar et al. (2010)**	**Álvarez-Alfageme et al. (2011) Romeis et al. (2012)**		**Hilbeck et al. (2012)**
Type of study	Tier 1a	Tier 1a	Tier 1a	Tier 1b	Tier 1a
Stage tested	1st to 4st instars, pupae	1st & 2nd instars	1st to 4th instars, pupae	1st & 2nd instars	1st to 4st instars, pupae
# insects tested	30	30	34-41	3	32
# replicates	4	3	1	15	3
Total # individuals tested	120 per treatment	90 per treatment	34-41 per treatment	45 per treatment	96 per treatment
Test material	Cry1Ab 5, 25 and 50 µg/ml diet	Cry1Ab 50 µg/ml diet	Cry1Ab 45 µg/ml diet	Prey fed maize MON 810	Cry1Ab
Route of exposure	Moth eggs coated with Cry1Ab	Artificial diet with Cry1Ab	Artificial diet with Cry1Ab	Spider mite (*Tetranychus urticae*)	Moth eggs coated with Cry1Ab & cotton balls moistened with Cry1Ab
Exposure duration	Continuous exposure 9-10 days	Continuous exposure 6 days	Discontinuous exposure 4 x 24 hours	Continuous exposure ~7 days	Discontinuous and continuous exposure 6 days
Positive (toxic) control	0	1	2	0	0
Endpoints	Mortality, development time and weight	Mortality	Mortality, development time and weight	Mortality, development time and weight	Mortality
Effect reported	Decreased survival of 1st instars at all concentrations, but less at the highest concentration tested; no effect on development time and weight	No effect on survival	No effect on survival, development time and weight	No effect on survival, development time and weight	Decreased survival when exposed continuously
Duration experiment	~16 days	6 days	~16 days	~7 days	6 days
Cry1Ab intake	Not quantified	Not quantified	Not quantified	Cry1Ab concentration quantified in prey and predator	Not quantified