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SCIENTIFIC OPINION

Application (Reference EFSA-GMO-NL-2007-37) for the placing on the market of the insect-resistant genetically modified maize MON89034, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto[1]

Scientific Opinion of the Panel on Genetically Modified Organisms

(Question No EFSA-Q-2007-042)

Adopted on 03 December 2008

PANEL MEMBERS*

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SUMMARY

This document provides an opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on genetically modified maize MON89034 (Unique Identifier MON-89Ø34-3) developed to provide resistance to certain insect pests.

In delivering its scientific opinion, the GMO Panel considered the new application EFSA-GMO-NL-2007-37, additional information provided by the applicant (Monsanto) and the scientific comments submitted by the Member States. The scope of application EFSA-GMO-NL-2007-37 is for food and feed uses, import and processing of maize MON89034 and all derived products, but excluding cultivation in the EU.

The GMO Panel assessed maize MON89034 with reference to the intended uses and the appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The scientific assessment included molecular characterisation of the

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

inserted DNA and expression of the new proteins. A comparative analysis of agronomic traits and composition was undertaken and the safety of the newly expressed proteins and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plan was undertaken.

Maize MON89034 was transformed by *Agrobacterium tumefaciens*-mediated gene transfer technology. Maize MON89034 contains the Cry1A.105 and the Cry2Ab2 expression cassettes (T-DNA I) but does not contain the *npt*II expression cassette (T-DNA II). The Cry1A.105 and the Cry2Ab2 expression cassettes confer resistance to certain insect pests.

The molecular characterisation data established that a single insert with one copy of the intact T-DNA I expression cassette is integrated in the maize genomic DNA. Appropriate analyses of the integration site including sequence determination of the inserted DNA and flanking regions and bioinformatic analysis have been performed. Bioinformatic analysis of junction regions demonstrated the absence of any potential new ORFs coding for known toxins or allergens. The expression of the genes introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of maize MON89034 does not raise any safety concern, and that sufficient evidence for the stability of the genetic modification was provided.

Based on the results of compositional analysis of samples from a representative range of environments, the GMO Panel concludes that forage and kernels of maize MON89034 are compositionally equivalent to those of the non-GM control and other conventional maize, except for the presence of the Cry1A.105 and Cry2Ab2 proteins. In addition, field trials did not show changes in phenotypic characteristics and agronomic performance except for the introduced traits.

No indications of adverse effects related to the exposure to the Cry1A.105 protein or the Cry2Ab2 protein were found in studies on acute oral toxicity in mice. There were no adverse effects in a 90-day feeding study with rats fed diets including kernels from maize MON89034. A feeding study on broiler chickens provided evidence of nutritional equivalence of maize MON89034 to conventional maize. In addition, the overall allergenicity of the whole plant is not changed. The GMO Panel is of the opinion that maize MON89034 is as safe as conventional maize. Maize MON89034 and derived products are unlikely to have any adverse effect on human and animal health in the context of the intended uses.

The application for maize MON89034 concerns food and feed uses, import and processing of maize MON89034 and all derived products, but excluding cultivation in the EU. There is therefore no requirement for scientific assessment of possible environmental effects associated with the cultivation of maize MON89034. Considering the scope of the application, not for cultivation, the GMO Panel is of the opinion that the likelihood of the spread and establishment of maize MON89034 is very low and that unintended environmental effects due to this maize will be no different from that of conventional maize varieties. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize MON89034. The monitoring plan provided by the applicant is in line with the EFSA Guidance Document and the Opinion of the GMO Panel on post-market environmental monitoring.

In conclusion, the GMO Panel considers that the information available for maize MON89034 addresses the scientific comments raised by the Member States and that the GM maize

MON89034 is as safe as its non genetically modified counterpart with respect to potential effects on human and animal health or the environment. Therefore the GMO Panel concludes that maize MON89034 is unlikely to have any adverse effect on human or animal health or on the environment in the context of its intended uses.

Key words: GMO, maize, MON89034, insect-resistance, Cry proteins, Cry1A.105, Cry2Ab2, human and animal health, environment, import, processing, food, feed, Regulation (EC) No 1829/2003.

TABLE OF CONTENTS

BACKGROUND

On 31 January 2007, EFSA received from the Competent Authority of The Netherlands an application (Reference EFSA-GMO-NL-2007-37), for authorisation of the insect-resistant genetically modified maize MON89034 (Unique Identifier MON-89Ø34-3), submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed (EC, 2003) for food and feed uses, import and processing.

After receiving the application EFSA-GMO-NL-2007-37 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States as well as the European Commission and made the summary of the dossier publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 13 August 2007, the applicant provided EFSA with additional information requested under completeness check (requested on 13 July and 7 August 2007) and on 24 August 2007 EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Regulation (EC) No 1829/2003 and Directive 2001/18/EC (EC, 2001) following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member State bodies had three months after the date of receipt of the valid application (until 24 November 2007) within which to make their opinion known.

The GMO Panel carried out a scientific assessment of genetically modified (GM) maize MON89034 taking into account the appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a).

On 27 November 2007, 21 January 2008, 27 February 2008 and 2 July 2008, the GMO Panel asked for additional data on maize MON89034. The applicant provided the requested information on 10 December 2007, 14 February 2008, 8 May 2008 and on 30 October 2008. After receipt and assessment of the full data package, the GMO Panel finalised its risk assessment of maize MON89034.

The GMO Panel carried out a scientific assessment of the GM maize MON89034 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into consideration the scientific comments of the Member States and the additional information provided by the applicant.

In giving its opinion on GM maize MON89034 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the receipt of the valid application. As additional information was requested by the EFSA GMO Panel, the time-limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, the EFSA opinion shall include a report describing the assessment of the food and feed and stating the reasons for its opinion and the information on which its opinion is based. This document is to be seen as the report requested

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

TERMS OF REFERENCE

The GMO Panel was requested to carry out a scientific assessment of the genetically modified maize MON89034 for food and feed uses and import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environments and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)e of Regulation (EC) No 1829/2003.

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

ACKNOWLEDGEMENTS

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ASSESSMENT

1. Introduction

The genetically modified (GM) maize MON89034 (Unique Identifier MON-89Ø34-3) was assessed with reference to its intended uses, taking account of the appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a). The risk assessment presented here is based on the information provided in the application EFSA-GMO-NL-2007-37 submitted in the EU including additional information from the applicant. In its evaluation the GMO Panel also considered the scientific comments that were raised by Member States on the present application.

2. Molecular Characterisation

2.1. Issues raised by the Member States

Comments from Member States are addressed in the table published as Annex G of the EFSA overall opinion on maize MON89034.

2.2. Evaluation of relevant scientific data

2.2.1. Transformation process and vector constructs

The aim was to generate maize plants producing the insecticidal proteins Cry1A.105 and Cry2Ab2. Maize MON89034 was developed through *Agrobacterium*-mediated transformation (strain ABI) of immature maize embryos using the binary plasmid vector PV-ZMIR245. Strain ABI also contains a helper plasmid that does not contain any T-DNA but allows for the transfer of T-DNA I and T-DNA II to the plant cells. PV-ZMIR245 contains two separate T-DNAs. The first T-DNA, designated as T-DNA I, contains the Cry1A.105 and the Cry2Ab2 expression cassettes. The Cry1A.105 protein is a modified Bt Cry1A protein with amino acid sequence identity to Cry1Ab, Cry1Ac and Cry1F proteins of 90.0%, 93.6 % and 76.7%, respectively. The Cry1A.105 protein consists substantially of domains I and II from Cry1Ab or Cry1Ac (these proteins share 100% amino acid sequence identity in domains I and II), domain III from Cry1F and the entire C-terminal domain of Cry1Ac protein. The Cry2Ab2 protein present in maize MON89034 is a member of the Cry2Ab class of proteins that share more than 95% amino acid sequence homology. It is a variant of the wild-type Cry2Ab2 protein isolated from *Bacillus thuringiensis* subsp. *kurstaki*. The second T-DNA, designated as T-DNA II, contains the *npt*II expression cassette that encodes the neomycin phosphotransferase enzyme that confers tolerance to certain antibiotics such as neomycin, kanamycin and paromomycin. The use of the two T-DNA approaches facilitates integration of the two different T-DNAs at genetic loci which can be segregated by conventional breeding. This allows the selection of plants which contain only T-DNA I and which lack the *npt*II marker gene.

2.2.1.1. T-DNA I

The expression cassette for the coding sequence of the Cry1A.105 protein is driven by a promoter from *Cauliflower mosaic virus* (CaMV) 35S RNA. The promoter contains a duplicated enhancer region and is designated promoter e35S. The expression cassette also contains the 5' untranslated leader of the wheat chlorophyll a/b binding protein (L-Cab), the intron from the rice actin (*ract*1) gene, the Cry1A.105 coding sequence optimised for

expression in monocots, and the 3' non-translated region of the coding sequence for wheat heat shock protein 17.3 (T-Hsp17), which terminates transcription and provides the signal for mRNA polyadenylation.

The *cry2Ab2* gene expression cassette that produces the Cry2Ab2 protein consists of the 35S promoter from *Figwort mosaic virus* (P-FMV), the first intron from the maize heat shock protein 70 gene (I-Hsp70). It contains a *cry2Ab2* coding sequence with a modified codon usage (CS-Cry2Ab2) fused to a chloroplast transit peptide region of maize ribulose 1,5-biphosphate carboxylase small subunit and its first intron (TS-SSU-CTP). The 3' non-translated region of the nopaline synthase (T-nos) coding region from *A. tumefaciens* T-DNA terminates transcription and directs polyadenylation.

2.2.1.2. T-DNA II

The *npt*II gene cassette that produces the NPTII protein consists of the promoter (P-35S) from the *cauliflower mosaic virus* (CaMV) 35S RNA. The sequence coding for the NPTII protein is followed by the 3' nontranslated region of the nopaline synthase (T-nos) coding region from *A. tumefaciens* that ends transcription and directs polyadenylation. The *npt*II gene, used as marker for the selection of transgenic plants, encodes the neomycin phosphotransferase II enzyme (NPTII) that inactivates certain aminoglycoside antibiotics such as neomycin, kanamycin and paromomycin.

2.2.2. Transgenic constructs in the genetically modified plant

Conventional breeding was used to isolate plants that contain the *cry1A.105* and the *cry2Ab2* expression cassettes (T-DNA I) but that do not contain the *npt*II expression cassette (T-DNA II). This was confirmed by molecular analysis.

Southern analyses were used to assess insert and copy number of the DNA inserted in maize MON89034. The insert number was evaluated by digesting the test and control DNA with *Nde*I, a restriction enzyme that does not cleave within T-DNA I. The number of copies of the T-DNA present was determined using the restriction enzyme *Ssp*I, which cleaves once within the insert. For the Southern analyses a conventional maize control was used which was F2 grains from a cross between lines designated LH172 and LH198. It is an appropriate control for the material used in the characterisation of maize MON89034, as the MON89034 material used for molecular characterisation included LH172 and LH198 in its breeding history.

Southern data indicate that i) maize MON89034 contains one copy of T-DNA I at a single locus; ii) *npt*II (T-DNA II) is absent and iii) vector backbone is absent.

The organisation of the elements within the insert in maize MON89034 was confirmed using PCR analysis by amplifying seven overlapping regions of DNA that span the entire length of the insert. The applicant has provided the sequence of MON89034 insert (9317 bp) and the 5' and 3' flanking maize genomic DNA. This analysis confirmed that both the *cry1A.105* and *cry2Ab2* coding sequences are identical to those of the corresponding genes in PV-ZMIR245. However, data indicate that the e35S promoter that regulates expression of the *cry1A.105* gene has been modified to produce a shorter promoter version, e35S89 (it lacks the duplicated enhancer element found in e35S). Furthermore, the right border region present in PV-ZMIR245 was replaced by a left border region. The explanation provided is that a recombination event has occurred either before or during the process of T-DNA transfer to the plant cell genome. This modification did not prevent expression of the Cry1A.105 protein to establish the trait.

A sequence comparison between the corresponding genomic region of conventional maize and the 5' and 3' flanking sequences from maize MON89034 indicated that the pre-insertion locus was preserved except for the deletion of 57 bp and addition of 10 bp.

A bioinformatic analysis was performed on genomic DNA sequence that flank the MON89034 insertion site to determine if any endogenous genes had been deleted and/or disrupted during the transformation event. By using the BLASTx algorithm, any potential peptide sequences bearing significant identity and similarity to known proteins can be detected. The BLASTx search returned no hits, indicating that the insertion of the MON89034 T-DNA I did not disrupt any known open reading frames. An updated (2008) bioinformatic analysis was performed using the BLASTn and BLASTx algorithms against the non-redundant public DNA database and the non-redundant public protein database. The data again indicated that no known endogenous maize genes in flanking DNA regions adjacent to the insert T-DNA I in maize MON89034 have been disrupted.

Bioinformatic analyses were also performed to assess the potential for allergenicity, toxicity, or bioactivity of putative polypeptides encoded by the 5' and 3' inserted DNA-maize genomic DNA junctions. Sequences spanning the 5' maize genomic DNA-inserted DNA junction and the 3' inserted DNA-maize genomic DNA junction were translated from stop codon to stop codon in all six reading frames. Putative polypeptides from each reading frame were compared to proprietary allergen, toxin, and public domain database sequences using bioinformatic tools. The FASTA sequence alignment tool was used to assess structural relatedness between the query sequences and any protein sequences in these databases. Structural similarities shared between each putative polypeptide with each sequence in the database were examined. In addition to structural congruence, each putative polypeptide was screened for short polypeptide matches using a pair-wise comparison algorithm. In these analyses, eight contiguous and identical amino acids were defined as immunologically relevant, where eight represents the typical minimum sequence length likely to represent an immunological epitope.

No biologically relevant structural congruence to allergens, toxins, or bioactive proteins was observed for any of the putative polypeptides. Furthermore, no short (eight amino acid) polypeptide matches were shared between any of the putative polypeptides and proteins in the allergen database.

2.2.3. Information on the expression of the insert

The levels of the Cry1A.105 and Cry2Ab2 proteins in various tissues of maize MON89034 were assessed by enzyme-linked immunosorbent assay (ELISA). Samples were analysed from five field trials in Argentina in 2004 and from five field trials conducted in the USA. in 2005. The trial locations represent the major maize growing region of these countries and provide a range of environmental conditions that would be encountered in the commercial production of maize.

Data from the 2004 and 2005 trials taken together showed that the ranges of Cry1A.105 levels were 27-850 µg/g dwt for overseason leaf (OSL-four stages), 17-110 µg/g dwt for overseason root, 6.2 -36 µg/g dwt for forage root, 9.4-48 µg/g dwt for senescent root, 23-570 µg/g dwt for whole plant, 20-130 µg/g dwt for silk, 6.1-16 µg/g dwt for pollen, 1.9-7 µg/g dwt for grain, 19-56 µg/g dwt for forage, 11-85 µg/g dwt for stover.

For the 2004 Argentina trial the means for Cry1A.105 protein levels across all sites were 2.6 µg/g dwt in grain, 30 µg/g dwt in forage, 7.7 µg/g dwt in pollen, 260 µg/g dwt in OSL-1, 200 µg/g dwt in OSL-4, 28 µg/g dwt in forage root, and 19 µg/g dwt in stover. In tissues harvested throughout the growing season, mean Cry1A.105 protein levels across all sites ranged from 160 – 260 µg/g dwt in leaf, 22 – 71 µg/g dwt in root, and 48 – 170 µg/g dwt in whole plant.

In the 2005 US trial the means for Cry1A.105 protein levels across all sites were 5.9 µg/g dwt in grain, 42µg/g dwt in forage, 12 µg/g dwt in pollen, 520 µg/g dwt in OSL-1, 120 µg/g dwt in OSL-4, 12 µg/g dwt in forage root, and 50 µg/g dwt in stover. In tissues harvested throughout the growing season, mean Cry1A.105 protein levels across all sites ranged from 72-520 µg/g dwt in leaf, 42-79 µg/g dwt in root, and 100-380 µg/g dwt in whole plant.

For the 2004 Argentina trail the means for Cry2Ab2 protein levels across all sites were 0.95 µg/g dwt in grain, 45 µg/g dwt in forage, 0.56 µg/g dwt in pollen, 120 µg/g dwt in OSL-1, 270 µg/g dwt in OSL-4, 31 µg/g dwt in forage root, and 44 µg/g dwt in stover. In tissues harvested throughout the growing season, mean Cry2Ab2 protein levels across all sites ranged from 120 – 270 µg/g dwt in leaf, 23 – 48 µg/g dwt in root, and 61 – 98 µg/g dwt in whole plant.

For the 2005 US trial the means for Cry2Ab2 protein levels across all sites were 1.3 µg/g dwt in grain, 38 µg/g dwt in forage, 0.64 µg/g dwt in pollen, 180 µg/g dwt in OSL-1, 160 µg/g dwt in OSL-4, 21 µg/g dwt in forage root, and 62 µg/g dwt in stover. In tissues harvested throughout the growing season, mean Cry2Ab2 protein levels across all sites ranged from 130-180 µg/g dwt in leaf, 26-58 µg/g dwt in root, and 39-130 µg/g dwt in whole plant.

2.2.4. Inheritance and stability of inserted DNA

Stability of the MON89034 insert was assessed by Southern analyses using material from multiple generations of the MON89034 breeding history. An additional non-GM comparator was used in stability analysis because the MON89034 (TI:BC1:F1xRP) generation has a different genetic background than the other generations. The MON89034 (TI:BC1:F1xRP) generation was in the process of being converted into commercial germplasm. The recurrent parent in this generation was the commercial inbred line 91DUQ1. Thus the non-GM comparator used was a commercial hybrid derived from 91DUQ1.

The stability of the MON89034 was confirmed using overlapping T-DNA I probes spanning the entire inserted DNA sequence. The absence of the *npt*II selectable marker gene was confirmed using overlapping probes spanning T-DNA II. The absence of plasmid PV-ZMIR245 backbone sequence across generations was confirmed using overlapping probes spanning the vector backbone of PV-ZMIR245. A second conventional maize control (referred to as conventional maize A) was used in these Southern blots to ensure that the genetic backgrounds of all the generations were accurately represented.

The presence of the MON89034 insert in the nuclear genome was demonstrated by Chi square analysis of the segregation results using ELISA to confirm the presence of the Cry proteins and PCR to detect the genes. The Chi square analysis of the segregation pattern, according to Mendelian genetics, was consistent with a single site of insertion into the maize nuclear DNA.

2.3. Conclusion

Appropriate analysis of the integration site, including flanking sequence and bioinformatic analysis, has been performed to characterise the transformation event MON89034. The expression of the Cry1A.105 and Cry2Ab2 proteins has been analysed sufficiently and the stability of the genetic modification has been demonstrated over several generations. The GMO Panel concludes that the molecular characterisation of the transformation event MON89034 does not indicate any safety concerns.

3. Comparative analysis

3.1 Issues raised by Member States

Comments from Member States are addressed in the table published as Annex G of the EFSA overall opinion on maize MON89034.

3.2 Evaluation of relevant scientific data

Having considered the information provided in the application and the Member States' comments, the GMO Panel requested from the applicant further data with respect to genetic relatedness of the controls used in field trials and safety studies to the genetically modified maize MON89034. The applicant provided the requested information.

3.2.1 Choice of comparator and production of material for the compositional assessment

In the compositional studies, the GM maize MON89034 was compared to the non-transgenic maize hybrid LH198 x LH172 (sometimes designated 1325023, 13250.23, or 50297-98), which is a conventional maize hybrid with background genetics similar to maize MON89034. In addition to the comparator, three commercially available maize hybrids were included in the field trial at each site, the commercial varieties being different in different trial sites. At each trial site there were three replicated blocks. The field trials were in the year 2004 carried out in the USA and in the season 2004-2005 in Argentina, each season/year at five different geographical sites under agronomical conditions representative of the respective geographical region. The 15 commercial maize varieties included in the field trials were used as reference material to estimate the naturally occurring variation in composition expected for the various analytes in conventional maize.

Materials for the compositional analyses were collected from each field trial site and constituted grains and forage.

3.2.2. Compositional analysis

Maize forage was analysed for proximates (protein, fat, ash, and moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), the minerals calcium and phosphorus, and carbohydrates by calculation. Grains were analysed for proximates, ADF, NDF, TDF (total dietary fibre), amino acids, fatty acids, vitamins (B_1, B_2, B_6, E, niacin, and folic acid), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), the anti-nutrients phytic acid and raffinose, and the secondary metabolites furfural, ferulic acid, and p-

coumaric acid. Also in this case carbohydrates were quantified by calculation. In total 9 components were analysed in forage and 68 in grain. The analytes vitamin E and raffinose were analysed only in material from field trials of 2005. These were the analytes recommended to be analyzed by OECD (2002). The GMO Panel found the set of data adequate. Each analyte was statistically analysed for possible difference between materials of maize MON89034 and the control collected from the same field trial site and across all sites (data from all sites combined). Several of the compounds analysed occurred at levels below the limit of quantification. When more than 50% of the analytical data points were below the limit of quantification, which was the case for 16 of the 77 compounds analysed, no statistical analyses were performed.

When the compositional data on materials from the 2004 field trials in the USA were analysed for each separate site, 44 statistically significant differences were observed. Of these differences 33 were observed at a single site only. Remaining statistical significances (for arachidic acid, carbohydrates, copper and iron) were observed at two or three of the five sites. For carbohydrate and iron, the difference was small and not consistent – in one case increased, in the other decreased. The arachidic acid level was slightly higher in maize MON89034 than in the control (0.38-0.41% vs 0.37-0.39%) at three of the five sites, but fell within the range of conventional varieties (0.32-0.47%). Copper levels were higher in MON89034 than in its control at two sites. Also in this case mean levels of MON89034 were well within the range of natural variation. When the compositional data on materials from the 2004 field trials in the USA were analysed across sites, the composition of maize MON89034 differed significantly from the control in relation to 3 of the 77 compounds investigated. These were the phosphorous level in forage, which was slightly increased in maize MON89034 (0.25 vs 0.21% dw) and only deviated significantly at one of the five trial sites, and the stearic acid and arachidic acid levels in grains, which both were increased in maize MON89034 (1.89 vs 1.82% and 0.39 vs 0.38% of total fat, respectively). Differences were not consistent between materials from different trial sites. All observed differences were small, and fell within the natural variation of conventional maize hybrids and data reported in the literature and data bases (ILSI, 2006). The Panel, therefore, concluded that the observed statistical differences in composition in 2004 had no biological relevance.

As the comparison of the level of the various key constituents in maize MON89034 and its control LH198 × LH172 did not reveal any statistically significant difference for constituents for which a food safety concern could be foreseen, the GMO Panel accepted that none of the field trial sites was replicated the second year.

Statistical evaluation of compositional data from the field studies performed in Argentina in the season 2004-2005 revealed five significant differences when the data was analysed across sites. These were eicosenoic acid (0.29 vs 0.30%), ferulic acid (1894 vs 1759 µg/g dw), stearic acid (1.84 vs 1.79%), manganese (6.81 vs 6.28 mg/kg dw) and vitamin B_2 (1.75 vs 1.94 mg/kg dw) in grain material. For eicosenoic acid and ferulic acid the statistical difference was not noted at any of the single sites when the statistical analysis was performed per site. Stearic acid was found to be increased at one of the five sites, whereas manganese was increased at two of five sites, and vitamin B_2 reduced at two of five sites. The differences were small and mean values and ranges of values for the studied compounds were within the range of natural variation. None of the statistically significant differences observed in the analyses performed per site indicated a need for further exploration. The Panel did not find any of the significant differences in the field studies in Argentina to be biologically relevant.

The GMO Panel considered the observed compositional differences between maize MON89034 and its comparator in the light of the field trial design, biological variation and the level of the studied compounds in conventional maize varieties, and concludes that maize MON89034 is compositionally equivalent to the non-GM counterpart except for the introduced traits.

3.2.3. Agronomic traits and GM phenotype

The applicant provided information on agronomic performance, phenotypic characteristics and natural ecological interaction of maize event MON89034 and maize controls with a comparable genetic background from field trials performed in the USA in 2004 and/or 2005. The control used was generally LH198 x LH172 (H1325023), but was the conventional maize hybrid DKC51-43 when the maize was grown in northern maize growing regions of the USA. The number of field trial sites varied from 1 to 18 depending on the specific character investigated. At each trial site, three to four conventional maize varieties were grown, resulting in 23 conventional maize hybrids being used to provide a range of values common to conventionally grown maize. The field studies gave information on germination, dormancy, emergence, vegetative growth, reproductive growth (including pollen characteristics, seed retention on the plant, phenotypic and agronomic characteristics), and plant interactions with insects, diseases and abiotic stressors (e.g. drought, wind, nutrient deficiency). The phenotypic characteristics evaluated were seedling vigour, early stand count, days to 50% pollen shed, days to 50% silking, stay green, ear height, plant height, dropped ears, stalk lodging, root lodging, final stand count, grain moisture, test weight, and yield. In the combined site analysis two statistically significant differences were noted between maize MON89034 and its control and these were plant height (2.14 vs 2.17 m) and stalk lodging (0.8 vs 2.4), but only for one of the two growing seasons. As the plant height of maize MON89034 also fell within the range of conventional maize hybrids and the difference to the control was small (less than 2%), the GMO Panel is of the opinion that this statistical finding is of no biological relevance. As the identified reduction in stalk lodging was inconsistent (observed in 2004 but not in 2005) and fell within the range observed for conventional maize hybrids, the Panel identified no biological relevance also to this finding.

Of the 11 insect categories, 12 disease categories and eight abiotic stressors evaluated in the studies of ecological interactions in 2004, only a few differences between maize MON89034 and its comparator were noted at particular growth stages and trial sites. No consistent differences were observed. In 2005 even more insect and disease categories and abiotic stressors were evaluated, but again no indication of a different response between maize MON89034 and its comparator was noted. Thus, no qualitative differences were observed in these ecological interactions between maize MON89034 and its control.

In the absence of consistent unexpected differences between the studied maize plants, the GMO Panel concluded that no other ecological interaction specific for maize MON89034 as compared to its control has been observed except for the introduced insect resistance traits.

3.3 Conclusion

Analyses carried out on materials from maize MON89034 and its closely genetically related comparator LH198 x LH172 indicate that they are compositionally and agronomically equivalent except for the introduced transgenic traits. The comparative analysis of maize

MON89034 provides no indications for unintended effects resulting from the genetic modification.

4. Food/Feed safety assessment

4.1. Issues raised by Member States

Comments from Member States are addressed in the table published as Annex G of the EFSA overall opinion on maize MON89034.

4.2. Evaluation of relevant scientific data

4.2.1. Product description and intended use

The scope of application EFSA-GMO-NL-2007-37 is for food and feed uses, import and processing of maize MON89034 and all derived products (e.g. starch, syrups, ethanol, maize oil, flakes, coarse and regular grits, coarse and dusted meal, flour, maize germ meal, maize gluten feed, condensed steep water and maize gluten meal).

The transgenic traits present in maize MON89034 result in the expression of the Cry1A.105 and Cry2Ab2 proteins, which are toxic to some lepidopteran species. The genetic modification is intended to improve agronomic performance only and it is not intended to influence the nutritional properties, the processing characteristics and overall use of maize as a crop.

4.2.2. Effects of processing

Since maize MON89034 has been found to be compositionally equivalent to the control maize and commercial maize hybrids, except for the newly expressed trait (see Section 3.2.2), the effect of processing on the constituents of maize MON89034 is not expected to be different compared to that on conventional maize hybrids.

A multitude of processes are used in maize processing, including temperature treatments, hydrolyses, soaking in slightly acidic water, and drying. Any of these methods are likely to influence degradation and/or denaturation of constituents, but there is no indication that they will influence maize MON89034 differently than conventional maize. To demonstrate the degradability of the Cry1A.105 and Cry2Ab2 proteins, the applicant studied the influence of heat (on average 204^{o}C for 15 minutes) on the stability of the proteins, as measured by immunodetectability The amount of detectable Cry1A.105 protein in dry-milled grain remaining after heat treatment was 78-94%, whereas it was 70-77% for the Cry2Ab2 protein. It should also be recognised that many maize products such as maize oil and maize starch are very low in protein content. The Panel concludes that representative food and feed products of maize MON89034 are expected to contain Cry1A.105 and Cry2Ab2 levels lower than those found in whole grain.

4.2.3. Toxicology

4.2.3.1 Cry1A.105 and Cry2Ab2 proteins used for safety assessment

Due to the low expression level of the Cry1A.105 and Cry2Ab2 proteins in maize MON89034 and the difficulties to isolate enough quantity of purified protein from the genetically modified maize, the safety studies with the newly expressed protein were conducted with Cry1A.105 and Cry2Ab2 proteins encoded by the *cry1A.105 and cry2Ab2* genes from *B. thuringiensis* and expressed in *Escherichia coli*. The structural similarity and functional equivalence of the Cry1A.105 and Cry2Ab2 proteins produced by *E. coli* to those produced in maize MON89034 was shown by confirmation of N-terminus intactness (Edman degradation or antibody detection), Western analysis, mobility in SDS-PAGE, MALDI-TOF mass spectrometry, glycosylation analysis and insect bioactivity bioassays. All these methods confirmed the equivalence of the bacterial and the plant Cry1A.105 and Cry2Ab2 proteins. Based on the identified similarity in structure, and functional equivalence between these proteins, the GMO Panel accepts the use of the *E.coli*-derived Cry1A.105 and Cry2Ab2 proteins for the safety testing as substitutes for the Cry1A.105 and Cry2Ab2 proteins expressed in maize MON89034. The bacterial proteins were also accepted as reference standards in the ELISA to estimate Cry1A.105 and Cry2Ab2 expression levels in various tissues of maize MON89034.

4.2.3.2 Toxicological assessment of expressed novel proteins

The newly introduced genes in maize MON89034 are derived from different strains of the soil bacterium *B. thuringiensis*. The chimeric *cry1A.105* gene and the *cry2Ab2* gene both code for proteins that are well known to be toxic to lepidopteran or lepidopteran/dipteran insects but are unknown to be toxic and allergenic to humans and animals.

The chimeric Cry1A.105 protein consists of several domains, domains I and II from Cry1Ab and Cry1Ac, domain III from Cry1F, and the C-terminal domain from Cry1Ac. The Cry1Ab and Cry1Ac proteins share 100% amino acid sequence homology with domains I and II, whereas the conformity between Cry1F and domain III is of lower degree. A phylogenetic study of the chimeric Cry1A.105 protein to other common Cry proteins showed the protein to be most genetically related to Cry1Ac proteins, with which it was 93.6% identical. Studies on insecticidal activity of Cry1A.105 further established functional similarity to Cry1Ac, Cry1Ab, and Cry1F proteins. Of these three Cry proteins, the GMO-Panel has previously found Cry1Ab and Cry1F to be safe when expressed in GM crops (EFSA, 2004; 2005a; 2005b). The Cry1Ac protein has been shown to be structurally similar to Cry1Ab and Cry1F proteins.

The amino acid sequence of the Cry2Ab2 protein expressed in maize MON89034 is 88% identical to the Cry2Aa protein produced by *B. thuringiensis* subsp. *kurstaki* that is frequently used in agriculture for control of insect pests. Use of this bacterial strain has been found safe. In general terms, there is an extensive body of knowledge on the safety in humans and animals of the use of Cry-protein-expressing *B. thuringiensis* (Bt) products in farming systems to reduce insect burden on crops, indicating no harm from these Bt products and their Cry proteins.

The potential for interactions between the Cry1A.105 and Cry2Ab2 proteins was studied in diet-incorporation bioassays using the European corn borer (*Ostrinia nubilalis*) and corn earworm (*Heliocoverpa zea*) as indicator organisms. As the toxic activity for lepidopteran insects was found to be additive, it was concluded that the presence of one protein did not

enhance the activity of the other. Additive toxicity in insect bioassays (*Heliothis virescens*, *Helicoverpa zea*, and *Spodoptera frugiperda*) have also been demonstrated for a chimeric Cry1Ab/Cry1Ac protein and a modified Cry 2Ab protein expressed in the transgenic cotton variety MON15985 (Greenplate et al., 2003).

(a) Acute toxicity testing

The applicant provided two single dose toxicity studies, each on 10 male and 10 female CD-1 mice gavaged with the proteins Cry1A.105 and Cry2Ab2, produced in *E. coli*., at doses of 2072 mg/kg body weight and 2198 mg/kg body weight, respectively. No indications of adverse effects related to the exposure to the Cry1A.105 protein or the Cry2Ab2 protein were found in these studies.

(b) Degradation in simulated digestive fluids

Digestion of the Cry1A.105 and Cry2Ab2 proteins were studied *in vitro* in simulated gastric fluid using SDS-PAGE gel staining and Western blot analysis. The SDS-PAGE gel staining showed that at least 99% of both the Cry1A.105 protein and the Cry2Ab2 protein produced in *E. coli* were degraded within 30 seconds in simulated gastric fluid assay containing pepsin. This finding was confirmed by Western blotting. After the same short period, less than 10% of the insecticidal activity remained.

When Cry2Ab2 was exposed to pepsin, no degradation fragments were formed whereas when Cry1A.105 was exposed to pepsin in simulated gastric fluid, a protein fraction with a size of around 4.5 kDa was transiently formed. However, after 20 minutes, this protein fraction could no longer be detected. The applicant characterized the fraction and showed that it contained four fragments, two being parts of the Cry1A.105 protein and having a size of around 40 amino acids, the other two being autocatalytic fragments of the pepsin molecule.

Both Cry proteins were also degraded by simulated intestinal fluid containing pancreatin. The Cry1A.105 protein was shown by Western blotting to be degraded within 5 minutes, producing several fragments. The largest of these fragment remained for up to 24 h. Such fragments have been observed after digestion of other Cry proteins known to be safe for humans. Similarly, 97.5% of the Cry2Ab2 protein was digested within 15 minutes in simulated intestinal fluid, with fragments appearing after around 5 minutes. Several smaller fragments appeared transiently at the 24 h time point. When Cry1A.105 fragments, which previously had been exposed to simulated gastric fluid, were subsequently exposed to simulated intestinal fluid did not survive longer than 30 seconds.

The *in vitro* digestion experiments demonstrate that the Cry1A.105 and Cry2Ab2 proteins are rapidly degraded by digestive enzymes. The Panel identified no degradation fragments that raise concern.

(c) Bioinformatic studies

Searches for amino acid sequence homology of the Cry1A.105 and Cry2Ab2 proteins expressed in maize MON89034 and the theoretical expression products of ORFs coding for putative fusion proteins in the regions flanking the inserts with amino acid sequences of toxic and public domain proteins described in databases (TOXIN5 and ALLPEPTIDES), indicated no sequence homology with known toxic proteins. A high degree of similarity was found only to other Cry proteins.

4.2.3.3. Toxicological assessment of new constituents other than proteins

No new constituent other than the Cry1A.105 and Cry2Ab2 proteins are expressed in maize MON89034 and no relevant changes in the composition of maize MON89034 were detected by the compositional analysis.

4.2.4. Toxicological assessment of the whole GM food/feed

The applicant provided a sub-chronic 90-day feeding study, performed according to OECD guideline (OECD, 2007), in which three groups of 20 Sprague-Dawley rats of strain Crl:CD® per sex were given a diet containing 33% maize grain (w/w). One of the groups received 33% MON89034, another 11% MON89034 (supplemented with 22% non-GM control maize), and the third 33% non-GM control maize (LH198 x LH172, also designed 50297-98). Diets were formulated to be nutritionally comparable to the Certified Rodent Labdiet. The composition of the diet and its quality, including herbicide residues and mycotoxin levels, were controlled by analysis. This design allowed data from historical controls given 33% maize grain to be used as an additional reference. The feed and water was available *ad libitum*.

No clinical relevant effects related to treatment were observed. One female animal, which was supplied with 33% MON89034 maize in the diet, died on study day 14. According to the study report, the microscopic examinations at necropsy revealed findings indicative of urinary tract obstruction, but the presence of 5 mL fluid in the thoracic cavity was considered to be the most clinically significant finding. The cause of death was undetermined, but it was most likely considered to be incidental and unrelated to consumption of the test diet.

There were no relevant differences in body weight, body weight gain or feed consumption between the groups. Of the 26 haematology, 18 serum chemistry and 14 urinalysis parameters determined for each sex and each dose, only the mean platelet count in females given the lower dose (11% MON89034) was statistically significantly different from the controls. As the value of this highly variable parameter was within the range recorded for this parameter in the historical control data, and there was no dose-response, and no alteration in related parameters, this finding, which did not occur in male animals, was not considered to be related to the exposure to maize MON89034. Organ weight determinations at necropsy showed no statistically significant differences between groups in males. Whereas no differences in absolute organ weights were found in females, a statistically significant reduced mean thyroid/parathyroid weight relative to final body weight (0.006g/100g vs 0.007/100g) was observed in females given the highest dose of maize MON89034 (33%) compared with the controls. However, the value was within the range of the historical control data, and there was no statistically significant alteration in thyroid/parathyroid weight relative to brain weight. Furthermore, microscopic analysis did not reveal histopathological alterations. Therefore the lower relative thyroid/parathyroid weight in females is considered to be an incidental finding.

Microscopic findings in organs and tissues were almost equally distributed and no statistically significant differences between males and females of the high dose group and the controls were noted. A numerically higher incidence of kidney alterations in females of the high dose group was attributable to two rats (one died at day 14 of unknown cause, the other survived to the end

of the study). The alterations in these two rats included minimal chronic progressive nephropathy, minimal/moderate transitional cell hyperplasia, minimal sub-acute inflammation and moderate hydronephrosis. The animal that died on day 14 additionally showed mild papillary necrosis and minimal tubular necrosis. Both rats had urinary bladder calculi and the study pathologist concluded that the lesions observed most likely were linked to these calculi. It seems unlikely that the urinary bladder calculi and associated kidney alterations could have been induced by the tested maize in 14 days. A low incidence of urinary bladder calculi is known to occur in this rat strain and may be considered a spontaneous finding in sub-chronic studies. According to historical control data supplied in the application, the incidence of urinary bladder calculi in high dose females in this study was also found in female control rats in previous studies conducted with CD rats in the same testing laboratory. The Panel therefore considers the urinary bladder calculi as well as the associated kidney alterations as incidental findings which were not related to administration of maize MON89034. The same applies to the nephroblastomas, a very rare tumour of the kidney, which were observed in two female animals of the control group.

4.2.5. Allergenicity

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

4.2.5.1. Assessment of allergenicity of the newly expressed proteins

The *cry2Ab2* gene and the gene sequence constituents of the chimeric *cry1A.105* gene originates from different strains of the soil bacterium *B. thuringiensis* that is unknown to be allergenic. When the amino acid sequences of the Cry1A.105 and Cry2Ab2 proteins in bioinformatic studies were compared with the sequences of known allergens available in an allergen database (AD8), no indication that the proteins resemble known allergens were obtained, i.e. no partial identity (>35%) between longer segments of the test proteins and known allergens using the FASTA algorithm, and no total identity between 8 consecutive amino acids large segments of the test proteins and known allergens using the ALLERGENSEARCH algorithm were found.

The potential allergenicity of the theoretical expression products of ORFs coding for putative fusion proteins in the regions flanking the inserts were considered (see Section 2.2.2.). No resemblance with allergens was found.

The studies on the degradation of the Cry1A.105 and Cry 2Ab2 proteins in simulated mammalian digestive fluids, which are also relevant for the assessment of potential allergenicity, have been described in Section 4.2.3.2. These studies did not identify any degradation fragments of potential concern. Based on the information available the GMO Panel considers it unlikely that the newly expressed Cry1A.105 and Cry 2Ab2 proteins are allergens.

4.2.5.2. Assessment of allergenicity of the whole GM plant

Rare cases of occupational allergy to maize dust or maize pollen allergy have been reported. Food allergy to maize is rare (Moneret-Vautrin *et al*., 1998), but IgE- binding proteins have been identified in maize flour (Pastorello *et al*., 2000; Pasini *et al*., 2002). Allergy to maize is detected in a minor fraction of the population of atopic patients. In addition, most individuals with a positive skin prick test (SPT) or having IgE antibodies against maize were suffering of respiratory allergy and only a few ones displayed a true food allergy upon oral challenge with maize products (Pasini et al., 2002; Jones et al., 1995). Therefore, oral sensitization to maize proteins is very rare.

The allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, for example through qualitative or quantitative modification of the pattern of expression of endogenous proteins. This issue does not appear relevant to the Panel since maize is not considered a major allergenic food, and possible over-expression of any endogenous protein, which is not known to be allergenic, would be unlikely to alter the overall allergenicity of the whole plant.

The GMO Panel concludes that the overall allergenicity of the whole plant is not changed.

4.2.6. Nutritional assessment of GM food/feed

The applicant provided a 42-day broiler (Ross × Ross 308) feeding study performed according to generally accepted guidelines (ILSI, 2003), and consisting of six treatment groups. One group received maize MON89034, another non-GM maize LH198 x LH172 (sometimes referred to as 13250.23) of comparable genetic background to MON89034, and four other groups commercially available non-GM maize varieties (ASGROW RX690, ASGROW RX772, DKC60-15 and DKC57-01).

Each treatment consisted of 50 male and 50 female broilers fed diets containing approximately 55% (w/w) maize grain in the starter diet and 59% maize grain in the grower/finisher diet. Diets were also formulated to be isocaloric based on nutrient analyses performed before diet formulation. Feed and water was given *ad libitum*.

All treatments resulted in comparable and rather low mortality rates. Chickens fed diets containing maize MON89034 showed no effect on total feed intake, feed conversion and growth across treatments. However, a small but statistically significant difference in adjusted feed conversion (a calculated parameter) in males was observed between broilers fed maize MON89034 and control broilers (1.59 vs 1.64 kg/kg). The adjusted feed conversion was 1.52, 1.53, 1.54 and 1.61 kg/kg for the four conventional maize varieties. Compared to three of the four conventional maize varieties, the adjusted feed conversion was not significantly different

in birds fed maize MON89034. There were no difference in chilled carcass, fat pad, breast, thigh, drum and wing weight, and no difference in percentage of moisture, protein, and fat in thigh and breast meat between broilers fed maize MON89034 and broilers fed the control maize. In the absence of any other treatment-related effects on performance, the Panel did not find the statistically significant difference in adjusted feed conversion to be of biological relevance.

Thus, the 42-day broiler feeding study showed that maize MON89034 is nutritionally equivalent to the non-GM comparator and conventional maize varieties.

4.2.7. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that maize MON89034 is any less safe than its non-GM comparator. In addition, maize MON89034 is, from a nutritional point of view, substantially equivalent to conventional maize. Therefore, and in line with the Guidance document (EFSA, 2006a), the GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

4.3. Conclusion

Based on the lack of adverse effects to mammals as experienced from using various *B. thuringiensis* strains, has resulted in the conclusion of a reasonable certainty of no harm from these Bt products and their Cry proteins.

The amino acid sequences of the transgenic Cry1A.105 and Cry2Ab2 proteins did not show any significant similarity with sequences of known toxins and allergens. Furthermore, they are rapidly degraded in simulated digestive fluids. In addition the transgenic Cry1A.105 and Cry2Ab2 proteins induced no adverse effects in acute oral toxicity studies in mice. A sub-chronic 90-day feeding study in rats with a diet containing 33% kernels of maize MON89034 revealed no treatment-related adverse effects. In addition, a nutritional feeding study on broiler chickens indicates that maize MON89034 is nutritionally equivalent to its comparator and conventional maize varieties. The feeding studies, therefore, support the conclusion of the compositional and agronomical comparison of maize MON89034 to its non-GM control that the genetic modification resulted in no unintended effects.

The GMO Panel is of the opinion that maize MON89034 is as safe as conventional maize and that the overall allergenicity of the whole plant is not changed. Maize MON89034 and derived products are unlikely to have any adverse effects on human and animal health in the context of its intended use.

5. Environmental risk assessment and monitoring plan

5.1. Issues raised by the Member States

Comments from Member States are addressed in the table published as Annex G of the EFSA overall opinion on maize MON89034.

5.2. Evaluation of relevant scientific data

The scope of application EFSA-GMO-NL-2007-37 is for food (e.g. starch, syrups, oil) and feed (e.g. maize gluten feed, maize gluten meal) uses, import and processing of maize MON89034 and does not include cultivation. Considering the intended uses of maize MON89034, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on GM maize MON89034 and with accidental release into the environment of MON89034 seeds during transportation and processing.

Maize MON89034 has been developed for protection against specific lepidopteran pests (*Ostrinia nubilalis*, *Spodoptera* ssp, *Agrotis ipsilon*). The insect resistance is achieved by expression of the Cry1A.105 and Cry2Ab2 proteins derived from *B. thuringiensis* subsp. *kurstaki* (see Section 2.2.1).

5.2.1. Potential unintended effects on plant fitness due to the genetic modification

Maize is highly domesticated and generally unable to survive in the environment without cultivation. Maize plants are not winter hardy in most regions of Europe, they have lost their ability to release seeds from the cob and they do not occur outside cultivated land or disturbed habitats in agricultural landscapes of Europe, despite cultivation for many years. In addition, there are no cross compatible wild relatives in Europe, and gene flow via pollen is largely restricted to neighbouring crops.

Insect resistance against certain lepidopteran pests, such as European Corn Borer (ECB) larvae (*Ostrinia nubilalis*), provides a potential advantage in cultivation under infestation conditions. However, survival of maize outside of cultivation in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, susceptibility to diseases and to cold climate conditions. Since these general characteristics of this GM maize are unchanged, the inserted insect trait is not likely to provide a selective advantage outside of cultivation in Europe. Therefore, it is considered very unlikely that volunteers of this GM maize, or its progeny, will differ from conventional maize varieties in their ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

The applicant carried out field trials at several locations in the USA in 2004 and 2005 and in Argentina in 2004. Information was provided on phenotypic characteristics and plant environment interactions of maize MON89034 compared with that of control maize. No biologically meaningful differences between the studied maize varieties were observed (see Section 3.2.3). The field data do not show increased invasiveness or enhanced weediness or fitness of maize MON89034 plants. In addition to the data presented by the applicant, the GMO Panel is not aware of any scientific report of increased spread and establishment of maize MON89034 and any change in survival capacity, including over-wintering.

Since maize MON89034 has no altered survival, multiplication or dissemination characteristics except in the presence of the specific target organisms, the GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this maize will not differ from that of conventional maize varieties.

5.2.2. Potential for gene transfer

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

5.2.2.1. Plant to bacteria gene transfer

Current scientific data (EFSA, 2004; EFSA, 2007) suggest that gene transfer from GM plants to microorganisms under natural conditions is extremely unlikely, and its establishment would occur primarily through homologous recombination in microorganisms.

In the case of accidental release and establishment of maize MON89034 in the environment, exposure of microorganisms to transgenic DNA derived from GM maize plants would take place during natural decay of GM plant material and/or pollen in the soil of areas where GM plants might establish.

Food and feed products derived from the GM maize could contain transgenic DNA. Therefore microorganisms in the digestive tract of humans and animals may be exposed to transgenic DNA.

The modified *cry1A.105* gene in maize MON89034 is under the control of the promoter e35S and leader for the *Cauliflower mosaic virus* (CaMV) 35S RNA containing a duplicated enhancer region. The *cry2Ab2* gene in maize MON89034 is under the control of the *Figwort mosaic virus* promoter 35S (P-FMV). Both promoters have limited, if any, activity in prokaryotic organisms (see sub-section 2.2.1.1). Genes under control of prokaryotic regulatory elements conferring related traits, as expressed in the GM plants, occur in certain microorganisms in natural environments.

The Cry1A.105 protein is a modified Bt Cry1A protein with amino acid sequence identity to Cry1Ab, Cry1Ac and Cry1F proteins of 90.0%, 93.6 % and 76.7%, respectively (see Section 2.2.1). The Cry1A.105 protein consists substantially of domains I and II from Cry1Ab or Cry1Ac (these proteins share 100% amino acid sequence identity in domains I and II), domain III from Cry1F and the entire C-terminal domain of Cry1Ac. The Cry2Ab2 protein present in MON89034 is a member of the Cry2Ab class of proteins that share more than 95% amino acid sequence homology. It is a variant of the wild-type Cry2Ab2 protein isolated from *B. thuringiensis* subsp. *kurstaki*.

Taking into account the origin and nature of the *cry1A.105* and *cry2Ab2* genes and the lack of selective pressure in the intestinal tract and the environment, the likelihood that horizontal gene transfer of the *cry1A.105* and *cry2Ab2* genes would confer selective advantage or increased fitness to microorganisms is very limited. For this reason it is very unlikely that genes from maize MON89034 would become transferred and established in the genome of microorganisms in the environment or human and animal digestive tract. In the very unlikely event that such horizontal gene transfer would take place, no adverse effects on human and animal health or the environment are expected, as no principally new traits would be introduced or expressed in microbial communities.

5.2.2.2. Plant to plant gene transfer

The extent of cross-pollination of other maize varieties will mainly depend on the scale of accidental release during transportation and processing. For maize, any vertical gene transfer is limited to other *Zea mays* plants as populations of sexually compatible wild relatives of maize are not known in Europe (OECD, 2003). The flowering of the sporadic GM maize plants originating from accidental release occurring during transportation and processing is unlikely to disperse significant amounts of GM maize pollen to other maize plants.

Insect resistance against certain lepidopteran pests, such as ECB larvae (*Ostrinia nubilalis*), provides an advantage in cultivation under infestation conditions. However, survival of maize outside cultivation in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, susceptibility to diseases and to cold climate conditions. Since these general characteristics of this GM maize are unchanged, the inserted trait is not likely to provide a selective advantage outside of cultivation in Europe. Therefore, as any other maize varieties, GM maize would only survive in subsequent seasons in the warmer regions of Europe and are not likely to establish feral populations under European environmental conditions (see Section 5.2.1.).

In conclusion, since maize MON89034 has no altered survival, multiplication or dissemination characteristics except when cultivated in the presence of the specific target organisms, the GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this maize is considered to be extremely low.

5.2.3. Potential interactions of the GM plant with target organisms

The maize MON89034 was developed to provide resistance to larvae of certain lepidopteran pests of maize. The modified Cry1A.105 and Cry2Ab2 proteins behave in a similar way to other Cry proteins and are pore-forming toxins producing ion channels in lipid membranes (Rausell *et al*., 2004; Bravo *et al*., 2007; Gomez *et al*., 2007; Pigott and Ellar, 2007).

The intended uses of maize MON89034 specifically exclude cultivation, so the environmental exposure is mainly limited to exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM maize as well as to accidental release into the environment of MON89034 seeds during transportation and processing and subsequently to potential occurrence of sporadic feral plants. Thus the level of exposure of target organisms to the Cry1A.105 and Cry2Ab2 proteins is likely to be extremely low and of no ecological relevance.

5.2.4. Potential interactions of the GM plant with non-target organisms

Considering the intended uses of maize MON89034, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM maize and with accidental release into the environment of GM seeds during transportation and processing.

The GMO Panel assessed therefore whether the Cry1A.105 and Cry2Ab2 proteins might potentially affect non-target organisms by entering the environment e.g. in manure and faeces from the gastrointestinal tracts mainly of animals fed on maize MON89034. Because of the selectivity of the Cry proteins, non-target organisms belonging to a similar taxonomic group as the target organisms are those most likely to be affected (OECD, 2007).

Data supplied by the applicant indicate that a limited amount of the Cry1A.105 and Cry2Ab2 proteins enters the environment due to expression (1.9 - 7.0 and 0.7 - 3.1 µg/g dry weight) in kernels. In addition, the data show that at least 99% of both the Cry1A.105 and Cry2Ab2 proteins produced in *E. coli* were degraded within 30 seconds in the simulated fluid assay containing pepsin. Both proteins were also degraded by simulated intestinal fluid containing pancreatin. Therefore most of the Cry proteins would be degraded by enzymatic activity in the gastrointestinal tract and only very low amounts of Cry1A.105 protein and Cry2Ab2 protein would remain intact to pass out in faeces. These data conformed to data from studies of related Cry proteins (Lutz *et al*., 2006; Lutz *et al*., 2005) and references therein which indicate that the majority of Cry proteins are degraded in the gastrointestinal tract.

Concerning the environmental exposure of Cry proteins in soils, Cry proteins can bind to humic acids, clays, and the organomineral complex found in soil which may give some protection from degradation (OECD, 2007). However, a number of studies provided data that there is no persistence and accumulation of Cry proteins from GM crops in soil (Head *et al*., 2002; Herman *et al.*, 2001; 2002; Dubelman *et al.*, 2005; Ahmad *et al.*, 2005; Baumgarte and Tebbe, 2005; Hopkins and Gregorich, 2005; Icoz and Stotzky 2007; Krogh and Griffiths, 2007).

In conclusion, exposure of soil and water environments to Cry toxins of maize MON89034 from disposal of animal wastes or accidental spillage of maize kernels is likely to be very low and localized. Thus exposure of potentially sensitive non-target organisms to the Cry1A.105 and Cry2Ab2 proteins is likely to be very low and of no biological relevance.

5.2.5. Potential interaction with the abiotic environment and biogeochemical cycles

This point was not considered an issue by the Member States nor by the GMO Panel because the level of exposure would be so low that potential effects on the abiotic environment and biogeochemical cycles are unlikely.

5.3. Monitoring

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

Monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific quality of the monitoring plan provided by the applicant (EFSA, 2006b). The potential exposure to the environment of maize MON89034 would be through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM maize or through accidental release into the environment of GM seeds during transportation and processing.

No specific environmental impact of this GM maize was indicated by the environmental risk assessment and thus no case-specific monitoring is required.

The general surveillance plan proposed by the applicant includes i) the description of an approach using operators (i.e. grain traders and maize processors) involved in the handling and

use of viable maize MON89034, to report to the applicants any potential unanticipated adverse effect of maize MON89034 on human health and the environment, ii) a coordinating system established by EuropaBio, iii) the use of networks of existing surveillance systems. The applicant will submit a general surveillance report on an annual basis. In case of adverse effects altering the conclusions of the environmental risk assessment, the applicant will immediately inform the European Commission.

The GMO Panel is of the opinion that the general approaches and measures of the post-market environmental monitoring plan proposed by the applicant are in line with the EFSA opinion on post-market environmental monitoring (EFSA, 2006b) as well as with the intended uses of maize MON89034 since the environmental risk assessment does not cover cultivation and identified no potential adverse environmental effects. The GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan. The GMO Panel advises that appropriate management systems should be in place to restrict seeds of maize MON89034 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

5.4. Conclusion

The scope of the application is for food and feed uses, import and processing of maize MON89034 and does not include cultivation. Considering the intended uses of maize MON89034, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the maize MON89034 and with accidental release into the environment of GM seeds during transportation and processing.

Maize is highly domesticated and not able to survive in the environment without cultivation. There are no indications of increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of MON89034 seeds during transportation and processing. Taking into account the scope of the application, both the rare occurrence of sporadic maize plants and the low levels of exposure through other routes indicate that the risk to target and non-target organisms is negligible.

The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize MON89034 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. Furthermore the GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel was requested to carry out a scientific risk assessment of the maize MON89034 for food and feed uses, import and processing of maize MON89034 and all derived products.

The GMO Panel is of the opinion that the molecular characterisation provided for the maize transformation event MON89034 is sufficient for the safety assessment. The bioinformatic analysis of the inserted DNA and flanking regions does not raise any safety concern. The expression of the genes introduced by genetic modification has been sufficiently analysed and

the stability of the genetic modification has been demonstrated over several generations. The GMO Panel considers that the molecular characterisation does not indicate any safety concern.

Comparative analysis has shown that maize MON89034 is compositionally and agronomically equivalent to conventional maize, except for the introduced transgenic traits. The risk assessment included an analysis of data from analytical studies, bioinformatic, and *in vitro* and *in vivo* studies. The GMO Panel concluded that maize MON89034 is as safe as its non-GM counterpart and that the overall allergenicity of the whole plant is not changed.

The application EFSA-GMO-NL-2007-37 concerns food and feed uses, import and processing of maize MON89034. There is therefore no requirement for scientific assessment of possible environmental effects associated with the cultivation of the GM maize. There are no indications of increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of MON89034 seeds during transportation and processing. Also, the low levels of environmental exposure through other routes indicate that the risk to target and non-target organisms is likely to be extremely low. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize MON89034.

In conclusion, the GMO Panel considers that information available for maize MON89034 addresses the comments raised by the Member States and considers it unlikely that maize MON89034 will have any adverse effect on human and animal health or on the environment in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of The Netherlands (VROM), received 31 January 2007, concerning a request for placing on the market of maize MON89034 in accordance with Regulation (EC) No 1829/2003.
2. Acknowledgement letter, dated 2 February 2007, from EFSA to the Competent Authority of The Netherlands (Ref. SR/KL/shv (2007) 1954259).
3. Letter from EFSA to applicant, dated 13 July 2007, with request for clarifications under completeness check (Ref. SR/SM/eb (2007) 2255040).
4. Letter from applicant, dated 18 July 2007, providing EFSA with clarifications required under completeness check.
5. Letter from EFSA to applicant, dated 7 August 2007, with request for further clarifications under completeness check (Ref. SR/SM/eb (2007) 2303901).
6. Letter from applicant, dated 13 August 2007, providing EFSA with an updated version of the application EFSA-GMO-NL-2007-37 submitted by Monsanto Europe S.A. under Regulation (EC) No 1829/2003:

   Part I – Technical dossier

   Part II – Summary

   Part III – Cartagena Protocol

   Part IV – Labelling and Unique Identifier

Part V – Samples and Detection

Part VI – Additional information for GMOs

7. Letter from EFSA to applicant, dated 24 August 2007, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2007-37, maize MON89034 submitted by Monsanto Europe S.A. under Regulation (EC) No 1829/2003 (Ref. SR/SM/shv (2007) 2328012).
8. Letter from EFSA to applicant, dated 29 August 2007, with request for additional information by CRL/JRC (ref. SR/KL/shv (2007) 2342536).
9. Letter from applicant to EFSA, dated 6 September 2007, providing EFSA with information.
10. Letter from EFSA to applicant, dated 19 October 2007, concerning the completeness of the application by CRL/JRC (ref. SR/SM/shv (2007) 2456054).
11. Letter from EFSA to applicant, dated 27 November 2007, with request for additional information (ref. SR/SM/shv (2007) 2530067).
12. Letter from applicant to EFSA, dated 6 December 2007, providing supplementary information.
13. Letter from EFSA to applicant, dated 21 January 2008, with request for additional information (ref. SR/SM/shv (2008) 2633518).
14. Letter from applicant to EFSA, dated 12 February 2008, providing supplementary information spontaneously.
15. Letter from applicant to EFSA, dated 13 February 2008, providing supplementary information.
16. Letter from EFSA to applicant, dated 27 February 2008, with request for additional information (Ref. SR/SM/shv (2008) 2717274).
17. Letter from applicant to EFSA, dated 16 April 2008, providing supplementary information spontaneously.
18. Letter from applicant to EFSA, dated 6 May 2008, providing supplementary information.
19. Letter from EFSA to applicant, dated 2 July 2008, with request for additional information (Ref. PB/SM/shv (2008) 3140198).
20. Letter from applicant to EFSA, dated 31 July 2008, providing partial supplementary information.
21. Letter from EFSA to applicant, dated 20 October 2008, with request for additional information (Ref. SR/SM/CP/shv (2008) 3383621).
22. Letter from applicant to EFSA, dated 29 October 2008, providing supplementary information.
23. Letter from EFSA to applicant, dated 25 November 2008, to re start the clock (Ref. PB/SM/shv(2008) 3482690)

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