SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-NL-2007-38) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON89034 x NK603 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto[1]

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

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ABSTRACT

This opinion reports on an evaluation of a risk assessment for placing on the market the genetically modified herbicide tolerant and insect resistant maize MON89034 x NK603 for food and feed uses, import and processing. Conventional breeding methods were used in the production of maize MON89034 x NK603 from inbred lines of the respective parental events. The structural integrity of the inserts in the single events as well as the phenotypes were retained in the hybrid. The expression levels of the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins in maize MON89034 x NK603 were demonstrated to be comparable with those of the single events. The comparative analysis of phenotypic, agronomic and compositional characteristics of this GM maize indicated equivalence with its non-GM counterpart and conventional maize except for the expression of the target proteins, providing resistance to certain lepidopteran pests and tolerance to glyphosate herbicide. The safety assessment identified no concerns regarding potential toxicity and allergenicity of maize MON89034 x NK603. A feeding study on broiler chickens confirmed the nutritional equivalence of this GM maize to non-GM counterpart and conventional maize. Considering the intended uses of maize MON89034 x NK603, which excludes cultivation within the European Union, no scientific assessment of potential environmental effects associated with cultivation of maize MON89034 x NK603 was required. In case of accidental release of viable maize grain of MON89034 x NK603 into the environment during transportation and processing, there are no indications of increased likelihood of establishment or survival of feral maize plants except in the presence of the herbicide. The GMO Panel concludes that maize MON89034 x NK603 is as safe as its non-GM counterpart with respect to effects on human and animal health and the environment.

KEY WORDS

GMO, maize, MON89034 x NK60, insect resistance, herbicide tolerance, risk assessment, food and feed safety, environment, import and processing, Regulation (EC) No 1829/2003.

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1 On request from the Competent Authority of The Netherlands for an application (EFSA-GMO-NL-2007-38) submitted by Monsanto, Question No EFSA-Q-2007-046, adopted on 9 September 2009.

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SUMMARY

Following the submission of an application (EFSA-GMO-NL-2007-38) under Regulation (EC) No 1829/2003 from Monsanto, the Panel on Genetically Modified Organisms was asked to deliver a scientific opinion on herbicide tolerant and insect resistant maize MON89034 x NK603 (Unique identifier MON-89Ø34-3 x MON-ØØ6Ø3-6) for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2007-38, additional information supplied by the applicant and scientific comments submitted by Member States. Further information from applications for placing the single maize events MON89034 and NK603 on the market under EU regulatory procedures was taken into account where appropriate. The scope of application EFSA-GMO-NL-20007-38 is for food and feed uses, import and processing of maize MON89034 x NK603 and all derived products, but excludes cultivation in the EU. The EFSA GMO Panel assessed maize MON89034 x NK603 with reference to the intended uses and 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) and the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events (EFSA, 2007a). The scientific assessment included molecular characterisation of the inserted DNA and expression of target proteins. A comparative analysis of agronomic traits and composition was undertaken, and the safety of the new proteins, as individual proteins and in combination, and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plan were undertaken.

Maize MON89034 x NK603 was produced by crosses between maize inbred lines containing maize MON89034 and NK603 events to combine the resistance trait to certain lepidopteran species in maize MON89034 and the tolerance to glyphosate in maize NK603.

The stability of the genetic modification has been demonstrated over several generations in the single events and Southern analysis has confirmed that the structural integrity of the single events was maintained in the hybrid MON89034 x NK603. Appropriate analyses of the integration sites in maize MON89034 and NK603, including flanking regions, were carried out. The bioinformatic analysis demonstrated the absence of any potential new ORFs coding for known toxins or allergens. The expression of the new proteins has been sufficiently analysed in MON89034 x NK603 and is comparable to expression in single events. The Cry1A.105 and Cry2Ab2 proteins expressed in the parental maize MON89034 and the CP4 EPSPS and CP4 EPSPS L214P protein expressed in maize NK603 have been assessed previously and no safety concerns have been identified. Furthermore, the results of the compositional analysis of grain and forage material of maize MON89034 x NK603 collected at field trials in Argentina during the season 2004-2005, indicated that, with the exception of the newly introduced proteins, maize MON89034 x NK603 is compositionally and agronomically equivalent to its non-GM counterpart. Based on the comprehensive data available, including responses of the applicant to questions posed by the GMO Panel, the Panel concluded that there was no indication that crossing maize MON89034 with maize NK603 results in an interaction of the newly expressed proteins affecting composition or agronomic characteristics. Furthermore, the nutritional properties of maize MON89034 x NK603 do not differ from its non-GM counterpart.

Given all the information provided, the GMO Panel concludes that interactions between the proteins expressed by the single events that might impact on food and feed safety are unlikely and that the nutritional properties of maize MON89034 x NK603 are not different from those of its non-GM counterpart. Therefore, the Panel considers that maize MON89034 x NK603 is as safe and as

nutritious as its non-GM counterpart and that the overall allergenicity of the whole plant is not changed.

Considering the intended uses of maize MON89034 x NK603, which exclude cultivation, there is no requirement for scientific assessment of possible environmental effects associated with the cultivation of this GM maize. In case of accidental release into the environment of maize MON89034 x NK603 viable grains during transportation and processing, there are no indications of increased likelihood of establishment and spread of feral maize plants. Also, the low levels of environmental exposure through other routes indicate that the risk to 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 x NK603.

In conclusion, the EFSA GMO Panel considers that the information available for maize MON89034 x NK603 addresses the scientific comments raised by Member States and that maize MON89034 x NK603 is as safe as its non-GM counterpart with respect to potential effects on human and animal health and the environment. The EFSA GMO Panel thus concludes that maize MON89034 x NK603 is unlikely to have any adverse effect on human and animal health or on the environment in the context of its intended uses.

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BACKGROUND

On 1st of February 2007, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands an application (Reference EFSA-GMO-NL-2007-38), for authorisation of the insect-resistant and herbicide-tolerant genetically modified maize MON89034 x NK603 (Unique Identifier MON-89Ø34-3 x MON-ØØ6Ø3-6), submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on genetically modified (GM) food and feed. After receiving the application EFSA-GMO-NL-2007-38 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed Member States and the European Commission, and made the summary of the application 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 19 July and 13 August 2007, EFSA received additional information requested under completeness check (requested on 13 July and 7 August 2007). 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 Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member State bodies had three months after the date of acknowledgement of the valid application (until 24 November 2007) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out a scientific assessment of the GM maize MON89034 x NK603 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. When carrying out the safety assessment, the EFSA GMO Panel took 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) and the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events (EFSA, 2007a), the scientific comments of Member States and the additional information provided by the applicant. Further information from applications for placing the single maize events MON89034 and NK603 on the market under EU regulatory procedures was taken into account where appropriate.

On 23 January 2008, 29 February 2008, 2 July 2008, 20 October 2008, 28 January 2009, and 2 February 2009 the EFSA GMO Panel requested from the applicant additional information. The applicant provided the requested information on 14 February 2008, 8 May 2008, 4 August 2008, 30 October 2008, 13 March 2009 and 3 June 2009. After receipt and assessment of the full data package the EFSA GMO Panel finalised its risk assessment on maize MON89034 x NK603.

The single maize events MON89034 and NK603 have been the subjects of earlier assessments and have received an EFSA opinion in favour of their authorisation (EFSA 2003a, b; EFSA, 2008; EFSA, 2009). Maize NK603 was authorised under Directive 2001/18/EC by Commission Decision 2004/643/EC (EC, 2004). The use of food and food ingredients from maize NK603 was authorised under the Regulation (EC) No 258/97 (EC, 1997) by Commission Decision 2005/448/EC (EC, 2005).

In giving its scientific opinion on GM maize MON89034 x NK603 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 acknowledgement of the valid application. As additional information was requested by the EFSA GMO Panel, the time limit of six 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, this scientific opinion 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 EFSA overall opinion in accordance with Articles 6(5) and 18(5).

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific risk assessment of maize MON89034 x NK603 for food and feed uses, 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/environment 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 a scientific opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA GMO Panel did also not consider 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 risk management.

ACKNOWLEDGEMENTS

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ASSESSMENT

1. Introduction

The genetically modified maize MON89034 x NK603 (Unique Identifier MON-89Ø34-3 x MON-ØØ6Ø3-6) was assessed with reference to its intended uses, taking 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) and the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events (EFSA, 2007a). The risk assessment presented here is based on the information provided in the application relating to maize MON89034 x NK603 submitted in the EU including additional information from the applicant and information on the single events, as well as scientific comments that were raised by the Member States.

2. Issues raised by Member States

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

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

The EFSA GMO Panel guidance document (EFSA, 2006a) states that when events have been combined by the interbreeding of existing approved GM lines, the need for further molecular analysis will depend, on a case-by-case basis, on the nature of the genetic modifications involved.

3.1.1. Method of production of maize MON89034 x NK603

Traditional breeding methods were used to produce maize MON89034 × NK603 and no new genetic modification was involved. The two inserts that are present in maize MON89034 × NK603 were derived from maize lines containing two independent single events: MON89034 and NK603. Each of these GM maize events was the subject of earlier safety evaluation and separate opinions for each of them have been published (EFSA, 2003a,b; 2008 and 2009). Maize MON89034 × NK603 combines the lepidopteran protection trait from maize MON89034 and the glyphosate tolerance trait from maize NK603.

3.1.2. Summary of the evaluation of the single events

Maize MON89034

Maize MON89034 was developed through *Agrobacterium*-mediated transformation of immature maize embryos using the binary plasmid vector PV-ZMIR245. 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 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 approach 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. 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. For the Southern analyses a non-GM counterpart, which was F2 grain from a cross between lines designated LH172 and LH198, was used. It is an appropriate control for the material used in the characterization 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.

Sequence analysis of the MON89034 insert and the 5' and 3' flanking maize genomic DNA confirmed that both the *cry1A.105* and *cry2Ab2* coding sequences are identical to those of the corresponding genes in PV-ZMIR245. Data indicate that the e35S promoter that regulates expression of the c*ry1A.105* gene has been modified to produce a shorter promoter version, e35S89 (which 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.

Bioinformatic analysis was performed on the 5' and 3' sequences flanking the MON89034 insertion site to determine if any endogenous genes had been deleted and/or disrupted during the transformation event. A BLASTx search returned no significant homologies, indicating that the insertion of the MON89034 T-DNA I did not disrupt any known open reading frames. An updated (February 2008) bioinformatics analysis was performed using BLASTn and BLASTx algorithms. The data again indicated that no known endogenous maize genes have been disrupted in flanking regions adjacent to insert T-DNA I in maize MON89034. Bioinformatic analysis also revealed no biologically relevant similarity to allergens, toxins, or bioactive proteins for any of the putative polypeptides that might be produced from the 5' and 3' flanking regions.

Stability of the MON89034 insert was confirmed by Southern analysis over multiple generations from the maize MON89034 breeding history. The presence of 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.

Maize NK603

Proprietary embryogenic maize cell culture AW x CW was the initial recipient of the introduced DNA by transformation using particle acceleration technology to develop the maize NK603 event. Conventional breeding methods were used to backcross plants generated from the initial transformation into a recurrent, desired inbred maize line with a genetic background of interest to the breeder.

Maize NK603 has been developed for tolerance to glyphosate by the introduction of a gene coding for glyphosate tolerant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). Particle acceleration was used to introduce a fragment of DNA isolated from the bacterial plasmid vector PV-ZMGT32. The plasmid vector contains two adjacent plant gene expression cassettes each containing a single copy of the CP4 *epsps* gene fused to chloroplast transit

peptide (CTP) sequences. In the first *ctp*2-CP4 *epsps* cassette the coding sequence is regulated by the rice actin promoter and a rice intron sequence introduced upstream of the *ctp* sequence. Expression of the second *ctp*2-CP4 *epsps* cassette is regulated by an enhanced 35S CaMV promoter and a maize intron derived from a gene encoding a heat shock protein. The vector also contains an *npt*II bacterial selectable marker gene for kanamycin resistance and an origin of replication (*ori*). A *Mlu*I restriction fragment of the PV-ZMGT32 plasmid vector, designated PV-ZMGT32L, was used for transformation and this fragment only contains the CP4 *epsps* plant gene expression cassettes. The *npt*II gene, as well as the *ori* gene, are not present in the fragment PV-ZMGT32L.

Southern analysis, PCR and DNA sequencing were used to show that the insert in maize NK603 is a single complete copy of PV-ZMGT32L. There is no detectable presence of plasmid DNA from outside of the vector fragment PV-TMGT32L. Both *ctp*2-CP4 *epsps* gene cassettes are intact within maize NK603. The sequence of the CP4 *epsps* gene from the first cassette in maize NK603 is identical to that in the original plasmid, whilst in the second inserted cassette the sequence of the CP4 *epsps* gene differs by two nucleotides from that in the original plasmid. These nucleotide changes result in one silent mutation (i.e. no amino acid modification) and one amino acid substitution of proline for leucine at amino acid position 214 (hence the gene is designated CP4 *epsps* L214P). Proteins derived from the CP4 *epsps* and CP4 *epsps l214p* genes were shown to be structurally and functionally equivalent.

The insert includes, at the 3' end, an additional 217 bp DNA fragment of the rice actin promoter. This fragment does not contain sequences needed for promoter activity. Next to this 217 bp fragment is a 305 bp region with homology to chloroplast DNA. Further sequencing of 5' and 3' flanking regions confirmed the sequences to be maize genomic DNA. Bioinformatics analysis did not indicate any interruption of know maize genes.

Bioinformatic analyses were carried out to assess the potential toxicity, allergenicity or pharmacological activity of putative polypeptides encoded at the 5' and 3' junctions of a segment of chloroplast DNA that is located downstream of the 3' end of the maize NK603. Similarly, a bioinformatics assessment was carried out on the potential toxicity, allergenicity or pharmacological activity of putative polypeptides, defined by the 5' junction of the NK603 insert and plant genomic DNA. The results of these 3' and 5' end bioinformatic analyses, which were updated in 2008, demonstrate that in the highly unlikely event that any of the junction polypeptides were translated, they do not share a sufficient degree of sequence similarity or identity to indicate that they are potentially toxic, allergenic or have other health implications.

RT-PCR data demonstrated that there was no detectable transcription into the NK603 insert from the maize genomic DNA sequence flanking the 3' end of the inserted DNA. However, the data did demonstrate that an RNA species could be detected that likely initiated in the promoter of the NK603 insert and preceded through the *nos* 3' transcriptional termination sequence continuing into the maize genomic DNA flanking the 3' end of the insert. Northern analyses with probes directed at potentially transcribed sequences downstream of the *nos* 3' element failed to detect the larger read-through transcript. Moreover, when these same blots were probed with a segment of the CP4 *epsps* coding region, only one band of 1.4 kb, the expected size of the CP4 *epsps* transcript terminating within the NOS terminator, was observed. This suggests that the read-through transcript accumulates at very low levels only detectable by a highly sensitive method such as RT-PCR. In the highly unlikely event that any of the junction polypeptides were translated, bioinformatic analyses revealed that they would not share a sufficient degree of sequence similarity or identity to potentially toxins or allergens.

Southern analysis provided data which were consistent with a single site of integration and confirmed the genetic stability of the inserted DNA in NK603. Segregation data for nine generations were provided including six generations of crossing and three generations of self pollination. Stability of the glyphosate tolerance trait was also confirmed.

3.1.3. Transgenic constructs in maize MON89034 x NK603

Maize MON89034 × NK603 has been produced by crossing inbred plants of MON89034 and NK603 using traditional breeding methods. F1 hybrid seed inherits the lepidopteran resistance trait from MON89034 and the glyphosate tolerance trait from NK603. Integrity of the individual inserts from MON89034 and NK603 in the MON89034 × NK603 hybrid was assessed using Southern analysis. Integrity of the MON89034 insert was determined using genomic DNA digested with *Ssp*I and probes consisting of approximately 2 kb of *cry*1A.105 and 1.2 kb of *cry*2Ab2. Integrity of NK603 insert was determined using genomic DNA digested with *Sca*I and *Msc*I and probes for coding sequence spanning the region of the CTP2/CP4 *epsps*. Southern analysis indicated that the integrity of the individual inserts was maintained in the MON89034 × NK603 hybrid.

3.1.4. Information on the expression of the inserts

The levels of the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins in various tissues of maize MON89034 × NK603 were assessed by validated enzyme-linked immunosorbent assay (ELISA). Tissue samples for analysis were collected from five field trials conducted in Argentina during 2004 (). The trials were located in the provinces of Buenos Aires, Cordoba and Santa Fe, which represent the major maize growing region of Argentina and provide a variety of environmental conditions. At each site, three replicated plots of MON89034 × NK603, MON89034 and NK603, as well as the non-GM counterpart, were planted using a randomized complete block field design. Young leaf, young root, over season whole plant 3 (OSWP-3), forage, forage-root, pollen, and grain tissues were collected from each replicated plot at all field sites. The samples from young leaf (over season leaf; OSL-1) and young root (over season root; OSR-1) were collected at the V2 – V4 growth stage (21 to 29 days after pollination) and the OSWP-3 samples were collected at the V10 – V12 growth stage (41 to 53 days after pollination).

Cry1A.105 protein levels

The mean Cry1A.105 protein levels in maize MON89034 × NK603 across all sites were 220 μg/g dwt in OSL-1, 66 μg/g dwt in OSR-1, 83 μg/g dwt in OSWP-3, 30 μg/g dwt in forage, 24 μg/g dwt in forage-root, 9.6 μg/g dwt in pollen and 3.1 μg/g dw in grain.

Cry2Ab2 protein levels

The mean Cry2Ab2 protein levels in maize MON89034 × NK603 across all sites were 140 μg/g dwt in OSL-1, 37 μg/g dwt in OSR-1, 72 μg/g dwt in OSWP-3, 33 μg/g dwt in forage, 27 μg/g dwt in forage-root, 0.66 μg/g dwt in pollen and 1.2 μg/g dwt in grain.

CP4 EPSPS protein levels

The mean CP4 EPSPS protein levels in maize MON89034 × NK603 across all sites were 240 μg/g dwt in OSL-1, 78 μg/g dwt in OSR-1, 210 μg/g dwt in OSWP-3, 74 μg/g dwt in forage, 48 μg/g dwt in forage root, 390 μg/g dwt in pollen and 8.1 μg/g dwt in grain.
Overall, the ranges across all sites for the Cry1A.105, Cry2Ab2 and CP4 EPSPS protein levels in maize MON89034 × NK603 were comparable to the corresponding ranges in either maize MON89034 or NK603.

3.1.5. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in events MON89034 and NK603 was demonstrated previously (EFSA 2008, 2009). In the maize MON89034 x NK603 inserts are combined. The

Southern data presented show that both events are present and that the structural integrity of each insert is retained. Furthermore, each of the traits has been conserved in this maize.

3.2. Conclusion

As conventional breeding methods were used in the production of maize MON89034 x NK603, no additional genetic modification was involved. Southern analyses demonstrated that the structural integrity of the inserts in maize MON89034 and NK603 were retained in maize MON89034 x NK603. The genetic stability of the integrated DNA has been demonstrated in the single events. Phenotypic analyses demonstrated that the traits were retained in the hybrid.
The expression levels of Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins in maize MON89034 x NK603 were measured in various tissues, including grains. Levels of expression in all tissues examined in maize MON89034 x NK603 have been demonstrated to be comparable with those of the single events.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

Having considered the information provided in the application and the Member States comments, as well as the information provided in applications to market the genetically modified parental maize varieties (MON89034 and NK603), the EFSA GMO Panel requested from the applicant further information with respect to the field trial design, including pesticide treatments of the GM maize and the non-GM counterpart maize, and the pedigree of the comparators used and their relatedness to maize MON89034 x NK630. The applicant provided the requested data.

4.1.1. Summary of the previous evaluation of the single events

The parental maize events MON89034 and NK603 have been assessed earlier for their composition relative to the composition of the corresponding non-modified maize varieties having comparable genetic backgrounds. The compounds studied were those recommended by OECD (2002). It was concluded that both genetically modified parental maize lines have a composition equivalent to conventional maize varieties (EFSA 2003a, 2003b, 2008, 2009). The main findings are summarised below.

Maize MON89034

Compositional data on grain and forage materials of maize MON89034 and the non-GM counterpart LH198 × LH172 from field trials in the USA in 2004 and Argentina the season 2004/2005 were statistically compared for all trial sites individually and across all trial sites. 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 non-GM counterpart in relation to three of the 77 compounds investigated. These were the phosphorus 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). Statistical evaluation of compositional data from the field trials in Argentina 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. In all cases differences were small, were sometimes not consistent and occurred only at a few single trial sites. The levels of these constituents fell within the natural variation of conventional maize varieties and data reported in the literature and data bases (ILSI, 2008). None of the statistically significant differences observed in the analyses performed per site indicated a need for further exploration. The Panel concluded that the observed statistical differences in composition in 2004 and 2004/2005 had no biological relevance and that maize MON89034 is compositionally equivalent to the non-GM counterpart except that it expresses the Cry1A.105 and Cry2Ab2 proteins.

Based on data obtained from field trials performed in USA in 2004 and/or 2005, the applicant also provided information on agronomic performance, phenotypic characteristics and parameters of natural ecological interaction of maize MON89034 and the non-GM counterpart with a comparable genetic background. The non-GM counterpart used was generally LH198 x LH172 (H1325023), but was the conventional maize variety DKC51-43 when the maize was grown in northern maize growing regions of USA. The number of field trial sites varied from 1 to 18 depending on the specific character investigated. At each trial site, three to 4 conventional maize varieties were grown, resulting in 23 conventional maize varieties 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, and plant interactions with insects, plant pathogens and abiotic stressors. 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 non-GM counterpart and these were plant height and stalk lodging, but only for one of the two growing seasons. As differences were relatively small (difference in plant height less than 2%) and both parameters of maize 89034 fell within the range of conventional maize varieties, the GMO Panel was of the opinion that this statistical finding is of no biological relevance. Of the considerable number of insect categories, disease categories and abiotic stressors evaluated in the studies of ecological interactions in 2004 and 2005, only a few differences between maize MON89034 and its comparator were noted and only at particular growth stages and trial sites. In the absence of consistent unexpected differences between maize MON89034 and its non-GM counterpart that is not linked to the introduced lepidopteran resistance trait, the GMO-panel concluded that maize MON89034 is agronomically and phenotypically equivalent to its non-GM counterpart and to conventional maize varieties, except for expressing the introduced insect protecting trait.

Maize NK603

Maize NK603 was compared with a non-GM maize counterpart (LH82 × B73) having a comparable genetic background to maize NK603 with regard to composition as well as phenotypic and agronomic chracteristics. The materials studied were obtained from replicated field trials in the USA (year 1998) and Europe (year 1999). The field trials included both plots with maize NK603 treated with the target herbicide glyphosate and plots untreated with this herbicide. With the exception of the glyphosate-tolerant trait, maize NK603 was found to be phenotypically and agronomically similar to its non-GM comparator. To evaluate the composition of maize NK603 a total of 44 different parameters, including proximates, amino- and fatty acids, minerals, vitamin E, phytic acid and trypsin inhibitor were analysed in grain. Forage was analysed for 7 parameters; proximates and neutral and acid detergent fibre. The levels of these constituents in maize NK603 were either within the ranges found in the non-GM maize control materials or within the ranges reported for these constituents and materials in published literature. No consistent compositional differences requiring further studies were found. A summary of the compositional data of maize NK603 is available in the open literature (Ridley *et al*., 2002). The GMO Panel concluded that NK603 maize is compositionally equivalent to conventional maize, except for the presence of the newly expressed CP4 EPSPS and CP4 EPSPS L214P proteins in maize NK603 (EFSA, 2003a, 2003b, 2009a).

The comparative phenotypic and agronomic assessment of maize NK603 to its non-GM controls were performed in nine field trials conducted in Germany (5) and France (4) between 2000 and 2002 according to Good Experimental Practice. In these studies three different hybrids with the NK603 transformation event (Crr0501RR, TW812H-A and DKC4445) and three appropriate non-GM counterparts (CRR0502, Monumental and DK440) were used. The parameters evaluated in these materials included growth and developmental characteristics, plant and ear morphology, plant health and vigour characteristics (including susceptibility to insect attack and to disease), susceptibility to applied pesticides, and agronomic performance, including forage and grain yield.

A few statistical significant differences between maize NK603 and its controls were observed at single trial sites, but these were not observed consistently. Thus, only two differences were noted in the five field trials in Germany. One of these was an increased yield (fresh weight) at one of the sites, the other an increased ear height at another site. In the French field trials, the opposite effect (reduction) on ear height was noted at one site. Other differences noted in the four French field trials were increased plant vigour of maize NK603 at one site, a reduced percentage of damaged ears at one site, an increased percentage of plants lacking ears at one site, and reduced early plant count at three of the four sites. When the latter difference was addressed specifically it was noted that the reduced early plant count most likely was due to reduced seed quality this year and not to the genetic modification per se. Other differences observed were small, not consistent and fell within the normal variation, and were not considered biologically meaningful in terms of pest potential or plant growth and development.

The lack of significant differences in morphology, reproductive maturity parameters, vigour, plant health, pest susceptibility, and weed potential between maize NK603 and its non-GM counterpart indicate that the overall fitness and survival characteristics of maize NK603 has not been altered. The GMO Panel found maize NK603 to be phenotypically and agronomically equivalent to its controls and conventional maize varieties.

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

In the compositional studies, the GM maize MON89034 × NK603 was compared to the non-transgenic maize LH198 x LH172 (also referred to as H1325023), which is a conventional maize variety with background genetics similar to maize MON89034 × NK603. In addition to the comparator, three conventional maize varieties were included in the field trial at each field trial site, the conventional varieties being different at the different trial sites. The field trials were performed under normal agronomic field conditions (only maize material carrying the NK603 event was exposed to glyphosate) at five different sites in Argentina the season 2004-2005. At each trial site each treatment was replicated three times. The 15 conventional maize varieties were used as reference material to determine a 99% confidence interval of each of the analytes analysed, that is, to illustrate 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. Additional materials were collected for expression analysis.

Also in the field studies performed to investigate agronomic, phenotypic and ecological aspects of the test material, maize LH198 x LH172 was used as non-GM counterpart material.

4.1.3. Compositional analysis

Maize forage of MON89034 x NK603 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, which corresponded to those suggested by OECD (2002). Each analyte was statistically analysed for whether a difference in the level occurred between forage and grain materials of maize MON89034 × NK603 and the non-GM counterpart collected from the same field trial site as well as across all sites (data from all sites combined). In cases when more than 50% of the analytical data points fell below the limit of quantification no statistical analyses were performed.

When the compositional data were analysed across sites, the composition of maize MON89034 × NK603 differed significantly from the non-GM counterpart in relation to six of the 77 compounds investigated. For three of these, forage ash (5.51 vs 4.98% dw), forage total fat (1.98 vs 2.45% total fatty acids), and grain acid detergent fibre (5.12 vs 5.66% dw), there were no significant difference observed at any of the single sites. The other three were grain total fat (3.47 vs 3.22% dw), which was increased at two of the five sites, grain stearic acid (1.88 vs 1.79% total fat), which was increased at three sites, and grain vitamin B2 (1.79 vs 1.94 mg/kg dw), which was reduced at one site. The observed differences were not large, fell within the variation observed for conventional maize varieties and data reported in the literature and in data bases (ILSI, 2008).

When the compositional data were analysed for each separate site, 14 analytes were found to differ between maize MON89034 × NK603 and the non-GM counterpart at one of the five field trial sites (and not across all sites). Neutral detergent fibre in forage was found to be significantly increased in maize MON89034 × NK603 at two of the five trial sites, and also in this case without any significant difference being observed when data across all sites were analysed. In these cases, the differences observed were small and fell within the natural variation, calculated from the analytical data of the conventional maize varieties included in the field studies, and data reported in the literature.

The GMO Panel considered the observed compositional differences between maize MON89034 × NK603 and its comparator in the light of the field trial design, measured biological variation and the level of the studied compounds in conventional maize varieties, and concludes that maize MON89034 × NK603 is compositionally equivalent to the non-GM counterpart and conventional maize varieties, except for the introduced trait.

4.1.4. Agronomic traits and GM phenotype

Previous studies have shown that with exception of the glyphosate-tolerance trait in maize NK603 and the lepidopteran resistant trait in maize MON89034, these genetically modified maize varieties are phenotypically and agronomically equivalent to conventional maize varieties (EFSA, 2003a, 2003b, 2008, 2009a).

The applicant provided information on agronomic performance, phenotypic characteristics and natural ecological interaction of maize MON89034 × NK603 and its non-GM counterpart. The data were obtained from field trials performed at five sites in Argentina the season 2004/2005. At each trial site three conventional maize varieties were fully randomized with the test materials.

Phenotypic characteristics investigated included seedling vigour, early stand count, 50% pollen shed and silking, staygreen, ear heights, plant heights, dropped ears, stalk lodging, root lodging, final stand count, grain moisture, test weight and yield. The only character that differed between the tested stacked genetically modified maize and the non-GM counterpart when the data was pooled across all five sites was 50% pollen shed which on average was 58 days (range 54.7-63.3 days) in MON89034 × NK603 and 57 days (range 54.7-60.7) in the non-GM counterpart. At the individual site analysis a difference was noted at two of the five trial sites. As the difference was small and fell within the

natural variation in 50% pollen shed among 15 conventional maize varieties (range 54.0-68.7 days), the GMO Panel interpreted the observed statistical difference in 50% pollen shed as biologically irrelevant. The seedling vigour could not be appropriately analysed with statistical methods due to lack of variability between trial sites. There were also some statistically significant differences in the individual site analysis, particularly at one of the trial sites, but none of the differences were marked or showed a consistent pattern. The GMO Panel concluded that the field studies devoted to agronomic performance and phenotypic characters identified no difference that is likely to be biologically meaningful.

4.2. Conclusion

Analysis carried out on materials from maize MON89034 × NK603 and its closely genetically related counterpart LH198 × LH172 (H1325023) indicate that they are compositionally and agronomically equivalent, except for the presence of the Cry1A.105, Cry2Ab2, CP4 EPSPS, and CP4 EPSPS L214 proteins in maize MON89034 × NK603. The comparison of maize MON89034 × NK603 to its non-GM counterpart provided no indication that stacking of the maize events MON89034 and NK603 results in compositional or agronomic changes.

5. Food/Feed safety assessment

5.1. Evaluation of relevant scientific data

Having considered the information provided in the application and the Member States comments, as well as the information provided in applications to market the genetically modified parental maize varieties (MON89034 and NK603), the EFSA GMO Panel requested from the applicant presentation of the expression data of Cry1A.105, Cry2Ab2 and CP4 EPSPS in various tissues of maize MON89034 x NK603, maize MON89034 and maize NK603 for each field trial site separately, and presentation of data in the 42-day broiler feeding study for each sex separately.

5.1.1. Summary of the previous evaluation of the single events

The Cry1A.105 and Cry2Ab2 protein(s) expressed in the parental maize MON89034, and the CP4 EPSPS and CP4 EPSPS L214P proteins expressed in the parental maize NK603 have already been assessed for their safety by the GMO Panel (EFSA, 2003a, 2003b, 2008, 2009a) and no safety concerns were identified.

Maize MON89034

Maize MON89034 expresses the Cry1A.105 and Cry2Ab2 proteins. *E.coli*-produced Cry1A.105 and Cry2Ab2 proteins were used for safety studies after it had been demonstrated experimentally that they are equivalent to those extracted from maize event MON89034. No toxicity of the Cry1A.105 and Cry2Ab2 proteins were observed in acute oral toxicity studies in mice. Both proteins were shown to be quickly degraded in simulated gastric fluid, and a little less quickly in simulated intestinal fluid. The amino acid sequence of Cry1A.105 and Cry2Ab2 showed no similarity to known toxins and allergens in bioinformatics studies. In a 90-day feeding study in rats with grain material from maize MON89034 no treatment-related adverse effects were observed, and a 42-day feeding study on broiler chickens showed that maize MON89034 is nutritionally equivalent to the non-GM counterpart and commercial maize varieties included in the study.

It was concluded 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 used.

Maize NK603

Maize NK603 expresses two versions of the CP4 EPSPS protein. *E.coli*-produced CP4 EPSPS and CP4 EPSPS L214P proteins were used for the safety studies after it had been demonstrated experimentally that these microbially produced proteins were equivalent to those extracted from maize plants carrying the event NK603. No toxicity of the CP4 EPSPS and CP4 EPSPS L214P proteins were observed in acute oral toxicity studies in mice. The CP4 EPSPS proteins were shown to be quickly degraded in simulated gastric fluid. Bioinformatics studies demonstrated that the CP4 EPSPS proteins show no homology to known toxic and allergenic proteins. A 13-week feeding study in rats with maize NK603 indicated no toxicity (Hammond *et al*., 2004), and a 42-day feeding study on broiler chickens showed that maize NK603 is as nutritionally wholesome as the non-GM counterpart (LH82 x B73) and commercial maize varieties included in the study (Taylor *et al*., 2003). The nutritional equivalence of maize NK603 to commercial maize varieties has been confirmed in feeding studies on Angus-continental cross steers, Holstein dairy cows and growing-finishing pigs of two breeds (Fischer *et al*., 2002; Erickson *et al*., 2003; Grant *et al*., 2003; Ipharraguerre *et al*., 2003; Hyun *et al*., 2004). These studies on experimental and farm animal supported the findings of the compositional analysis of no change in composition beyond the intended expression of the CP4 EPSPS and CP4 EPSPS L214P proteins.

5.1.2. Product description and intended use

The scope of application EFSA-GMO-NL-2007-38 is for food and feed uses, import and processing of maize MON89034 × NK603, and thus include all derived products (e.g. starch, syrups, ethanol, maize oil, flaking, coarse and regular grits, coarse and dusted meal, flour, maize germ meal, maize gluten feed, condensed steep water and maize gluten meal).

The genetic modification present in maize MON89034 × NK603 result in the expression of the Cry1A.105 and Cry2Ab2 proteins, which are toxic to certain lepidopteran species, and the CP4 EPSPS and CP4 EPSPS L214P proteins, which renders the stacked maize hybrid tolerant to glyphosate-containing herbicides. The genetic modification is intended to improve agronomic performance only, and is not intended to influence the nutritional properties, the processing characteristics and overall use of maize as a crop. Maize MON89034 × NK603 will most likely be imported mixed with other genetically modified maize varieties or with conventional maize varieties.

5.1.3. Effect of processing

Since maize MON89034 × NK603 has been found to be compositionally equivalent to the non-GM counterpart and to conventional maize, except for the newly expressed Cry1A.105, Cry2Ab2, CP4 EPSPS, and CP4 EPSPS L214P proteins (see Section 4.1.3), the effect of processing on maize MON89034 x NK603 is not expected to be different compared to that of conventional maize.

A multitude of processes are used in maize processing, including temperature treatments, hydrolyses, soaking in slightly acidic water, and drying. Any of such methods are likely to influence degradation and/or denaturation of constituents, but there is no indication that they will influence maize MON89034 x NK603 differently than conventional maize.

5.1.4. Toxicology

5.1.4.1. Toxicological assessment of expressed novel proteins in maize MON89034 x NK603

No new genes in addition to those occurring in the parental maize varieties have been introduced in maize MON89034 x NK603. Analysis of the newly expressed proteins in various tissues of maize

MON89034 x NK603, MON89034 and NK603 revealed comparable expression levels in the stacked hybrid as compared to the expression levels in the parental events. The GMO Panel considered all the data available for maize MON89034 x NK603 and for the single events, including information provided by the applicant in response to questions of the Panel, and considers that interactions between the single events that might impact on food and feed safety are unlikely. f

5.1.4.2. Toxicological assessment of new constituents other than proteins

No new constituent other than the Cry1A.105, Cry2Ab2, CP4 EPSPS, and CP4 EPSPS L214P proteins are expressed in maize MON89034 × NK603 and no relevant changes in the composition of maize MON89034 × NK603 were detected by the compositional analysis.

5.1.4.3. Toxicological assessment of the whole GM food/feed

The genetically modified parental maize events MON89034 and NK603 have previously been found to be as safe as conventional maize for human and animal consumption (EFSA, 2003a, 2003b; 2008, 2009a). These safety studies included 90-day feeding studies in rats, which did not raise concern. A molecular characterization undertaken on maize MON89034 x NK603 identified no altered stability of the events (see section 3.1.5) when these were brought together by crossing, and expression analysis of the Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins revealed no change in protein expression levels that could raise concerns for human and animal health. As the composition of maize MON89034 x NK603 is comparable with that of non-GM maize varieties and the single events and also no indication for interaction between the single events was found, the GMO Panel is of the opinion that no additional safety studies on animals are required.

5.1.5. 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).

5.1.5.1. Assessment of allergenicity of the newly expressed proteins

The proteins present in the maize MON89034 x NK603 have been assessed previously and it was found unlikely that they are allergenic (EFSA, 2003a, 2003b, 2008, 2009a). Based on the information provided, the GMO Panel considers it unlikely that potential interactions occur that might change the allergenicity of the newly expressed proteins.

5.1.5.2. Assessment of allergenicity of the whole GM plant

The issue of a potential increased allergenicity of maize MON89034 x NK603, as compared to the parental GM maize events and conventional maize varieties, does not appear relevant to the Panel since maize is not considered a common allergenic food. Food allergies to maize are of low frequency and mainly occur in populations of specific geographic areas. Rare cases of occupational allergy to maize dust have been reported. There is no reason to expect that the use of maize MON89034 x NK603 will significantly increase the intake and exposure to maize. Therefore a 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 or the allergy risk for consumers.

5.1.6. Nutritional assessment of GM food/feed

The parental maize varieties, MON89034 and NK603, have individually been assessed for their nutritional wholesomeness and found to be as nutritious as conventional maize (EFSA, 2003a, 2003b, 2008, 2009a).

The applicant provided a 42-day broiler feeding study performed according to generally accepted guidelines (ILSI, 2003), and consisting of eight treatment groups. One group of chickens received maize MON89034 × NK603, another group maize LH198 x LH172 (H1325023; a non-GM maize with comparable background genetics to maize MON89034 × NK603), and the other six groups conventional non-GM maize varieties (Pioneer 32B33, Garst 8371, Midland 7B15, NK N72-J5, Nc + 5411 and DKC61-50).

Each treatment consisted of 50 male and 50 female broilers (10 birds/pen; pens in a randomised complete block design) fed isocaloric diets containing approximately 58% (w/w) maize grain in the starter diet and 59.5% maize grain in the grower/finisher diet ad libitum.

There were no differences in total feed intake, live bird weight at end of study, and unadjusted and adjusted feed conversion in broiler chickens fed diets with maize MON89034 × NK603, the appropriate non-GM counterpart or the conventional maize varieties. There was also no difference between treatments on performance, carcass, and meat quality parameters of the broiler chickens, except marginally increased wing and thigh weights in males (7 and 14g, respectively). These differences were not considered biologically meaningful to the Panel. The mortality rate was comparable after all treatments, being around 1% in the group receiving maize MON89034 × NK603, which is a very low death rate for broiler chickens in feeding studies. The mortality was randomly distributed in treatment groups without any relationship to treatment. Thus, the 42-day broiler feeding study showed that maize MON89034 × NK603 is nutritionally equivalent to the non-GM comparator and to conventional maize varieties.

5.1.7. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that maize MON89034 × NK603 is any less safe than its non-GM comparator, the parental GM maize lines, and conventional maize varieties. In addition, no biologically relevant agronomic and compositional changes were identified in maize MON89034 × NK603, and the maize is from a nutritional point of view 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.

5.2. Conclusion

The Cry1A.105 and Cry2Ab2 proteins expressed in maize MON89034 and the CP4 EPSPS and CP4 EPSPS L214P proteins expressed in maize NK603 have been assessed previously and no safety concerns for humans and other organisms were identified. Similarly, both maize MON89034 and maize NK603 were in the previous assessments found to be as safe as their non-genetically modified counterparts with respect to potential effects on human and animal health.

Given all the information provided, the GMO Panel concluded that interactions between the single events that might impact on food and feed safety of maize MON89034 x NK603 are unlikely.

The nutritional properties of maize MON89034 x NK603 has been studied in a feeding study with broiler chickens. The study confirms the conclusion from the compositional comparison, which indicated that the nutritional properties of maize MON89034 x NK603 are no different from those of conventional maize.

In conclusion the GMO Panel considers that maize MON89034 x NK603 is as safe and as nutritious as its non-GM counterpart and that the overall allergenicity of the whole plant is not changed. Maize MON89034 x NK603 is unlikely to have any adverse effect on human and animal health in the context of its intended uses.

6. Environmental risk assessment and monitoring

6.1. Evaluation of relevant scientific data

The scope of application EFSA-GMO-NL-2007-38 is for food and feed uses, import and processing of maize MON89034 x NK603 and does not include cultivation. Considering the intended uses of maize MON89034 x NK603, the environmental risk assessment is concerned with exposure through manure and faeces from the gastrointestinal tracts of animals fed GM maize MON89034 x NK603 (e.g. maize gluten feed, maize gluten meal) and with accidental release of maize MON89034 x NK603 viable grains into the environment during transportation and processing.

As the scope of the present application excludes cultivation, environmental concerns within the EU related to the use of glyphosate herbicides on maize MON89034 x NK603 do not apply.

Maize MON89034 x NK603 has been developed for protection against specific lepidopteran pests (*Ostrinia nubilalis*, *Spodoptera* ssp, *Agrotis ipsilon*) and for tolerance to glyphosate. The insect resistance is achieved by expression of the Cry1A.105 and Cry2Ab2 proteins derived from *Bacillus thuringiensis* subsp. *kurstaki* in maize MON89034 and tolerance to glyphosate in maize NK603 is conferred by the 5-enolpyruvylshikimate-3-phosphate synthase genes from *Agrobacterium* sp. strain CP4 (CP4 EPSPS and EPSPS L214P).

The Cry1A.105 protein is a modified Bt Cry1A protein with amino acid sequence identity to Cry1Ab, Cry1Ac and Cry1F proteins. 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 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 *B. thuringiensis* subsp. *kurstaki*.

6.1.1. Evaluation of the single events

The assessed dossiers for maize MON89034 (application EFSA-GMO-NL-2007-37 under Regulation (EC) No 1829/2003) and maize NK603 (notification C/ES/00/01 under Directive 2001/18/EC and a notification under Article 4 of Novel Food Regulation (EC) No 258/97) concerned import and processing of these maize events for food and feed uses. The EFSA GMO Panel was of the opinion that maize MON89034 and NK603 are as safe as conventional maize and that their placing on the market, for import and processing for food and feed uses, is unlikely to have an adverse effect on human or animal health or, in that context, on the environment (EFSA, 2003a, 2003b, 2008).

A post-market environmental monitoring plan, including general surveillance of maize NK603, was proposed by the applicant and accepted by the EFSA GMO Panel (EFSA, 2003a, 2003b). The EFSA GMO Panel also agreed with the post-market environmental monitoring plan provided by the applicant for maize MON89034 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects (EFSA, 2008).

6.1.2. Environmental risk assessment

6.1.2.1. 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.

The herbicide tolerance trait can only be regarded as providing a selective advantage for the GM maize plant where and when glyphosate herbicides are applied. Similarly, 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 plant pathogens and frost. Since these general characteristics of this GM maize are unchanged in maize MON89034 x NK603, herbicide tolerance and insect resistance are not likely to provide a selective advantage outside of cultivation or other areas where the herbicides are applied in Europe. Even if herbicides are applied on these plants, this will not change their ability to survive over seasons. Therefore, it is considered very unlikely that maize MON89034 x NK603 will differ from conventional maize varieties in their ability to survive until subsequent seasons or to establish feral populations under European environmental conditions.

In addition to the field trials carried out with the single GM maize MON89034 and NK603 events (EFSA, 2003a, 2003b, 2008, 2009a), field trials with maize MON89034 x NK603 were carried out by the applicant at 5 locations in Argentina in 2004. Information was provided on phenotypic characteristics and plant environment interactions of maize MON89034 x NK603 compared with that of control maize. No biologically meaningful differences between the studied maize varieties were observed (see Section 4.1.4). The field data provided in the application do not show a change in fitness and invasiveness or weediness, except when glyphosate herbicides are applied and/or under infestation conditions of specific target organisms. In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased spread and establishment of maize MON89034 x NK603 and of any change in survival capacity, including over-wintering.

Since maize MON89034 x NK603 has no altered survival, multiplication or dissemination characteristics except when glyphosate herbicides are applied and/or under infestation conditions of specific target organisms, the EFSA 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 maize MON89034 or NK603, or that of conventional maize varieties.

6.1.2.2. 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.

(a) Plant to bacteria gene transfer

Current scientific knowledge (see EFSA, 2009b for further details) suggests that gene transfer from GM plants to microorganisms under natural conditions is extremely unlikely, and that its establishment would occur primarily through homologous recombination in microorganisms. *Cry1A.105*, *cry2Ab2* and *cp4 epsps* genes, as expressed in maize MON89034 x NK603, are derived from bacteria). As the functional genes are already present in microorganisms in the natural environment, homologous recombination and acquisition of these genes by microorganisms will not alter the gene pool of the natural microbial community.

In addition, 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.

Transgenic DNA is a component of many food and feed products derived from GM maize. Therefore, microorganisms in the digestive tract of humans and animals (domesticated animals and other animals feeding on fresh and decaying GM plant material) may be exposed to transgenic DNA although DNA becomes degraded in the human or animal digestive tract.

In the case of accidental release and establishment of maize MON89034 x NK603 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 establish.

Taking into account the microbial origin and nature of the *cry1A.105*, *cry2Ab2* and *cp4 epsps* genes and the lack of selective pressure in the intestinal tract and the environment, the likelihood that horizontal gene transfer of the *cry1A.105*, *cry2Ab2* and *cp4 epsps* genes would result in increased fitness on microorganisms or other selective advantage is very small. For this reason it is very unlikely that genes from maize MON89034 x NK603 would become 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 either from the single events or from an interaction between the events should any co-transference occur.

(b) 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 (Eastham and Sweet, 2002; OECD, 2003).

The flowering of occasional feral 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. Field observations performed on GM maize volunteers in Spain revealed that maize volunteers had a low vigour, rarely had cobs and produced pollen that cross-pollinated neighbour plants only at low levels (Palaudelmàs *et al*., 2009).

Herbicide tolerance and insect resistance provide agronomic and selective advantages in areas where the specific herbicides are applied and/or under infestation conditions of the specific target organisms. However survival of maize outside cultivation in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, susceptibility to plant pathogens and frost. Since these general characteristics of this GM maize are unchanged in maize MON89034 x NK603, herbicide tolerance and insect resistance are not likely to provide selective advantages outside cultivation and other areas where the herbicides are applied in Europe. Therefore, as for any other maize varieties, GM maize plants would only survive in subsequent seasons in the warmer regions of Europe and are not likely to establish feral populations under European environmental conditions.

In conclusion, since maize MON89034 x NK603 has no altered survival, multiplication or dissemination characteristics except in the presence of glyphosate herbicides and/or the target pest infestation conditions, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this maize is will not differ from that of maize MON89034 or NK603, or of conventional maize varieties.

6.1.2.3. 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 x NK603 specifically exclude cultivation, so the environmental exposure to the GM maize plants is limited to the accidental release of maize MON89034 x NK603 viable grains into the environment during transportation and processing.

The EFSA GMO Panel considers that it would need successful establishment and spread of high numbers of maize MON89034 x NK603 for enabling any significant interaction with target organisms, which is very unlikely.

Environmental exposure to Cry1A.105 and Cry2Ab2 proteins is otherwise limited mainly to manure and faeces from the gastrointestinal tracts of animals fed maize MON89034 x NK603. Most Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only a very low amount of Cry proteins would remain intact to pass out in faeces (Einspanier *et al*., 2004, Ahmad *et al*., 2005, Lutz *et al*., 2005, Lutz *et al*., 2006, Wiedemann *et al*., 2006, Guertler *et al*., 2008). It can thus be concluded that the level of exposure of target organisms to the Cry1A.105 and Cry2Ab2 proteins is likely to be extremely low and of no biological relevance.

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

Considering the intended uses of maize MON89034 x NK603, the environmental risk assessment is concerned with exposure through manure and faeces from the gastrointestinal tracts of animals fed the GM maize and with accidental release of GM maize viable grains into the environment during transportation and processing.

The EFSA GMO Panel assessed therefore whether the Cry1A.105 and Cry2Ab2 proteins might potentially affect non-target organisms by entering the environment through manure and faeces from the gastrointestinal tracts of animals fed maize MON89034 x NK603. Due to the selectivity of the Cry proteins, non-target organisms most likely to be affected by the Cry1A.105 and Cry2Ab2 proteins are those belonging to a similar taxonomic group as that of the target organisms.

Data supplied by the applicant indicate that a limited amount of the Cry1A.105 and Cry2Ab2 proteins enters the environment due to expression (mean value of 3.1 and 1.2 µg/g dry weight respectively) 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, meaning that only very low amounts of Cry1A.105 and Cry2Ab2 proteins would remain intact to pass out in faeces (Einspanier *et al*., 2004, Ahmad *et al*., 2005, Lutz *et al*., 2005, Lutz *et al*., 2006, Wiedemann *et al*., 2006, Guertler *et al*., 2008). There would subsequently be further degradation of the Cry proteins in the manure and faeces due to microbial processes.

While Cry proteins can bind to clay minerals and humic substances in soil, thereby reducing their availability to microorganisms for degradation, a number of studies revealed that there is no persistence and accumulation of Cry proteins from GM crops in soil (Herman *et al*., 2001, Head *et al*., 2002, Herman *et al*., 2002, Hopkins and Gregorich, 2003, Ahmad *et al*., 2005, Baumgarte and Tebbe, 2005, Dubelman *et al*., 2005, de Vaufleury *et al*., 2007, Icoz and Stotzky, 2008).

Considering the scope of the application (that excludes cultivation) and the intended uses of maize MON89034 x NK603, it can be concluded that the level of exposure of potentially sensitive non-target organisms to the Cry1A.105 and Cry2Ab2 proteins expressed in maize MON89034 x NK603 in combination with the CP4 EPSPS protein is likely to be very low and of no biological relevance.

6.1.2.5. Interactions with the abiotic environment and biogeochemical cycles

Considering the scope of the application and the intended uses of maize MON89034 x NK603 and due to the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

6.1.3. Post-market environment monitoring

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

Monitoring is also 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 x NK603 would be through manure and faeces from the gastrointestinal tracts of animals fed the GM maize or through accidental release of maize MON89034 x NK603 viable grains into the environment 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 (1) the description of an approach involving operators (federations involved in maize import and processing), reporting to applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of the information recorded by the various operators (Lecoq *et al*., 2007; Windels *et al*., 2008); and (3) the use of networks of existing surveillance systems. The applicant proposes a general surveillance report on an annual basis and a final report at the end of the consent.

The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MON89034 x NK603 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan. The EFSA GMO Panel advises that appropriate management systems should be in place to prevent seeds of maize MON89034 x NK603 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

6.2. Conclusion

The scope of the application is for food and feed uses, import and processing of maize MON89034 x NK603 and excludes cultivation. Considering the intended uses of maize MON89034 x NK603, the environmental risk assessment is concerned with exposure through manure and faeces from the gastrointestinal tracts of animals fed the maize MON89034 x NK603 and with accidental release of MON89034 x NK603 viable grains into the environment during transportation and processing.

There are no indications of increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of maize MON89034 x NK603 viable grains during transportation and processing for food and feed uses. Only extremely low levels of gene transfer to other maize plants are predicted with no adverse effects. Taking into account the scope of the application, both the rare occurrence of maize plants and low levels of GM plants and Cry proteins exposure through other routes indicate that the risk to non-target organisms is considered negligible.

The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of maize MON89034 x NK603 since the environmental risk assessment excluded 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.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out a scientific risk assessment of the maize MON89034 x NK603 for food and feed uses, import and processing.

The EFSA GMO Panel is of the opinion that the molecular characterisation provided for maize MON89034 x NK603 produced by conventional breeding is sufficient for the safety assessment. The bioinformatic analysis of the inserted DNA and the flanking regions of the single events MON89034 and NK603 do not raise any safety concern. The expression of the newly introduced proteins in MON89034 x NK603 has been sufficiently analysed and proved to be comparable to the expression in the single events. The stability of the genetic modifications has been demonstrated over several generations in the single events, and Southern analysis confirmed that the structural integrity of the inserts was maintained in the hybrid. The EFSA GMO Panel considers that the molecular characterisation does not indicate any safety concern.

The results of the comparative analysis indicates that maize MON89034 x NK603 is compositionally, agronomically and phenotypically equivalent to its non-GM counterpart except for the presence of the Cry1A.105, Cry2Ab2 and CP4 EPSPS proteins in maize MON89034 x NK603.

The Cry1A.105 and Cry2Ab2 proteins expressed in the parental maize line MON89034 and the CP4 EPSPS proteins expressed in the NK603 parental maize line have been assessed previously and no safety concerns were identified. Given all the information provided, the Panel concludes that interactions between the single events that might impact on food and feed safety are unlikely.

The nutritional value of maize MON89034 x NK603 has been studied in a feeding study with broiler chickens and this study indicates that the nutritional properties of maize MON89034 x NK603 is no different from those of its non-GM counterpart.

Considering the intended uses of maize MON89034 x NK603, which exclude cultivation, there is no requirement for scientific assessment of possible environmental effects associated with the cultivation of this GM maize. In case of accidental release into the environment of maize MON89034 x NK603 viable grains during transportation and processing, there are no indications of increased likelihood of establishment or survival of feral maize plants. Also, the low levels of environmental exposure through other routes indicate that the risk to 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 x NK603.

The EFSA GMO Panel advises that appropriate management systems should be in place to prevent seeds of maize MON89034 x NK603 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, the EFSA GMO Panel considers that information available for maize MON89034 x NK603 addresses the outstanding questions raised by the Member States and considers it unlikely

that maize MON89034 x NK603 will have any adverse effect on human and animal health or on the environment in the context of its proposed uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the MS, dated 1 February 2007, concerning a request for placing on the market of genetically modified maize MON89034 x NK603 in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 8 February 2007, from EFSA to the Competent Authority of the Netherlands.

3. Letter from EFSA to applicant, dated 13 July 2007, requesting additional information under completeness check.

4. Letter from applicant to EFSA, dated 19 July 2007, providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 7 August 2007, requesting further clarifications under completeness check.

6. Letter from applicant to EFSA, dated 13 August 2007, providing the information requested under completeness check.

7. Letter from EFSA to applicant, dated 24 August 2007, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2007-38, maize MON89034 x NK603 submitted by MONSANTO under Regulation (EC) No 1829/2003.

8. Letter from EFSA to applicant, dated 10 September 2007, requesting additional information (Joint Research Centre) and stopping the clock.

9. Letter from applicant to EFSA, dated 10 December 2007, providing additional information.

10. Letter from EFSA to applicant, dated 19 December 2007, maintaining the clock stopped.

11. Letter from EFSA to applicant, dated 7 January 2008, restarting the clock.

12. Letter from EFSA to applicant, dated 23 January 2008, requesting additional information and stopping the clock (2).

13. Letter from applicant to EFSA, dated 14 February 2008, providing additional information.

14. Letter from EFSA to applicant, dated 29 February 2008, requesting additional information and stopping the clock (3).

15. Letter from applicant to EFSA, dated 7 April 2008, providing the timeline for submission of response.

16. Letter from applicant to EFSA, dated 17 April 2008, providing additional information.

17. Letter from EFSA to applicant, dated 19 December 2008, restarting the clock.

18. Letter from EFSA to applicant, dated 28 January 2009, requesting additional information and stopping the clock (4).

19. Letter from EFSA to applicant, dated 2 February 2009, requesting additional information and maintaining the clock stopped.

20. Letter from applicant to EFSA, dated 10 February 2009, providing the timeline for submission of response.

21. Letter from applicant to EFSA, dated 6 March 2009, providing the timeline for submission of response.

22. Letter from applicant to EFSA, dated 13 March 2009, providing additional information.

23. Letter from applicant to EFSA, dated 20 March 2009, providing the timeline for submission of response.

24. Letter from applicant to EFSA, dated 3 June 2009, providing additional information.

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