SCIENTIFIC OPINION

Scientific Opinion on an application (EFSA-GMO-NL-2009-65) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Dow AgroSciences and Monsanto[1]

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

European Food Safety Authority (EFSA), Parma, Italy

This scientific opinion, published on 15th October 2010, replaces the earlier version published on 27th September 2010[4].

ABSTRACT

This scientific opinion is an evaluation of a risk assessment for placing on the market the genetically modified (GM) insect resistant and herbicide tolerant maize MON 89034 × 1507 × NK603 for food and feed uses, import and processing. Maize MON 89034 × 1507 × NK603 was produced by conventional crossing methods and the F1 plant is hemizygous for all newly introduced traits. The maize contains *cry1A.105*, *cry2Ab2*, *cry1F*, *pat*, CP4 *epsps* and CP4 *epsps l214p* genes conferring resistance against certain lepidopteran target pests and tolerance to glufosinate-ammonium- and glyphosate-based herbicides. The maize events MON 89034, 1507 and NK603, crossed together to create maize MON 89034 × 1507 × NK603, behave as independent genetic loci. The F_2 grain harvested from maize MON 89034 × 1507 × NK603 and assessed for safety is expected to contain a

[1] On request from the Competent Authority of the Netherlands on an application (EFSA-GMO-NL-2009-65) submitted by Dow AgroSciences and Monsanto, Question No EFSA-Q- 2009-00413, adopted on 8 September 2010.

[2] Panel members: Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Patrick du Jardin, Gerhard Flachowsky, Lieve Herman, Huw Jones, Sirpa Kärenlampi, Jozsef Kiss, Gijs Kleter, Harry Kuiper, Antoine Messéan, Kaare Magne Nielsen, Joe Perry, Annette Pöting, Jeremy Sweet, Christoph Tebbe, Atte Johannes von Wright, and Jean-Michel Wal. Correspondence: gmo@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Molecular characterisation, Food and Feed and Environment for the preparatory work on this scientific opinion: Boet Glandorf and EFSA's staff members Yann Devos (ENV), Zoltán Divéki (MC), Christina Ehlert (CC) and Antonio Fernández Dumont (FF) for the support provided to this EFSA scientific output.

[4] In sections Abstract and Summary on pages 1 and 3, respectively, the word "coleopteran" has been deleted from the original scientific opinion since the introduced traits do confer resistance against certain lepidopteran target pests but not against coleopteran. The changes do not affect the overall conclusions of the scientific opinion. To avoid confusion, the original version of the scientific opinion has been removed from the website and it is available upon request.

Suggested citation: EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on application (EFSA-GMO-NL-2009-65) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Dow AgroSciences and Monsanto. EFSA Journal 2010; 8(9):1782. [34 pp.]. doi:10.2903/j.efsa.2010.1782. Available online: www.efsa.europa.eu/efsajournal.htm

mixture of MON 89034 × 1507 × NK603 and all sub-combinations of the individual events which will be imported and processed for food and feed uses. The EFSA GMO Panel has evaluated the risk assessment with respect to safety concerns which might arise through any potential combination of the following events MON 89034, 1507 and NK603 in maize MON 89034 × 1507 × NK603 and in its segregating progeny.

Molecular analyses indicated that the structure of the inserts in the single events was retained in maize MON 89034 × 1507 × NK603. Updated bioinformatic analyses of the flanking sequences and the open reading frames spanning the insert-plant DNA junctions did not raise any safety concern. Levels of the newly expressed proteins in maize MON 89034 × 1507 × NK603 were demonstrated to be comparable with those of the single events. Comparative analyses established that maize MON 89034 × 1507 × NK603 is compositionally, phenotypically and agronomically comparable to its conventional counterpart and equivalent to commercial maize varieties, except for the newly expressed proteins. The safety assessment identified no concerns regarding potential toxicity and allergenicity of maize MON 89034 × 1507 × NK603. Considering the intended uses of maize MON 89034 × 1507 × NK603, which excludes cultivation within the European Union, no scientific assessment of potential environmental effects associated with cultivation of this maize was required. In case of accidental release of viable grains from maize MON 89034 × 1507 × NK603 into the environment during transportation and processing, there are no indications of an increased likelihood of establishment or survival of feral maize plants, except in the presence of glufosinate-ammonium- and/or glyphosate-based herbicides and/or under infestation of target pests. It is highly unlikely that the recombinant DNA will be transferred and establish itself in the genome of bacteria in the environment or human and animal digestive tracts. In conclusion, the EFSA GMO Panel considers that the information available for maize MON 89034 × 1507 × NK603 addresses the scientific comments raised by the Member States and concludes that the maize MON 89034 × 1507 × NK603, assessed in this application, is as safe as its conventional counterpart and other appropriate comparators with respect to potential effects on human and animal health and the environment. In addition, the EFSA GMO Panel is of the opinion that crossing of single maize events MON 89034, 1507 and NK603 to produce maize MON 89034 × 1507 × NK603 does not result in interactions between the events which would affect the safety of maize MON 89034 × 1507 × NK603 with respect to potential effects on human and animal health and on the environment, in the context of its intended uses. Based on data provided for maize stack MON 89034 × 1507 × NK603, the single maize events (MON 89034, 1507 and NK603), and for the two double stacks 1507 × NK603 and MON 89034 × NK603, the EFSA GMO Panel is of the opinion that there is no biological reason to expect that any of the other sub-combinations of the individual events as present in its segregating progeny would raise a safety concern. The EFSA GMO Panel concludes that maize MON 89034 × 1507 × NK603 is unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses.

KEY WORDS

GMO, maize (*Zea mays*), MON 89034 x 1507 x NK603, insect resistant, herbicide tolerant, risk assessment, food and feed safety, environmental safety, food and feed uses, import and processing, Regulation (EC) No 1829/2003.

SUMMARY

Following the submission of an application (EFSA-GMO-NL-2009-65) under Regulation (EC) No 1829/2003 from Dow AgroSciences and Monsanto, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of insect resistant and herbicide tolerant genetically modified (GM) maize MON 89034 × 1507 × NK603[5] and all sub-combinations of the individual events as present in its segregating progeny[6] for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2009-65, additional information supplied by the applicants, the scientific comments submitted by the Member States, and relevant scientific publications. Further information from applications for placing on the market under EU regulatory procedures the single maize events MON 89034, 1507 and NK603 and the two double stacks 1507 × NK603 and MON 89034 × NK603 was taken into account. The scope of application EFSA-GMO-NL-2009-65 is for food and feed uses, import and processing of maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny, and all derived products, but excludes cultivation in the EU. The EFSA GMO Panel evaluated maize MON 89034 × 1507 × NK603 with reference to the intended uses and appropriate principles describe in its Guidance Documents for the risk assessment of GM plants and derived food and feed, and for the risk assessment of GM plants containing stacked transformation events. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of the corresponding proteins. An evaluation of the comparative analyses of composition, agronomic and phenotypic traits was undertaken, and the safety of the new proteins, both individually and in combination, and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional quality. An evaluation of environmental impacts and the post-market environmental monitoring plan was undertaken.

The single maize events MON 89034, 1507 and NK603, and two sub-combinations of these events 1507 x NK603 and MON 89034 x NK603 were the subject of separate earlier risk assessment evaluations by the EFSA GMO Panel. No new genes in addition to those occurring in maize MON 89034, 1507 and NK603 have been introduced in maize MON 89034 × 1507 × NK603. Maize MON 89034 × 1507 × NK603 was produced by conventional crossing of the single maize events, to combine in the same stack resistance against certain lepidopteran target pests and tolerance to glufosinate-ammonium- and glyphosate-based herbicides.

Molecular analysis confirmed that maize MON89034, 1507 and NK603 inserts are present and that their structures are retained in maize MON 89034 × 1507 × NK603. The result of the updated bioinformatic analyses of the flanking sequences and the open reading frames spanning the insert-plant DNA junctions did not reveal a safety concern. The overall levels of the Cry1A.105, Cry2Ab2, Cry1F, PAT and CP4 EPSPS proteins, were comparable to those of the respective single maize events MON 89034, 1507 and NK603.

Based on the results of comparative analyses, the EFSA GMO Panel concludes that maize MON 89034 × 1507 × NK603 is compositionally, phenotypically and agronomically comparable to its conventional counterpart and equivalent to commercial maize varieties, except for the presence of Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and CP4EPSPS L214P proteins in maize MON 89034 × 1507 × NK603. Based on the assessment of the data available, including the additional information provided by the applicants in response to the EFSA GMO Panel's question regarding

[5] Unique Identifier MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-ØØ6Ø3-6

[6] Sub-combinations of these events exclude all single events. The sub-combination not previously evaluated by the EFSA GMO Panel is MON 89034 × 1507; Sub-combinations previously evaluated by the EFSA GMO Panel are MON 89034 × NK603 and 1507 × NK603

maize MON 89034 × 1507 × NK603, its conventional counterpart and data on the single maize events, the EFSA GMO Panel is of the opinion that crossing of maize MON 89034, 1507 and NK603 does not result in interactions between the single maize events which causes compositional, phenotypical or agronomic changes. The safety of Cry1A.105 and Cry2Ab2 proteins expressed in maize MON 89034, the Cry1F and PAT proteins expressed in maize 1507, and the CP4 EPSPS and CP4 EPSPS L214P proteins expressed in maize NK603 have been assessed for their safety previously and no safety concerns were identified for humans and animals. Regarding the safety and nutritional properties of food and feed products derived from maize MON 89034 × 1507 × NK603, the EFSA GMO Panel considers it unlikely that interactions between the single maize events will occur that may impact on the food and feed safety and nutritional properties of maize MON 89034 × 1507 × NK603. The EFSA GMO Panel bases this consideration on the known functional characteristics of the newly expressed proteins and on the outcomes of the comparative analysis of compositional, phenotypic and agronomic characteristics of maize MON 89034 × 1507 × NK603. In addition, the EFSA GMO Panel considers it unlikely that the overall allergenicity of maize MON 89034 × 1507 × NK603 has been altered. In conclusion, the EFSA GMO Panel is of the opinion that maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny are as safe and as nutritious as its conventional counterpart and commercial maize varieties, and concludes that this maize and derived products are unlikely to have adverse effects on human and animal health in the context of its intended uses.

The application EFSA-GMO-NL-2009-65 concerns food and feed uses, import and processing, but excludes cultivation in the EU. Therefore, there is no requirement for scientific assessment of possible environmental effects associated with the cultivation of maize MON 89034 × 1507 × NK603. There are no indications of an increased likelihood of establishment and spread of feral maize plants in case of accidental release into the environment of viable grains from maize MON 89034 × 1507 × NK603 (including all sub-combinations of the individual events as present in its segregating progeny) during transportation and processing, except in the presence of glufosinate-ammonium- and/or glyphosate-based herbicides and/or under infestation of target pests. Taking into account the scope of the application, the rare occurrence of feral maize plants and the low levels of exposure through other routes, the risk to non-target organisms is extremely low. It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts. The scope of the post-market environmental monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicants in the general surveillance plan. The EFSA GMO Panel recommends that appropriate management systems should be in place to restrict seeds of maize MON 89034 × 1507 × 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 the information available for maize MON 89034 × 1507 × NK603 addresses the scientific comments raised by the Member States and concludes that the maize MON 89034 × 1507 × NK603, assessed in this application, is as safe as its conventional counterpart and other appropriate comparators with respect to potential effects on human and animal health and the environment. In addition, the EFSA GMO Panel is of the opinion that crossing of single maize events MON 89034, 1507 and NK603 to produce maize MON 89034 × 1507 × NK603 does not result in interactions between the events which would affect the safety of maize MON 89034 × 1507 × NK603 with respect to potential effects on human and animal health and on the environment, in the context of its intended uses. Based on data provided for maize stack MON 89034 × 1507 × NK603, the single maize events (MON 89034, 1507 and NK603), and for the two double stacks 1507 × NK603 and MON 89034 × NK603, the EFSA GMO Panel is of the opinion that there is no biological reason to expect that any of the other sub-combinations of the individual events as present in its segregating progeny would raise a safety concern. The EFSA GMO Panel concludes that maize MON 89034 ×

1507 × NK603 is unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses.

TABLE OF CONTENTS

BACKGROUND

On 6 February 2009, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands an application (Reference EFSA-GMO-NL-2009-65) for authorisation of the insect resistant and herbicide tolerant genetically modified (GM) maize MON 89034 × 1507 × NK603[7] for food and feed uses, import and processing, submitted by Dow AgroSciences and Monsanto within the framework of Regulation (EC) No 1829/2003 on GM food and feed. After receiving the application EFSA-GMO-NL-2009-65 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed the 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 21 April 2009, 22 April 2009, 3 June 2009, 25 June 2009 and 16 July 2009, EFSA received additional information requested under completeness check (requested on 19 March 2009, 13 May 2009 and 23 June 2009). On 6 August, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

Concerning grain, in their letter of 26 May 2010, the applicants confirmed that the scope of this application covers the F_2 grain produced by hybrid F_1 maize MON 89034 × 1507 × NK603[8]. The applicants indicated that the single maize events MON 89034, 1507 and NK603 crossed together to create maize MON 89034 × 1507 × NK603, behave as independent genetic loci. Since maize grain is the product of fusion of gametes formed after segregation of genetic components according to Mendelian laws, the F_2 grain harvested from maize MON 89034 × 1507 × NK603 and imported into the EU, is expected to contain a mixture of maize MON 89034 × 1507 × NK603 (42.2%), three triple stacks MON 89034 × 1507, MON 89034 × NK603[9] and 1507 × NK603[10] (14.1% each), single events (4.7% each) and negative segregant grain (1.6%). This mixture is referred to hereafter as "segregating progeny".

Each event present in maize MON 89034 × 1507 × NK603 has been previously assessed by EFSA without raising safety concerns (EFSA, 2003a,b, 2004a, 2005a,b, 2006c, 2008, 2009b,c,d). The EFSA GMO Panel Guidance Document for the risk assessment of genetically modified plants containing stacked transformation events (EFSA, 2007) states that a single risk assessment of a stack could cover all combinations with fewer of these events, if the single events have been risk assessed. The risk assessment should focus on the intactness and stability of events combined by crossing, the expression of the traits, and the potential interactions between the stacked events. Therefore, EFSA was asked to deliver a scientific opinion on the safety of insect resistant and herbicide tolerant GM maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny[11].

EFSA made the valid application available to the Member States and the European Commission, and consulted nominated risk assessment bodies of the Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member States had three months after the date of acknowledgement of the valid application (6 November 2009) within which to make their opinion known.

[7] Unique Identifier MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-ØØ6Ø3-6

[8] The F_1 hybrid maize plants of MON 89034 × 1507 × NK603 are hemizygous for all newly introduced traits

[9] Previously assessed by EFSA (2009)

[10] Previously assessed by EFSA (2006)

[11] For regulatory purposes, the term "sub-combinations of the individual events as present in its segregating progeny" as used throughout this opinion, excludes all single events, although it is recognised that the latter will also occur in the F_2 grain harvested from maize MON 89034 × 1507 × NK603. The unique identifiers of these sub-combinations are MON-89Ø34-3 × DAS-Ø15Ø7-1; MON-89Ø34-3 × MON-ØØ6Ø3-6 and DAS-Ø15Ø7-1 × MON-ØØ6Ø3-6

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out an evaluation of the scientific risk assessment of the GM maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny, 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 evaluation, the EFSA GMO Panel took into account the principles described in the EFSA GMO Panel Guidance Documents for the risk assessment of GM plants and derived food and feed (EFSA, 2006a) and for the risk assessment of GM plants containing stacked transformation events (EFSA, 2007), the scientific comments of the Member States, and the additional information provided by the applicants. Further information from applications for placing the single maize events MON 89034, 1507 and NK603 as well as the maize double stacks 1507 x NK603 and MON 89034 x NK603 on the market under EU regulatory procedures was taken into account (EFSA, 2003a,b, 2004a, 2005a,b, 2006c, 2008, 2009b,c,d).

The EFSA GMO Panel requested from the applicants additional information on 28 October 2009, 29 January 2010 and 22 March 2010. The applicants provided the requested information on 23 December 2009, 4 February 2010 and 12 April 2010, respectively. Additional information was provided by the applicants on 5 October 2009 and 3 February 2010 (spontaneously submitted). On 2 and 26 July 2010 EFSA requested additional information from the applicants. The applicants provided the requested information on 19 and 30 July 2010.

In giving its scientific opinion on maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny, to the European Commission, Member States and the applicants, 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 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 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 MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny, 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 labeling 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.

ASSESSMENT

1. Introduction

The GM maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny were evaluated with reference to its intended uses, taking into account the principles described in the EFSA GMO Panel Guidance Documents for the risk assessment of GM plants and derived food and feed (EFSA, 2006a) and for the risk assessment of GM plants containing stacked transformation events (EFSA, 2007). The evaluation of the risk assessment presented here is based on the information provided in the application relating to maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny submitted in the EU, including additional information provided by the applicants as well as scientific comments submitted by the Member States and relevant scientific publications. Further information from applications for placing on the market under EU regulatory procedures of the single maize events MON 89034, 1507 and NK603 as well as the maize double stacks 1507 × NK603 and MON 89034 × NK603 was taken into account (EFSA, 2003a,b, 2004a, 2005a,b, 2006c, 2008, 2009b,c,d).

2. Issues raised by Member States

The scientific comments raised by the Member States are addressed in Annex G of the EFSA overall opinion[12] and have been considered in this scientific opinion.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Method of production of maize MON 89034 × 1507 × NK603

Maize MON 89034 × 1507 × NK603 was produced by conventional crossing and no new genetic modification was involved. The three inserts that are present in maize MON 89034 × 1507 × NK603 were derived from maize lines containing the single events: MON 89034, 1507 and NK603. Each of these GM maize events was the subject of an earlier opinion of the EFSA GMO Panel (EFSA, 2003a,b, 2004a, 2005a,b, 2008, 2009b,c).

The Maize MON 89034 × 1507 × NK603 assessed in this application is hemizygous for all newly introduced genes and was produced from a cross between homozygous MON 89034 × NK603 in inbred line HCL301 and homozygous 1507 in inbred line 5XH751. It is mainly the F_2 grain produced by maize MON 89034 × 1507 × NK603 and not other plant components that will be imported into the EU. The applicants indicated that the inserts of the events behave as independent genetic loci[13]. The F_2 grain produced by self fertilisation of maize MON 89034 × 1507 × NK603 imported to the EU is expected to contain a mixture of maize MON 89034 × 1507 × NK603 (42.2%), three double stacks MON 89034 × 1507, MON 89034 × NK603, 1507 × NK603 (14.1% each), single events (4.7% each) and negative segregant grain (1.6%).

3.1.2. Summary of the evaluation of the single maize events

Maize MON 89034

[12] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2009-00413
[13] Additional information sent by the applicant on 2 April 2010

Maize MON 89034 was developed through *Agrobacterium tumefaciens* (renamed *Rhizobium radiobacter*)-mediated transformation using the binary plasmid vector PV-ZMIR245 containing two separate T-DNAs. One T-DNA, designated as T-DNA I, contains the *cry1A.105* and the *cry2Ab2* expression cassettes providing increased resistance to certain lepidopteran target pests such as European corn borer (*Ostrinia nubilalis*), fall armyworm (*Spodoptera* ssp), black cutworm (*Agrotis ipsilon*) and corn earworm (*Helicoverpa zea*). The other T-DNA, designated as T-DNA II, contains the *npt*II expression cassette that encodes neomycin phosphotransferase that confers tolerance to certain antibiotics such as neomycin and kanamycin. The use of the two-T-DNA approach facilitates integration of the two different T-DNAs at genetic loci which can be segregated by breeding. Conventional crossing was used to isolate plants that contain the *cry1A.105* and the *cry2Ab2* expression cassettes (T-DNA I), but do not contain the *npt*II expression cassette (T-DNA II).

Molecular characterisation data established that maize MON 89034 contains a single copy of T-DNA I and that T-DNA II and vector backbone sequences are absent (EFSA, 2008). The structure of the insert in maize MON 89034 was determined by Southern analyses and DNA sequencing. Data indicate that the *Cauliflower mosaic virus e35S* promoter that regulates expression of the *cry1A.105* gene has been truncated, and that the T-DNA right border region has been replaced by a T-DNA left border region. Sequence comparison between the flanking regions of the maize MON 89034 and the corresponding genomic region of conventional maize indicated that the pre-insertion locus was preserved, except for the deletion of 57 bp and the addition of 10 bp. Updated bioinformatic analyses indicate that no known endogenous maize coding sequences or regulatory sequences have been disrupted by the insert. Updated bioinformatic analyses also revealed no biologically relevant similarities to allergens or toxins for any of the putative peptides that might be produced from open reading frames (ORFs) spanning the junction regions. Southern analyses of maize MON 89034 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations.

Maize 1507

Maize 1507 was generated by particle bombardment. As a result of the genetic modification, maize 1507 expresses a truncated *cry1F* gene from *Bacillus thuringiensis* subsp. *aizawai*, conferring resistance to certain lepidopteran target pests such as the European corn borer, and the *pat* gene from *Streptomyces viridochromogenes* that renders it tolerant to glufosinate-ammonium-based herbicides.

Molecular analyses showed that maize 1507 contains one copy of the DNA fragment used for transformation (containing the *cry1F* and phosphinothricin acetyltransferase (*pat)* genes) and additional partial fragments of the *cry1F* and *pat* genes and that these fragments are present at a single locus in the nuclear genome (EFSA, 2004a, 2005a,b, 2009c). The structure of the insert in maize 1507 was determined by Southern analyses and DNA sequencing. A published study from Morisset *et al.* (2009) showed that the 35S promoter of event 1507 contains a single nucleotide difference compared to the reported sequence of the DNA fragment used for transformation. Following a request from the EFSA GMO Panel, the applicant has clarified that this difference was present in plants at early stages of product development and is present in all 1507 maize lines and stacks that have been evaluated by the EFSA GMO Panel. Updated bioinformatic analyses confirmed that, in addition to the intact genes, the insert in maize 1507 includes DNA sequences originating from the fragment used for transformation as well as maize chloroplast DNA sequences (EFSA, 2004a). Analyses of DNA sequences flanking both ends of the insert showed that they are maize genomic DNA. Updated bioinformatic analyses of these flanking sequences suggest that the insert in maize 1507 is flanked by a putative RIRE2 retrotransposon (downstream) and a Huck1 retrotransposable element (upstream). Transcript and bioinformatic analyses of ORFs spanning all junction regions between genomic and insert DNA, as well as junction regions between partial fragments of *cry1F* and *pat* genes were performed and no novel putative proteins with sequence similarity to known toxins or allergens were identified. Southern analyses of maize 1507 and maintenance of the phenotype indicated genetic and phenotypic stability of the event over multiple generations.

Maize NK603

Maize NK603 was generated by particle bombardment. As a result of the genetic modification NK603 is tolerant to glyphosate-based herbicides due to the expression of the CP4 *epsps* gene from *Agrobacterium* sp. strain CP4 (CP4 EPSPS and CP4 EPSPS L214P, a variant of CP4 EPSPS containing a proline residue at position 214 instead of leucine).

The DNA fragment used for transformation contains two adjacent gene expression cassettes each consisting of a copy of the CP4 *epsps* gene, one under the control of rice actin promoter, the other under the control of CaMV 35S promoter. The structure of the insert in NK603 was determined by Southern analysis and DNA sequencing. These analyses showed that NK603 contains a single insert and that one of the CP4 *epsps* expression cassettes contains a point mutation which results in a single amino acid change in the CP4 EPSPS protein at position 214 (L214P). CP4 EPSPS L214P was shown to be structurally and functionally equivalent to CP4 EPSPS. The insert includes, at the 3' end, an additional 217 bp DNA fragment of the rice actin promoter lacking sequences needed for promoter activity. Next to this fragment is a 305 bp region with similarity to chloroplast DNA. These rearrangements and the insertion of chloroplast DNA do not lead to new traits and are not considered to pose a safety risk. In the unlikely event that a new peptide or protein is produced as a consequence of the insertion event, updated bioinformatic analysis of the ORFs spanning the junction regions showed that these would have no similarity to known toxins or allergens.

An updated BLASTn analysis of the flanking DNA sequences identified a similarity to a genomic clone including the maize P2 protein gene, a myb-related transcription factor. However, the aligned interval is limited to 109 bp of the query sequence (reconstructed pre-insertion site) and the sequence similarity is located 3' to the protein-coding part of the P2 gene sequence deposited in GenBank. This is in line with the BLASTx analysis which failed to find similarity to any known protein from maize. Altogether, these results are not indicative of the interruption of any known endogenous protein-coding sequences and do not raise a safety concern.

Segregation data for nine generations (six generations of crossing and three generations of self-pollination) of line NK603 have demonstrated the stability of the inserted DNA.

3.1.3. Transgene constructs in maize MON 89034 × 1507 × NK603

The integrity of the individual inserts present in maize MON 89034 × 1507 × NK603 was investigated using Southern analyses[14]. This involved the use of DNA probes specific for the MON 89034, 1507 and NK603 inserts and restriction enzyme digestions informative of the structure of all events, including the junctions with the host genomic DNA. The predicted DNA hybridization patterns from each single event were retained in maize MON 89034 × 1507 × NK603, demonstrating that integrity of the transgene inserts was maintained.

3.1.4. Information on the expression of the inserts

The levels of the newly expressed proteins Cry1A.105, Cry2Ab2, Cry1F, PAT and CP4 EPSPS in MON 89034 × 1507 × NK603 were analysed by validated enzyme-linked immunosorbent assays (ELISA)[15]. Tissue samples for analyses were collected from five field trials conducted in the USA during 2006. The trials were located within the major maize-growing regions of the USA and provided a variety of environmental conditions. Each trial included appropriate comparators (see section 4.1.2) and protein expression levels were determined in leaves, roots, forage, whole plants, pollen and grain. The plants were treated with glyphosate (NK603 and MON 89034 × 1507 × NK603) and/or glufosinate-ammonium (1507 and MON 89034 × 1507 × NK603).

[14] Technical Dossier/ Section D2
[15] Technical Dossier/ Section D3

The scope of the application covers food and feed uses and import and processing of maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny and excludes cultivation. Therefore, protein expression data related to the grain (F_2 generation) produced by maize MON 89034 × 1507 × NK603 are considered the most relevant and are summarized in Table 1. Levels of proteins in the grain (F_2 generation) produced by maize MON 89034 × 1507 × NK603 are comparable to those in the single events, although the mean level of Cry1A.105 was lower in maize MON 89034 compared to maize MON 89034 × 1507 × NK603. The levels of the newly expressed proteins do not pose safety concern (see also section 5.1.4.1, 5.1.5.1 and 6.1.2). The same conclusions were reached by the EFSA GMO Panel for the assessed lower combinations (EFSA, 2006c, 2009d).

Table 1: Summary of protein levels in grain produced by maize MON 89034 × 1507 × NK603, MON 89034, 1507 and NK603 (µg/g dry weight)

		MON 89034 × 1507 × NK603	MON 89034	1507	NK603
Cry1A.105	mean	4.5	2.8	NA	NA
	range	[3.4 – 5.8]	[1.7 – 3.5]		
Cry2Ab2	mean	5.1	5.6	NA	NA
	range	[1.9 – 6.3]	[2.7 – 7.1]		
Cry1F	mean	3.0	NA	3.2	NA
	range	[2.4 – 4.1]		[2.4 – 4.6]	
PAT	mean	< LOD	NA	< LOD	NA
	range				
CP4 EPSPS	mean	8.5	NA	NA	6.7
	range	[5.4 – 11]			[4.9 – 8.8]

NA: not assayed, LOD: limit of detection

3.1.5. Inheritance and stability of inserted DNA

The genetic stability of the inserted DNA in the single events MON 89034, 1507 and NK603 was demonstrated previously (EFSA, 2003a,b, 2004a, 2005a,b, 2008, 2009b,c). Southern analyses data show that the three events are present in maize MON 89034 × 1507 × NK603 and that the structure of each insert is retained[16].

3.2. Conclusion

Maize MON 89034 × 1507 × NK603 was produced by conventional crossing, no additional genetic modification was involved. Southern analyses demonstrated that the structures of maize events MON 89034, 1507, and NK603 were retained in maize MON 89034 × 1507 × NK603. Updated bioinformatic analyses of the flanking sequences and the ORFs spanning the insert-plant DNA

[16] Technical Dossier/ Section D5

junctions did not indicate any safety concern. The levels of Cry1A.105, Cry2Ab2, Cry1F, PAT and CP4 EPSPS of maize MON 89034 × 1507 × NK603 have been demonstrated to be comparable with those of the single events. Molecular characterisation data do not indicate safety concerns arising from combining the single events MON 89034, 1507 and NK603 to produce the maize stack MON 89034 × 1507 × NK603. Based on these data it is also unlikely that safety concerns could arise from the segregating progeny of maize MON 89034 × 1507 × NK603.

The EFSA GMO Panel concludes that the molecular characterisation does not indicate a safety concern.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

4.1.1. Summary of previous evaluations of the single events

Maize MON 89034

Forage and grain of maize MON 89034 and its conventional counterpart were obtained from field trials carried out in the USA in 2004 and in Argentina for the season 2004-2005. Both cultivation periods included field trials at five different locations, all being representative of the maize growing regions of the respective countries. The trials used agronomic practices representative of the respective regions. In addition to maize MON 89034 and its conventional conventional counterpart, a total of fifteen commercial maize varieties were included in the field trial to estimate the naturally occurring variation in composition expected for the various analytes in conventional maize. In the field trials performed to study the agronomic and phenotypic characteristics, in total twenty-three commercial maize varieties were used to describe the natural variation in studied parameters.

With regard to agronomic and phenotypic characteristics, no consistent differences were observed between maize MON 89034 and its conventional counterpart grown in the various field trials. With regard to compositional analyses, statistical differences between maize MON 89034 and its conventional counterpart were identified, but these were not consistently found across the different field trial sites. All of the observed differences were small and fell within the natural variation found in the commercial maize varieties grown in the study. Furthermore, the composition of maize MON 89034 fell within the natural variation as reported in the literature and in the ILSI crop composition database (ILSI, 2006).

Based on these data and in line with its previous opinion (EFSA, 2008), the EFSA GMO Panel considers, that maize MON 89034 does not differ from its conventional counterpart with regard to compositional, phenotypic and agronomic characteristics and is equivalent to commercial maize varieties, except for the newly expressed Cry1A.105 and Cry2Ab2 proteins.

Maize 1507

The whole crop and grain of maize 1507 and its conventional counterpart were collected for compositional analysis from field trials. These field trials were performed during three seasons and at different locations (six locations in Chile (1998-1999), three locations in France and Italy (1999), and six locations in France, Italy and Bulgaria (2000)). GM maize plants in the Chilean field trials were all treated with glufosinate-ammonium-based herbicides, while those in the European field trials were split into treated and untreated groups. Based on the results of compositional analysis of samples from three seasons and a representative range of environments, the EFSA GMO Panel concluded that forage and grain of maize 1507 are compositionally equivalent to those of conventional maize, except for the presence of Cry1F and PAT proteins in maize 1507.

In addition, field trials carried out over several seasons and at different locations (USA in 1999, France, Italy, and Bulgaria in 2000, Spain in 2002) did not indicate any unexpected changes to agronomic and phenotypic characteristics (EFSA, 2005a,b).

Based on these data and in line with its previous opinions (EFSA, 2004a; 2005a,b; 2009c), the EFSA GMO Panel considers that maize 1507 does not differ from its conventional counterpart with regard to compositional, phenotypic and agronomic characteristics and is equivalent to commercial maize varieties, except for the newly expressed Cry1F and PAT proteins.

Maize NK603

Maize NK603 was compared to a conventional counterpart with regard to chemical composition and phenotypic and agronomic characteristics. The materials studied were obtained from field trials in the USA (1998) and in Europe (1999). The field trials included both plots with maize NK603 treated with the target herbicide glyphosate and plots untreated with this herbicide. The levels of fifty-one compositional parameters in grain, and seven compositional parameters in forage of maize NK603 were either within the ranges found in the non-GM maize control material 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 EFSA GMO Panel concluded that maize NK603 is compositionally equivalent to its conventional counterpart and to conventional maize varieties, except for the presence of the newly expressed CP4 EPSPS and CP4 EPSPS L214P proteins in maize NK603 (EFSA, 2003a,b, 2009b).

Field trials performed at altogether nine locations in Germany and France between 2000 and 2002 were used for the comparative assessment of phenotypic and agronomic characteristics of maize NK603 and appropriate non-modified control maize varieties. These investigations showed that, with the exception of the glyphosate-tolerance characteristic of maize NK603, this GM maize is phenotypically and agronomically equivalent to the conventional counterpart and to conventional maize varieties.

Based on these data and in line with its previous opinions (EFSA, 2003a,b, 2009b), the EFSA GMO Panel considers that maize NK603 does not differ from its conventional counterpart with regard to compositional, phenotypic and agronomic characteristics and is equivalent to commercial maize varieties, except for the newly expressed CP4 EPSPS and CP4 EPSPS L214P proteins.

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

Given the outcomes of the risk assessment of the single maize events and the fact that compositional data on the single maize events grown during multiple seasons have already been assessed by the EFSA GMO Panel, the Panel considers the data from one season as sufficient for the evaluation of the maize stack MON 89034 × 1507 × NK603, and in line with its guidance on the assessment of stacked events (EFSA, 2007).

In the comparative compositional, agronomic and phenotypic studies, maize MON 89034 × 1507 × NK603 was compared to the non-transgenic conventional counterpart XE6001, which is a conventional maize with background genetics similar to maize MON 89034 × 1507 × NK603. The field trials were performed under normal agronomic conditions (glyphosate-based and glufosinate ammonium herbicides were given only to maize MON 89034 × 1507 × NK603) at five separate field trial locations in the major maize growing regions of North America in 2006: two sites in Illinois, two sites in Iowa and one site in Nebraska. The treatments were replicated three times at each field trial site. The tested maize MON 89034 × 1507 × NK603 was grown side-by-side to its conventional counterpart and to three commercial maize varieties used as reference material in a randomised complete block design. Due to strong winds at the time of pollen shed of the maize, pollination from

neighbouring plants resulted in unacceptable presence of unintended traits in two of the three control replicates at one of the sites in Illinois. Therefore, the data on maize MON 89034 × 1507 × NK603 and its conventional counterpart from this field trial site were discarded. In total 14 different commercial maize varieties[17] were used to establish the natural variation in measured parameters as control studies revealed that also one of the intended reference lines grown at one of the trial sites in Illinois contained unacceptable high levels of an unintended trait. Materials for the compositional analyses were collected from each field trial site and constituted grains and forage harvested from plots of maize MON 89034 × 1507 × NK603, the conventional counterpart and commercial maize varieties. The agronomic treatment of MON 89034 × 1507 × NK603 included spraying with glyphosate-based and, 1-2 weeks later, spraying with glufosinate-ammonium herbicides. The comparator was not sprayed with these herbicides[18]. The EFSA GMO Panel accepted that no studies were performed with maize MON 89034 × 1507 × NK603 not being sprayed with glyphosate-based and glufosinate ammonium herbicides, as the influence of these herbicides on the compositional parameters had been studied in the single maize events MON89034, 1507 and NK603.

4.1.3. Compositional analysis

Maize forage was analysed for proximates (protein, fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), the minerals calcium and phosphorus, whereas carbohydrates was quantified by calculation. Grains were analysed for proximates, ADF, NDF, total dietary fiber (TDF), amino acids, fatty acids, vitamins and vitamin precursors (A, B_1, B_2, B_6, E, folic acid and niacin), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, and zinc), the anti-nutrients phytic acid and raffinose, and the secondary metabolites ferulic acid, and p-coumaric acid. Also in this case carbohydrates were quantified by calculation. 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[19] of the 78 compounds analysed, no statistical analyses were performed for these parameters. In total 9 components were statistically analysed in forage and 53 in grain, which corresponded to parameters suggested by OECD (2002). The statistical analysis compared the levels of each analyte in forage or grain of maize MON 89034 × 1507 × NK603 with the levels in the conventional counterpart. The statistical analysis was performed both on analytical data from materials collected at each field trial site, and on data from all locations combined.

When the compositional data were analysed across sites, the composition of maize MON 89034 × 1507 × NK603 forage did not differ from that of forage from the control maize. In grains, the level of 12 of the 53 analysed components in maize MON 89034 × 1507 × NK603 were found to be significantly different from the level in the maize XE6001 conventional counterpart not applying multiple comparison adjustment. The constituents that differed across sites were 16:0 palmitic acid, 18:0 stearic acid, 18:1 oleic acid, 18:3 linolenic acid, 20:0 arachidic acid, 22:0 behenic acid, ferulic acid, calcium, manganese, potassium, folic acid, and vitamin E. The magnitudes of all differences were small and were with one exception not consistent. Adopting a Dunnett's multiple comparison test adjustment in the statistical analysis resulted in only six of the previous statistically significant differences remaining significant: 18:0 stearic acid (8% increase in the GM maize: 2.09% vs 1.93% total fatty acids); 18:3 linolenic acid (5% increase in the GM maize: 1.01% vs 0.96% total fatty acids); 20:0 arachidic acid (5% increase: 0.42% vs 0.40% total fatty acids); ferulic acid (17% increase: 1729 vs 1478 mg/kg dw); calcium (9% reduction: 35.98 vs 39.67 mg/kg dw); and manganese (9%

[17] Garst 8424, NC+4822, Pioneer 34N43, Moews 3744, Moews 3765, Pioneer 33K39, Burrus 625, Burrus 645, DKC 61-50, Golden Harvest H8920, Golden Harvest H8991, DKC 61-42, DKC 63-78 and Pioneer 33N29

[18] Technical Dossier/ Section D7.2

[19] More than 50% of the analytical data points were below the limit of quantification for 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, sodium, and furfural (2-furaldehyde).

reduction: 5.68 vs 6.22 mg/kg dw). Whereas the increased levels of stearic acid was observed at all of the individual field trial sites, the increased level of linolenic acid and arachidic acid, and the reduced level of calcium was observed at three sites, and the altered level of manganese, and ferulic acid at a single site. For all plant constituents showing altered levels, the magnitude of the alteration were modest, and the amounts observed were always within the range observed for the commercial maize varieties, and within the range in levels reported in the scientific literature and in the ILSI Crop Composition Database (ILSI, 2006). In conclusion, the only trend consistently found at all field trial sites was the 8% increase in 18:0 stearic acid level in grains. The level in maize MON 89034 × 1507 × NK603, 2.09% of total fatty acids, could be compared to the range in stearic acid levels in the maize reference lines, 1.38-2.38 % of total fatty acids. The EFSA GMO Panel considered none of these statistical differences to be of biological relevance.

In addition, the levels of four grain parameters (moisture, total dietary fiber, leucine, and tryptophan) that did not differ in level between maize MON 89034 × 1507 × NK603 and XE6001 in the analysis across sites, were statistically significantly different in these maize materials at two or more sites in the individual site analysis. Seventeen constituents in grain and two in forage occurred at different levels in maize MON 89034 × 1507 × NK603 and XE6001 at a single field trial site. With three exceptions differences were small. The exceptions were an around 25% increased level of raffinose, an around 25% reduced level of NDF, and a 311% increased level of copper at the field trial site in Nebraska. The increased value for raffinose was 0.11% dw in maize MON 89034 × 1507 × NK603, which could be compared to the 95% confidence interval of the levels observed in the 14 reference lines (0.03-0.23% dw). The reduced value for NDF 9.87% dw was also within the 95% confidence interval of the levels in the reference lines (6.2-15-5% dw). The deviating copper data in the material from Nebraska was due to increased levels in all three replicates of maize MON 89034 × 1507 × NK603, one of the samples having a particularly high level. All three values were outside the range in copper content of maize defined by the reference maize varieties. While the source and identity of the contaminant is unknown, a possible explanation for the anomalous copper levels in the maize MON 89034 × 1507 × NK603 grain samples from the Nebraska site could be inadvertent contamination during sample collection.

Although the level of p-coumaric acid in maize MON 89034 × 1507 × NK603 (64 mg/kg dw) was not significantly different from the level in the conventional counterpart XE6001 (46 mg/kg dw), the level of this secondary metabolite in maize XE6001 was below the range in average p-coumaric acid content defined by the reference maize varieties. It was also lower than the values for p-coumaric acid reported in maize by the ILSI Crop Composition Database (2006), which give the range 53.4-576.2 mg/kg dw. Apparently the control of the present investigation will increase that range, whereas maize MON 89034 × 1507 × NK603 fell within the earlier reported range[20].

The EFSA GMO Panel considered the observed compositional differences between grain produced by maize MON 89034 × 1507 × NK603 and its conventional counterpart in the light of the field trial design, measured biological variation and the level of the studied compounds in commercial maize varieties, and concludes that the only compositional difference between maize MON 89034 ×1507 × NK603 and its conventional counterpart, except for the intended expression of the Cry1A.105, Cry1F, Cry2Ab2, PAT, CP4 EPSPS and CP4 EPSPS L214P proteins, is a slightly increased level of 18:0 stearic acid in maize MON 89034 × 1507 × NK603. The EFSA GMO Panel is of the opinion that the level of stearic acid in the maize MON 89034 × 1507 × NK603, assessed in this application, is within the range normally observed in commercial maize varieties, and that the maize MON 89034 × 1507 × NK603, therefore, has a composition comparable to its conventional counterpart and equivalent to commercial maize varieties, except for the newly introduced traits.

[20] Technical Dossier/ Section D7.3 & Additional info December 2009

4.1.4. Agronomic traits and GM phenotype

Previous studies have shown that with exception of the insect resistance in maize MON 89034, the insect resistant and glufosinate-ammonium herbicide tolerant traits in maize 1507 and the glyphosate-based herbicide tolerance trait in maize NK603, these GM maize are agronomically and phenotypically equivalent to their conventional counterparts (EFSA, 2003a,b, 2004a, 2005a,b, 2008, 2009b,c).

In the present application, the applicants provided information on agronomic performance, and phenotypic and ecological characteristics of maize MON 89034 × 1507 × NK603 as compared to XE6001 maize obtained from field trials in the USA 2006, the same field trials that produced material for the compositional comparison (see section 4.1.2.). The agronomic, phenotypic and ecological parameters in these field trials included early stand count, seedling vigour, days to 50% pollen shed, days to 50% silking, ear height, plant height, staygreen, dropped ears, final stand count, stalk lodging, root lodging, grain moisture, test weight, yield, and effects of insect, disease, and abiotic stressors.

In the combined-site analysis of the data, only grain moisture differed between maize MON 89034 × 1507 × NK603 (25.0%) and the XE6001 control maize (23.0%). However, these grain moisture values compared well with grain moisture in commercial maize varieties which ranged between 19.9% and 28.0%. The other 13 phenotypic characteristics were equivalent in these two types of maize.

Thirty-four symptoms of ecological stress were studied (11 related to insects, 15 to diseases, and 8 to abiotic stressors). Only one difference was noted between maize MON 89034 × 1507 × NK603 and the control maize and that was the incidence of northern corn leaf blight at one of the field trial sites, at one time during the growth period. Whereas the symptoms in maize MON 89034 × 1507 × NK603 was slight, the symptoms in the control were moderate. In the absence of consistent unexpected differences between the studied maize plants, the EFSA GMO Panel concludes that no biologically relevant agronomic and phenotypic differences specific for maize MON 89034 × 1507 × NK603 as compared to its conventional counterpart and commercial varieties have been observed except for the introduced herbicide tolerance and insect resistance traits.

4.2. Conclusion

Based on the results of a comparative analysis of data in the present application, the EFSA GMO Panel concludes that maize MON 89034 × 1507 × NK603, as assessed in this application, is compositionally, phenotypically and agronomically comparable to its conventional counterpart and equivalent to commercial maize varieties, except for the presence of the newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and CP4EPSPS L214P) in maize MON 89034 × 1507 × NK603. Based on the assessment of the data available including data on the F_2 grain, the EFSA GMO Panel is of the opinion that crossing maize MON 89034, 1507 and NK603 to produce maize MON 89034 × 1507 × NK603 does not result in interactions between the single maize events which cause compositional, agronomic or phenotypic changes that would raise a safety concern.

Furthermore, based on all data available, the EFSA GMO Panel is of the opinion that the same conclusions can be extended to any sub-combinations of individual events as present in its segregating progeny.

5. Food/Feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Summary of the previous evaluation of the single events

Maize MON 89034

Maize MON 89034 expresses the Cry1A.105 and Cry2Ab2 proteins. *Escherichia coli*-produced Cry1A.105 and Cry2Ab2 proteins were used for safety studies after it had been demonstrated experimentally that they were equivalent to those that are present in maize expressing the event MON 89034. 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 marginally less rapidly in simulated intestinal fluid. In bioinformatics studies, the amino acid sequence of Cry1A.105 and Cry2Ab2 neither showed similarity to proteins that are known to be allergens nor toxic to humans or other animals (EFSA, 2008).

In a 90-day feeding study in rats with grain material from maize MON 89034 (33% of the feed), no treatment-related adverse effects were observed, and a 42-day feeding study on broiler chickens (55-59% of the feed) showed that maize MON 89034 does not differ nutritionally from its conventional counterpart and is equivalent to commercial maize varieties included in the study (EFSA, 2008).

The EFSA GMO Panel concluded that maize MON 89034 is as safe as conventional maize and that the overall allergenicity of the whole plant is not changed. Maize MON 89034 and derived products are unlikely to have any adverse effects on human and animal health in the context of its intended use (EFSA, 2008).

Maize 1507

Maize 1507 expresses the Cry1F and PAT proteins. A trypsinised *Pseudomonas fluorescens*-produced Cry1F protein, identical to the truncated Cry1F protein expressed in 1507 maize, except for a phenylalanine instead of a leucine at position 604 and a C-terminal extention with seven amino acids residues (606-612: Ala-Glu-Tyr-Asp-Leu-Glu-Arg), was used for the safety testing instead of the maize-produced truncated Cry1F after it had been demonstrated experimentally that it was functionally equivalent to that present in maize 1507. Similarly a PAT microbial protein was used for safety studies after it had been demonstrated experimentally that it was equivalent to the enzyme present in maize 1507. Similarly a PAT microbial protein was used for safety studies after it had been demonstrated experimentally that it was equivalent to the enzyme present in maize 1507. No toxicity of the Cry1F and PAT proteins were observed in acute oral toxicity studies in mice. No oral toxicity of maize 1507 was observed in a rat study where the experimental animals were fed *ad libitum* a diet containing up to 33% maize 1507. In addition, nutritional data comprising target animal feeding studies with whole maize grains on broilers and dairy cows indicate that maize 1507 is nutritionally equivalent to conventional maize cultivars. The allergenicity risk of the Cry1F and PAT proteins has been already expressed in maize 1507 was found to be low and alteration in the allergenicity of the whole crop does not appear relevant to the Panel since maize is not considered a common allergenic food. The GMO Panel concluded that the studies available support the findings of the molecular characterization and the compositional analysis and indicates maize 1507 to be as safe as its conventional counterparts (EFSA, 2004a; 2005a,b; 2009c).

Maize NK603

E.coli-produced CP4 EPSPS and CP4 EPSPS L214P proteins were used for toxicity studies after it had been demonstrated experimentally that these microbially produced proteins were equivalent to those extracted from maize 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 toxicity study in rats with 33% of the diet being maize NK603 indicated no toxicity, and a 42-day feeding study on broiler chickens with 57-63% of the diet being maize NK603 showed equivalent nutritional wholesomeness to the non-GM control maize and commercial maize varieties included in the study. The nutritional equivalence of maize NK603 to commercial maize varieties were confirmed in feeding studies on Angus-continental cross steers, Holstein dairy cows and growing-finishing pigs of two breeds. These studies on experimental and farm animal supported the findings of the compositional analysis which showed no change in composition beyond the intended expression of the CP4 EPSPS and CP4 EPSPS L214P proteins. The EFSA GMO Panel concluded that maize NK603 is as safe as conventional maize (EFSA, 2003a,b, 2009b).

5.1.2. Product description and intended use

The scope of application EFSA-GMO-NL-2009-65 is for food and feed use, import and processing of maize MON 89034 × 1507 × NK603 and all derived products.

The transgenic traits present in maize MON 89034 × 1507 × NK603 result in the expression of the Cry1A,105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and CP4 EPSPS L214P proteins. These traits are intended to improve the agronomic performance only and are not intended to influence the nutritional properties, the processing characteristics and overall use of maize as a crop. Thus, all food, feed and processed products derived from maize MON 89034 × 1507 × NK603 will be the same as those from commercial maize. The primary use of maize is for animal feed, but it is also processed into valuable food products, including e.g. starch, syrups, ethanol and oils, which all have low protein content.

5.1.3. Effect of processing

Since maize MON 89034 × 1507 × NK603 is compositionally comparable to its conventional counterpart and equivalent to commercial maize varieties (see Section 4.2), except for the newly expressed proteins, the effect of processing on maize MON 89034 × 1507 × NK603 is not expected to be different compared with conventional maize.

5.1.4. Toxicology

5.1.4.1. Toxicological assessment of expressed novel proteins

No new genes in addition to those occurring in maize MON 89034, 1507 and NK603 have been introduced in maize MON 89034 × 1507 × NK603. The Cry1A.105 and Cry2Ab2 proteins expressed in maize MON 89034, the Cry1F and PAT proteins expressed in maize 1507, and the CP4 EPSPS and CP4 EPSPS L214P proteins expressed in maize NK603 have been assessed for their safety previously using biochemical, *in silico*, *in vitro* and *in vivo* methodology (EFSA, 2003a, 2003b, 2004a, 2005a, 2005b, 2008, 2009b,c) and no safety concerns were identified for humans and/or animals. The EFSA GMO Panel is not aware of any new information that would change these conclusions.

While the EFSA GMO Panel has considered the bioinformatic studies in the published EFSA scientific opinions on the single maize events MON89034 (2008), 1507 (2004a; 2005a,b; 2009c) and NK603 (2003a,b; 2009b), the applicants provided updated bioinformatics studies using the FASTA search algorithm to compare the amino acid sequence of the Cry1A.105, Cry2Ab2, CP4 EPSPS, and CP4 EPSPS L214P proteins with proteins known to be toxic and retrieved from the databases. While Cry1A.105 and Cry2Ab2, as expected, showed various degrees of similarity with other insecticidal Cry proteins, they did not demonstrate any sequence similarity (and inferred structural similarity) with proteins that are toxic to humans and animals. The CP4 EPSPS and CP4 EPSPS L214P proteins

showed highest identity with the toxic anion resistance protein, but the identity was low and did not raise concern. The amino acid sequence of the Cry1F and PAT proteins expressed in maize 1507 was compared with the amino acid sequences of toxic proteins in an updated database already when the GMO Panel gave their opinion on the renewal of marketing of maize 1507 (EFSA, 2009c). The outcomes of these bioinformatics-supported comparisons with updated databases did not show any relevant similarities. In addition, the EFSA GMO Panel is not aware of any other new information that would change the conclusions of its previous opinions.

Determination of the levels of the newly expressed proteins in grain produced by maize MON 89034 × 1507 × NK603 showed comparable levels to those in the respective single maize events (MON 89034, 1507, NK603) (see section 3.1.4). Based on the known function characteristics and modes of action of the newly expressed proteins Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and CP4EPSPS L214P, the EFSA GMO Panel considers it unlikely that interactions between these newly expressed proteins would occur that would raise any safety concern.

5.1.4.2. Toxicological assessment of new constituents other than proteins

No new constituents other than the Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and CP4EPSPS L214P proteins are expressed in maize MON 89034 × 1507 × NK603. Moreover, no biologically relevant changes in the composition of maize MON 89034 × 1507 × NK603 were detected in the compositional analysis. Therefore, a toxicological assessment of new constituents is not applicable.

5.1.4.3. Toxicological assessment of the whole GM food/feed

As described in section 5.1.1., which summaries the evaluation of the single events that in this application on maize MON 89034 × 1507 × NK603 have been brought together by conventional crosses, the EFSA GMO Panel has found the single maize events to be as safe as their conventional counterparts for human and animal consumption (EFSA, 2003a,b, 2004a, 2005a,b, 2008, 2009b,c). No new genes in addition to those present in maize MON 89034, 1507 and NK603 have been introduced in maize MON 89034 × 1507 × NK603. In the current assessment, the molecular characterization identified no changes in the structural integrity of the inserts in maize MON 89034 × 1507 × NK603 compared to these in the respective single events (section 3.1.3.), and the protein expression levels in maize MON 89034 × 1507 × NK603 were shown to be comparable to those in the respective single maize events (section 3.2). Moreover, the composition of maize MON 89034 × 1507 × NK603 was found to be comparable to its conventional counterpart and equivalent to commercial maize varieties (section 4.1.3). The EFSA GMO Panel considered all the data available for maize MON 89034 × 1507 × NK603, and for the newly expressed proteins Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and CP4EPSPS L214P, and is of the opinion that interactions between the maize events that might impact on the food and feed safety of maize MON 89034 x 1507 x NK603 are unlikely.

The EFSA GMO Panel does not consider additional animal safety studies with the whole GM food/feed necessary.

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 Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and CP4EPSPS L214P proteins present in maize MON 89034 × 1507 × NK603 have been assessed previously and it was found unlikely that they are allergenic (EFSA, 2003a, 2003b, 2004a, 2005a, 2005b 2009b,c). As the databases with allergenic proteins have been updated after some of the previous bioinformatics studies on newly expressed proteins in maize MON 89034 × 1507 × NK603 were performed, the applicants provided updated bioinformatics studies using the FASTA and ALLERGENSEARCH search routines to compare the amino acid sequence of the Cry1A.105, Cry2Ab2, CP4 EPSPS, and CP4 EPSPS L214P proteins with the amino acid sequences of known allergenic proteins stored in the Allergen Database version 8.0. The bioinformatics search identified no sequences of the newly expressed proteins being at least 35% identical to allergenic proteins over an 80 amino acids large sliding window. There were also no contiguous 8 amino acid long polypeptide matches shared between the query proteins and allergenic proteins. Based on the information provided, the GMO Panel considers it unlikely that these newly expressed proteins are allergenic.

5.1.5.2. Assessment of allergenicity of the whole GM plant

The issue of a potential increased allergenicity of maize MON 89034 × 1507 × NK603, as compared to the single maize events MON 89034, 1507 and NK603, and to conventional maize varieties, does not appear relevant to the EFSA GMO Panel since maize is not considered a common allergenic food. However rare cases of occupational allergy to maize dust have been reported in the literature (Bardana, 2008, Jeebhay and Quirce, 2007). The EFSA GMO Panel is also aware that a few cases of food allergy to maize have been reported in some geographically restricted areas where maize is a common food and that the major maize allergens have then been identified. In the context of the present application the EFSA GMO Panel considers it unlikely that any interactions between the newly expressed proteins and metabolic pathways of maize would alter the pattern of expression of endogenous proteins/potential allergens and thereby significantly change the overall allergenicity of the whole plant. In addition, given all the available information, the EFSA GMO Panel sees no reason to expect that the use of GM maize MON 89034 × 1507 × NK603 would significantly increase the intake and exposure to maize.

5.1.6. Nutritional assessment of GM food/feed

The nutritional equivalence of maize hybrids containing the single maize events MON 89034, 1507 and NK603 to their conventional counterpart and other appropriate comparators have already been established (EFSA, 2003a, 2003b, 2004a; 2005a, 2005b, 2008, 2009b,c).

The applicants provided a 42-day feeding study with broiler chickens to analyse the nutritional value of grains from maize MON 89034 × 1507 × NK603, in relation to grains from its conventional counterpart and one commercial maize variety. However, this study was not considered by the EFSA GMO Panel because of relevant deviations from Good Agricultural Practice (e.g. ILSI 2007), in particular dehydration among the birds and high animal losses in the first week of the study.

Based on the outcome of the comparative compositional, phenotypic and agronomic analysis of maize MON 89034 × 1507 × NK603 in relation to its conventional counterpart (see section 4.2), the EFSA GMO Panel does not consider a nutritional feeding study with the whole GM food/feed necessary.

5.1.7. Post-market monitoring of GM food/feed

An evaluation of the risk assessment concluded that no data have emerged to indicate that maize MON 89034 × 1507 × NK603 is not as safe as its conventional counterpart. In addition, no biologically relevant agronomic and compositional changes were identified in maize MON 89034 × 1507 × NK603

as compared to its conventional counterpart, with exception of the newly expressed (Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS and CP4EPSPS L214P). Given the intended use of maize MON 89034 × 1507 × NK603, the overall intake or exposure to maize is not expected to be changed. Therefore, and in line with the guidance document (EFSA, 2006a), the GMO Panel is of the opinion that post-market monitoring of the food/feed derived from maize MON 89034 × 1507 × NK603 is not necessary.

5.2. Conclusion

The Cry1A.105 and Cry2Ab2 proteins expressed in maize MON 89034, the Cry1F and PAT proteins expressed in maize 1507, and the CP4 EPSPS and CP4 EPSPS L214P proteins expressed in maize NK603 have been assessed for their safety previously (EFSA, 2003a,b, 2004a, 2005a,b, 2009b,c) and no safety concerns were identified for humans and animals. Furthermore, the whole maize crop carrying the single maize events MON 89034, 1507 and NK603 have been found safe for these organisms. No new genes in addition to those occurring in maize MON 89034, 1507 and NK603 have been introduced in maize MON 89034 × 1507 × NK603. Based on the molecular characteristics of these events (see section 3.2), the known functional characteristics and modes of action of the newly expressed proteins and the outcomes of the comparative analysis of compositional, phenotypic and agronomic characteristics of maize MON 89034 × 1507 × NK603, the EFSA GMO Panel considers it unlikely that interactions between the maize events will occur that would impact on the food and feed safety and nutritional properties of maize MON 89034 × 1507 × NK603. In addition, the EFSA GMO Panel considers it unlikely that the overall allergenicity of maize MON 89034 × 1507 × NK603 has been altered.

Based on data on the maize stack MON 89034 × 1507 × NK603, data on the single maize events MON 89034, 1507 and NK603, and the two double stacks 1507 × NK603 and MON 89034 × NK603, the EFSA GMO Panel identified no biological reason to expect that any of the other stacks of these single events as present in the segregating progeny would raise a safety concern. In conclusion, the EFSA GMO Panel is of the opinion that maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny are as safe and as nutritious as its conventional counterpart and commercial maize varieties, and concludes that this maize and derived products are unlikely to have any adverse effects on human and animal health, in the context of its intended uses.

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

The scope of the application is for food and feed uses, import and processing of maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny, and does not include cultivation. Considering the proposed uses of maize MON 89034 × 1507 × NK603, the environmental risk assessment is concerned with the exposure through manure and faeces from animals fed grain (F_2 generation) produced by maize MON 89034 × 1507 × NK603 and with the accidental release into the environment of viable grains from maize MON 89034 × 1507 × NK603 (which include its segregating progeny) during transportation and processing.

6.1.1. Evaluation of single maize events and stacked maize events

In its previous scientific opinions, the EFSA GMO Panel was of the opinion that the single maize events MON 89034, 1507 and NK603 and stacked maize events 1507 × NK603 and MON 89034 × NK603 are as safe as their conventional counterparts, and that the placing on the market of maize MON 89034, 1507, NK603, 1507 × NK603 and MON 89034 × NK603 for food and feed uses, import

and processing is unlikely to have an adverse effect on human or animal health, or on the environment (EFSA, 2003a,b; 2004a, 2005a,b, 2006c, 2008, 2009b,c,d). Furthermore, post-market environmental monitoring plans for maize MON 89034, 1507, NK603, 1507 × NK603 and MON 89034 × NK603, including general surveillance, were proposed by the applicants and considered in line with the EFSA GMO Panel scientific opinion on post-market environmental monitoring (EFSA, 2006b).

6.1.2. Environmental risk assessment

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

Maize is highly domesticated and generally unable to survive in the environment without management intervention. Maize plants are not winter hardy in many 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 cultivation, maize volunteers may arise under some environmental conditions (mild winters). Observations made on cobs, cob fragments or isolated grains shed in the field during harvesting indicated that grains may survive and overwinter in some regions, resulting in volunteers in subsequent crops. The occurrence of maize volunteers was reported in Spain and other European regions (e.g., Gruber *et al.*, 2008). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs *et al.*, 2009).

Previous field trials have shown that there are no indications of altered fitness of the single maize events MON 89034, 1507 and NK603 and the stacked maize events 1507 × NK603 and MON 89034 × NK603 as compared to their conventional counterparts. In addition to the field trials carried out with the single maize events and double stacked maize events (EFSA, 2003a,b; 2004a, 2005a,b, 2006c, 2008, 2009b,c,d), a series of field trials with maize MON 89034 × 1507 × NK603 were conducted across four USA corn belt locations in 2006. Information on phenotypic and agronomic characteristics was provided to assess the agronomic performance of maize MON 89034 × 1507 × NK603 in comparison with its conventional counterpart. These field trial data did not show changes in plant characteristics that indicate altered fitness and invasiveness of maize MON 89034 × 1507 × NK603 plants, though there is a potential for enhanced biomass production when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests. Based on the available data on the single events and the stacked maize events 1507 × NK603, MON 89034 × NK603 and MON 89034 × 1507 × NK603, the EFSA GMO Panel considers it very unlikely that the segregating progeny of MON 89034 × 1507 × NK603 would have any increased persistence and invasiveness in EU receiving environments. In addition, the EFSA GMO Panel is not aware of any scientific report of increased establishment, spread or any change in survival capacity including overwintering of maize MON 89034 × 1507 × NK603, or maize with comparable properties such as single maize events and sub-combinations of maize MON 89034 × 1507 × NK603.

The herbicide tolerance traits can only be regarded as providing a potential agronomic advantage for maize MON 89034 × 1507 × NK603 plants and all sub-combinations of these events expressing the herbicide tolerance genes where and when glufosinate-ammonium- and/or glyphosate-based herbicides are applied. Similarly, insect resistance against certain lepidopteran target pests provides a potential agronomic advantage in cultivation under infestation by target pests. However, survival of maize plants outside cultivation or other areas where glufosinate-ammonium- and/or glyphosate-based herbicides could be applied in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens, herbivores and cold climatic conditions. Since these general characteristics are unchanged in maize MON 89034 × 1507 × NK603, herbicide tolerance and insect resistance are not likely to provide a selective advantage outside cultivation in Europe. Therefore, it is considered very unlikely that maize MON 89034 × 1507 × NK603 and its segregating progeny will differ from conventional maize varieties in their ability to

survive until subsequent seasons or to establish feral populations under European environmental conditions.

Since maize MON 89034 × 1507 × NK603 has no altered survival, multiplication or dissemination characteristics, except when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the accidental release into the environment of viable grains from maize MON 89034 × 1507 × NK603 (which include all sub-combinations of the individual events as present in its segregating progeny) will not differ from that of the single maize events MON 89034, 1507 and NK603, the stacked maize events 1507 × NK603 and MON 89034 × NK603, or from that of conventional maize varieties.

6.1.2.2. Gene transfer

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

(a) Plant to bacteria gene transfer

Genomic DNA is a component of many food and feed products derived from maize. It is well documented that DNA present in food and feed becomes substantially degraded in the process of digestion in human or animal gastrointestinal tracts. However, a low level of exposure of fragmented products of the ingested DNA, including their recombinant fraction of such DNA, to microorganism in the digestive tracts of humans, domesticated animals, and other animals feeding on maize MON 89034 × 1507 × NK603 grains is expected.

Current scientific knowledge indicates that horizontal gene transfer of non-mobile DNA fragments between unrelated organisms (such as plants to microorganisms) is extremely unlikely to occur under natural conditions (see EFSA, 2009a for further details). The concentration of extracellular DNA fragments in gastrointestinal tracts is relatively low and most bacteria lack competence to take up and recombine foreign DNA.

The *cry1A.105*, *cry2Ab2*, *cry1F*, *pat* and CP4 *epsps* genes in maize MON 89034 × 1507 × NK603 are all derived from bacterial genes. Thus, in theory, the *cry1A.105*, *cry2Ab2*, *cry1F*, *pat* and CP4 *epsps* genes of the recombinant DNA inserts could provide sufficient DNA similarity for homologous recombination to take place in environmental bacteria. However, as discussed further below, such hypothesised horizontal gene transfer event is not likely to be maintained in bacterial populations due to a predicted lack of efficient expression and no identified selective advantage for gene transfer recipients in the unlikely case of their expression.

In case of non-homologous recombination into environmental bacterial genomes, it is unlikely that recombinant genes (*cry1F* and CP4 *epsps*) regulated by eukaryotic plant promoters in maize MON 89034 × 1507 × NK603 would be expressed. The *cry1A.105*, *cry2Ab2* and CP4 *epsps* genes are regulated by plant virus promoters. The activity of plant virus promoters in unrelated organisms such as bacteria cannot be excluded, but in the unlikely event that the above mentioned genes and regulatory elements are taken up by bacteria, no selective advantage is anticipated, because *cry*, *pat* and *epsps* genes are already occurring in various bacterial species in the environment. Thus, the hypothesised low level exposure of bacterial communities to the maize MON 89034 × 1507 × NK603 (and all sub-combinations of the individual events as present in its segregating progeny) *cry1A.105*, *cry2Ab2*, *cry1F*, *pat* and/or CP4 *epsps* genes must be seen in the context of the natural occurrence and level of exposure to alternative sources of genetically diverse *cry*, *pat* and *epsps* genes to which bacterial communities are naturally exposed.

The wide environmental presence of genetically diverse natural variants of the recombinant DNA coding sequences, the use of regulatory sequences optimised for expression in eukaryotes, and the absence of an identified plausible selective advantage that would be provided to receiving bacteria, suggest it is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts (EFSA, 2009a).

(b) Plant to plant gene transfer

The extent of cross-pollination to other maize varieties will mainly depend on the scale of accidental release during transportation and processing, and on successful establishment and subsequent flowering of this GM maize plant. 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 maize volunteers after GM maize cultivation 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 and when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests. Even though the occurrence of some GM maize plants outside cropped area have been reported in Korea due to grain spillage during import, transportation, storage, handling and processing (Kim *et al.*, 2006; Lee *et al.*, 2009; Park *et al.*, 2010), survival of maize plants outside cultivation in Europe is mainly limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens, herbivores and frost. Since these general characteristics are unchanged in maize MON 89034 × 1507 × NK603 and its segregating progeny, herbicide tolerance and insect resistance are not likely to provide selective advantages outside cultivation or other areas where glufosinate-ammonium- and/or glyphosate-based herbicides could be applied and/or under infestation by target pests in Europe. Therefore, as for any other maize varieties, GM maize plants would only survive in subsequent seasons in warmer regions of Europe and are not likely to establish feral populations under European environmental conditions.

The EFSA GMO Panel takes into account that this application does not include cultivation of maize MON 89034 × 1507 × NK603 within the EU so that the likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low. However, in countries cultivating maize MON 89034 × 1507 × NK603 and producing seed for export, there is a potential for admixture in seed production and thus the introduction of GM seeds through this route. Hence, it is important that appropriate management systems are in place to restrict seeds of maize MON 89034 × 1507 × NK603 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, maize MON 89034 × 1507 × NK603 and its segregating progeny have no altered survival, multiplication or dissemination characteristics, except when glufosinate-ammonium- and/or glyphosate-based herbicides are applied, and/or under infestation by target pests. The EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes, resulting from imports of this maize and its segregating progeny in Europe, will not differ from that of the single maize events MON 89034, 1507 and NK603, or from that of conventional maize varieties.

6.1.2.3. Interactions of the GM plant with target organisms

The intended uses of maize MON 89034 × 1507 × NK603 specifically exclude cultivation and the environmental exposure to maize MON 89034 × 1507 × NK603 and all sub-combinations of these events is limited to the accidental release of 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 MON 89034 × 1507 × NK603 plants or their segregating progeny to enable any significant interaction with target organisms, which is very unlikely.

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

The intended uses of maize MON 89034 × 1507 × NK603 specifically exclude cultivation and the environmental exposure to maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny, is limited to the accidental release of 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 MON 89034 x 1507 x NK603 plants or their segregating progeny to enable any significant interaction with non-target organisms, which is very unlikely.

In addition, the EFSA GMO Panel evaluated whether the Cry1A.105, Cry2Ab2 and Cry1F proteins might potentially affect non-target organisms by entering the environment through manure and faeces from animals fed maize MON 89034 × 1507 × NK603. Due to the specific insecticidal selectivity of the Cry protein, non-target organisms most likely to be affected by the Cry1A.105, Cry2Ab2 and Cry1F proteins belong to the same or closely related taxonomic groups as those of the target organisms.

Data supplied by the applicants suggest that only low amounts of the Cry1A.105, Cry2Ab2 and Cry1F proteins enter the environment due to low expression in grains. Moreover, these Cry proteins are degraded by enzymatic activity in gastrointestinal tracts of animals fed on GM maize or derived feed products (see section 5.1.1), meaning that only low amounts of these proteins would remain intact to pass out in faeces. This has been demonstrated for Cry1Ab (Einspanier *et al.*, 2004; Lutz *et al.*, 2005, 2006; Wiedemann *et al.*, 2006; Guertler *et al.*, 2008; Paul *et al.*, 2010). It is expected that there would subsequently be further degradation of Cry proteins in the manure and faeces due to intrinsic microbial proteolytic activity. Therefore, exposure of soil and aquatic environments to the Cry1A.105, Cry2Ab2 and Cry1F proteins from disposal of animal wastes or accidental spillage of maize grains is likely to be very low and localised. While Cry proteins may bind to a certain degree to clay minerals or humic substances in soil, thereby reducing their availability to microorganisms for degradation, there are no indications of persistence and accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky, 2008).

Considering the scope of the application (that excludes cultivation) and the intended uses of maize MON 89034 × 1507 × NK603, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1A.105, Cry2Ab2 and Cry1F proteins is likely to be very low and of no ecological relevance.

6.1.2.5. Interactions with the abiotic environment and biochemical cycles

Considering the scope of the application (that excludes cultivation) and the intended uses of maize MON 89034 × 1507 × 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 environmental 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 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 EFSA GMO Panel gives its opinion on the scientific quality of the monitoring plan provided by the applicants (EFSA, 2006b). The potential exposure to the environment of maize MON 89034 × 1507 × NK603 would be mainly through manure and faeces from animals fed grain produced by maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregated progeny, and/or through accidental release into the environment of GM maize grains during transportation and processing.

No specific environmental impact of maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny was indicated by the environmental risk assessment and thus no case-specific monitoring is required.

The general surveillance plan proposed by the applicants includes (1) the description of an approach involving operators (federations involved in maize import and processing), reporting to the 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; and (3) the use of networks of existing surveillance systems (Lecoq *et al.*, 2007; Windels *et al.*, 2008). The applicants propose 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 applicants is in line with the intended uses of maize MON 89034 × 1507 × NK603 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicants in the general surveillance plan.

6.2. Conclusion

The scope of the application includes food and feed uses, import and processing of maize MON 89034 × 1507 × NK603 and excludes cultivation. Considering the intended uses of maize MON 89034 × 1507 × NK603, the environmental risk assessment is concerned with indirect exposure mainly through manure and faeces from animals fed grain produced by maize MON 89034 × 1507 × NK603 and with the accidental release into the environment of viable grains from maize MON 89034 × 1507 × NK603 (which includes its segregating progeny) during transportation and processing.

There are no indications of an increased likelihood of establishment and spread of feral maize plants in case of accidental release into the environment of viable grains from maize MON 89034 × 1507 × NK603 grains (which includes the segregating progeny of maize MON 89034 × 1507 × NK603) during transportation and processing, except in the presence of glufosinate-ammonium- and/or glyphosate-based herbicides and/or under infestation by target pests. Taking into account the scope of the application, both the rare occurrence of feral maize plants and low levels Cry1A.105, Cry2Ab2 and Cry1F protein exposure in maize MON 89034 × 1507 × NK603 grains or through other routes indicate that the risk to non-target organisms is extremely low. It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts.

The scope of the monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny, 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 applicants in the general surveillance plan.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel was requested to carry out an evaluation of a scientific risk assessment for food and feed uses, import and processing in accordance with Regulation (EC) No 1829/2003 of maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny. The EFSA GMO Panel evaluated MON 89034 × 1507 × NK603, which has been produced by conventional crossing of maize lines containing the single maize events MON 89034, 1507 and NK603. All single maize events MON 89034, 1507 and NK603 as well as the stacked maize events 1507 × NK603 and MON 89034 × NK603 have been evaluated by the EFSA GMO Panel (EFSA, 2003a,b, 2004a, 2005a,b, 2006c, 2008, 2009b,c,d). In evaluating maize MON 89034 × 1507 × NK603 the EFSA GMO Panel considered the application EFSA-GMO-NL-2009-65, additional information provided by the applicants, scientific comments submitted by the Member States, and relevant scientific publications. Further information from applications for placing the single maize events MON 89034, 1507 and NK603, as well as the stacks 1507 × NK603 and MON 89034 × NK603 on the market under EU regulatory framework was taken into account (EFSA, 2003a,b, 2004a, 2005a,b, 2006c, 2008, 2009b,c,d).

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for maize MON 89034 × 1507 × NK603 are sufficient. The results of the bioinformatic analyses of the inserted DNA and the flanking regions of the single events MON 89034, 1507 and NK603 do not raise safety concern. The levels of Cry1A.105, Cry2Ab2, Cry1F, PAT and CP4 EPSPS proteins in maize MON 89034 × 1507 × NK603 have been sufficiently analysed and the stability of the genetic modification has been demonstrated. The EFSA GMO panel considers that the molecular characterisation does not indicate a safety concern.

Based on the results of comparative analysis it was concluded that maize MON 89034 × 1507 × NK603 is compositionally and agronomically comparable to its conventional counterpart and equivalent to commercial maize varieties, except for the newly expressed proteins. The Cry1A.105 and Cry2Ab2 proteins expressed in maize MON 89034, the Cry1F and PAT expressed in maize line 1507, and the CP4 EPSPS proteins expressed in maize NK603 have been assessed previously and no safety concerns were identified. The assessment of the Cry1A.105, Cry2Ab2, CP4 EPSPS, CP4 EPSPS L214P proteins regarding toxicity and allergenicity were updated with new bioinformatic studies. Given all the information provided, the Panel concludes that interactions between the Cry1A.105, Cry2Ab2, Cry1F, PAT, CP4 EPSPS, and CP4 EPSPS L214P proteins in maize MON 89034 × 1507 × NK603 that might impact on food and feed safety are unlikely. The EFSA GMO Panel considers that maize MON 89034 × 1507 × NK603 is as safe and as nutritious as its conventional counterpart and commercial maize varieties and that the overall allergenicity of the whole plant is not changed. In conclusion, the EFSA GMO Panel is of the opinion that maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny are as safe and as nutritious as the conventional counterpart and commercial maize varieties, and concludes that this maize and derived products are unlikely to have adverse effects on human and animal health, in the context of its intended uses.

Considering the intended uses of maize MON 89034 × 1507 × 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 viable grains from maize MON 89034 × 1507 × NK603 during transportation and processing, there are no indications of

an increased likelihood of establishment and spread of feral maize plants, except in the presence of glufosinate-ammonium- and/or glyphosate-based herbicides and/or under infestation by target pests. In addition, the low levels of environmental exposure to these GM maize plants and the Cry1A.105, Cry2Ab2 and Cry1F proteins through other routes indicate that the risk to non-target organisms is extremely low. It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts. The scope of the post-market environmental monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 × 1507 × NK603 and all sub-combinations of the individual events as present in its segregating progeny.

The EFSA GMO Panel recommends that appropriate management systems should be in place to restrict seeds of maize MON 89034 × 1507 × 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 the information available for maize MON 89034 × 1507 × NK603 addresses the scientific comments raised by the Member States and concludes that the maize MON 89034 × 1507 × NK603, assessed in this application, is as safe as its conventional counterpart and other appropriate comparators with respect to potential effects on human and animal health and the environment. In addition, the EFSA GMO Panel is of the opinion that crossing of single maize events MON 89034, 1507 and NK603 to produce maize MON 89034 × 1507 × NK603 does not result in interactions between the events which would affect the safety of maize MON 89034 × 1507 × NK603 with respect to potential effects on human and animal health and on the environment, in the context of its intended uses. Based on data provided for maize stack MON 89034 × 1507 × NK603, the single maize events (MON 89034, 1507 and NK603), and for the two double stacks 1507 × NK603 and MON 89034 × NK603, the EFSA GMO Panel is of the opinion that there is no biological reason to expect that any of the other sub-combinations of the individual events as present in its segregating progeny would raise a safety concern. The EFSA GMO Panel concludes that maize MON 89034 × 1507 × NK603 is unlikely to have adverse effects on human and animal health and the environment, in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of The Netherlands, received 6 February 2009, concerning a request for placing on the market of maize MON 89034 × 1507 × NK603 submitted jointly by Dow AgroSciences and Monsanto Europe under Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 25 February 2009, from EFSA to the Competent Authority of The Netherlands (Ref. PB/KL/shv (2009) 3692902).

3. Letter from EFSA to applicant, dated 19 March 2009, requesting additional information under completeness check (Ref. PB/CE/md (2009) 3816687).

4. Letter from applicant to EFSA, received 21 April 2009, providing additional information under completeness check.

5. Letter from applicant to EFSA, received 21 April 2009, providing additional information under completeness check.

6. Letter from EFSA to applicant, dated 13 May 2009, requesting additional information under completeness check (Ref. PB/KL/CE/shv (2009) 3942700).

7. Letter from applicant to EFSA, received 3 June 2009, providing additional information under completeness check.

8. Letter from EFSA to applicant, dated 23 June 2009, requesting additional information under completeness check (Ref. PB/KL/CE/mt (2009) 4055969).

9. Letter from applicant to EFSA, received 25 June 2009, providing additional information under completeness check.

10. Letter from EFSA to applicant, dated 6 August 2009, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2009-65, maize MON 89034 × 1507 × NK603 submitted jointly by Dow AgroSciences and Monsanto Europe under Regulation (EC) No 1829/2003 (Ref. PB/KL/CE/lg (2009) 4190310).

11. Letter from applicant to EFSA, received 25 September 2009, providing supplementary information spontaneously.

12. Letter from applicant to EFSA, received 6 October 2009, providing additional information spontaneously.

13. Letter from EFSA to applicant, dated 28 October 2009, requesting additional information and stopping the clock (Ref. PB/KL/AFD/ls (2009) 4395645).

14. Letter from EFSA to applicant, dated 29 October 2009, requesting clarifications on the additional information sent spontaneously by the letter received 25 September 2009 (Ref. PB/KL/NP/ZD/lg (2009) 4390891).

15. Letter from applicant to EFSA, received 23 December 2009, providing additional information.

16. Letter from EFSA to applicant, dated 29 January 2010, requesting clarifications concerning additional information provided (Ref. PB/KL/AFD/ls (2010) 4607524).

17. Letter from applicant to EFSA, received 2 February 2010, providing additional information related to the spontaneous supplementary information received on 25 September 2009.

18. Letter from EFSA to applicant, received 4 February 2010, providing clarifications.

19. Letter from EFSA to applicant, dated 8 March 2010, restarting the clock (Ref. PB/KL/AFD/lg (2010) 4698190).

20. Letter from EFSA to applicant, dated 22 March 2010, requesting additional information and stopping the clock (Ref. PB/KL/AFD/mt (2010) 4735624).

21. Letter from applicant to EFSA, received 7 April 2010, providing additional information.

22. Mail from EFSA to applicant, dated 22 April 2010, requesting clarifications concerning additional information provided.

23. Letter from applicant to EFSA, received 3 May 2010, providing clarifications.

24. Letter from EFSA to applicant, dated 25 May 2010, restarting the clock (Ref. PB/KL/AFD/shv (2010) 4888683).

25. Letter from applicant to EFSA, received 16 June 2010, providing clarifications on the scope of the application.

26. Letter from applicant to EFSA, received 24 June 2010, providing additional information.

27. Letter from EFSA to applicant, dated 2 July 2010, requesting additional information and stopping the clock (Ref. PB/KL/mt (2010) 4972267).

28. Letter from EFSA to applicant, dated 12 July 2010, requesting additional information concerning the supplementary information submitted spontaneously on 25 September 2009 and 2 February 2010 (Ref. PB/KL/NP/shv (2010) 4951463).

29. Letter from applicant to EFSA, received 19 July 2010, providing additional information.

30. Letter from EFSA to applicant, dated 26 July 2010, requesting additional information and maintaining the clock stopped (Ref. PB/KL/NP/CE/mt (2010) 5017819).

31. Letter from applicant to EFSA, received 30 July 2010, providing additional information.

32. Letter from EFSA to applicant, dated 19 August 2010, restarting the clock (Ref. PB/KL/AFD/lg (2010) 5063258).

REFERENCES

Bardana EJ, Jr., 2008. 10. Occupational asthma. Journal of Allergy and Clinical Immunology, 121, S408-411; quiz S421CAC, 2003. Codex principles and guidelines on foods derived from biotechnology. Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation, Rome.

Eastham K, Sweet J, 2002. Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer, European Environment Agency,

EFSA, 2003a. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference CE/ES/00/01) for the placing on the market of herbicide-tolerant genetically modified maize NK603, for import and processing, under Part C of Directive 2001/18/EC from Monsanto (Question No EFSA-Q-2003-003). The EFSA Journal, 10, 1-13.

EFSA, 2003b. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of foods and food ingredients derived from herbicide-tolerant genetically modified maize NK603, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No 258/97 by Monsanto (Question No EFSA-Q-2003-002). EFSA Journal 9, 1-14.

EFSA, 2004a. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/NL/00/10) for the placing on the market of insect-tolerant genetically modified 1507 maize, for import and processing, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds. The EFSA Journal (2004) 124, 1-18.

EFSA, 2004b. Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. The EFSA Journal, 48, 1-18.

EFSA, 2005a. Opinion of the Scientific Panel on Genetically Modified Organisms on an application (reference EFSA-GMO-NL-2004-02) for the placing on the market of insect-tolerant genetically modified 1507 maize, for food use, under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International/Mycogen Seeds. The EFSA Journal (2005) 182, 1-22.

EFSA, 2005b. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507 for import, feed and industrial processing and cultivation, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds. The EFSA Journal (2005) 181, 1-33.

EFSA, 2006a. Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. The EFSA Journal 99, 1-100.

EFSA, 2006b. Opinion of the Scientific Panel on GM Organisms on the Post Market Environmental Monitoring (PMEM) of GM plants. The EFSA Journal 319, 1-27.

EFSA, 2006c. Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-UK-2004-05) for the placing on the market of insect-protected and glufosinate and glyphosate-tolerant genetically modified 1507 x NK603 maize, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred and Mycogen Seeds, The EFSA Journal (2006) 355, 1-23.

EFSA, 2007, Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events The EFSA Journal 512, 1-5.

EFSA, 2008. Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-NL-2007-37) for the placing on the market of the insect-resistant genetically modified maize MON 89034, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto, The EFSA Journal (2008) 909, 1-30.

EFSA, 2009a. Statement of EFSA on the consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants". The EFSA Journal 1108 1-8.

EFSA, 2009b. Scientific Opinion of the Panel on Genetically Modified Organisms on an application (Reference EFSA-GMO-NL-2005-22, EFSA_GMO-RX-NK603) for the placing on the market of the genetically modified glyphosate tolerant maize NK603 for cultivation, food and feed uses, import and processing and for renewal of the authorization of maize NK603 as existing products, both under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal (2009) 1137, 1-50.

EFSA, 2009c. Scientific Opinion of the Panel on Genetically Modified Organisms on an application (EFSA-GMO-RX-1507) for renewal of authorisation for the continued marketing of existing products produced from maize 1507 for feed use, under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International, Inc./Mycogen Seeds. The EFSA Journal (2009) 1138, 1-11.

EFSA, 2009d. EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on application (EFSA-GMO-NL-2007-38) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON 89034 × NK603 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2009; 7(9): 1320, 1-29.

Einspanier R, Lutz B, Rief S, Berezina O, Zverlov V, Schwarz W, Mayer J, 2004. Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgenic maize. European Food Research and Technology 218, 269-273.

Gruber S, Colbach N, Barbottin A, Pekrun C, 2008. Post-harvest gene escape and approaches for minimizing it. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 3, 1-17.

Guertler P, Lutz B, Kuehn R, Meyer HHD, Einspanier R, Killermann B, Albrecht C, 2008. Fate of recombinant DNA and Cry1Ab protein after ingestion and dispersal of genetically modified maize in comparison to rapeseed by fallow deer (*Dama dama*). European Journal of Wildlife Research 54, 36-43.

Icoz I, Stotzky G, 2008. Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biology and Biochemistry 40, 559-586.

ILSI (2006) International Life Sciences Institute Crop Composition Database Version 3.0. Available from: http://www.cropcomposition.org

Jeebhay MF and Quirce S, 2007. Occupational asthma in the developing and industrialised world: a review. International Journal of Tuberculosis and Lung Disease, 11, 122-133.

Kim CG, Yi H, Park S, Yeon JE, Kim DY, Kim DI, Lee KH, Lee TC, Paek IS, Yoon W, Jeong S-C and Kim HM, 2006. Monitoring the occurrence of genetically modified soybean and maize around cultivated fields and at a grain receiving port in Korea. Journal of Plant Biology, 49, 218-298.

Lecoq E, Holt K, Janssens J, Legris G, Pleysier A, Tinland B, Wandelt C, 2007. General surveillance: roles and responsibilities the industry view. Journal of Consumer Protection and Food Safety 2(S1), 25-28.

Lee B, Kim C-G, Park J-Y, Park KW, Kim H-J, Yi H, Jeong C-C, Yoon WK, Kim HM, 2009. Monitoring the occurrence of genetically modified soybean and maize in cultivated fields and along the transportation routes of the Incheon Port in South Korea. Food Control 20, 250-254.

Lutz B, Wiedermann S, Einspanier R, Mayer J, Albrecht C, 2005. Degradation of Cry1Ab protein from genetically modified maize in the bovine gastrointestinal tract. Journal of Agricultural and Food Chemistry 53, 1453-1456.

Lutz B, Wiedermann S, Albrecht C, 2006. Degradation of transgenic Cry1Ab DNA and protein in Bt-176 maize during the ensiling process. Journal of Animal Physiology and Animal Nutrition 90, 116-123.

Morisset D, Demisar T, Gruden K, Vojvoda J, Stebih D, Zel J (2009) Detection of genetically modified organisms – closing the gaps. Nature Biotechnology 27(3): 700-701.

OECD, 2002. Consensus Document on Compositional Considerations for New Varieties of Maize (Zea mays): Key Food and Feed Nutrients, Anti-nutrients and Secondary Plant Metabolites. Series on the Safety of Novel Foods and Feeds, No. 6, Organisation for Economic Co-operation and Development (OECD), Paris.

OECD, 2003. Consensus Document on the biology of Zea mays subsp. mays (Maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2003)11), No. 27, 1-49.

Palaudelmàs M, Peñas G, Melé E, Serra J, Salvia J, Pla M, Nadal A, Messeguer J, 2009. Effect of volunteers on maize gene flow. Transgenic Research 18, 583-594.

Park KW, Lee B, Kim C-G, Kim DY, Park J-Y, Ko EM, Jeong S-C, Choi KH, Yoon WK, Kim HM, 2010. Monitoring the occurrence of genetically modified maize at a grain receiving port and along transportation routes in the Republic of Korea. Food Control 21, 456-461.

Paul V, Guertler P, Wiedemann S, Meyer HHD, 2010. Degradation of Cry1Ab protein from genetically modified maize (MON810) in relation to total dietary feed proteins in dairy cow digestion. Transgenic Research, DOI:10.1007/s11248-009-9339-z.

Wiedemann S, Lutz B, Kurtz H, Schwarz FJ, Albrecht C, 2006. In situ studies on the time-dependent degradation of recombinant corn DNA and protein in the bovine rumen. Journal of Animal Science 84, 135-144.

Windels P, Alcalde E, Lecoq E, Legris G, Pleysier A, Tinland B, Wandelt C, 2008. General surveillance for import and processing: the EuropaBio approach. Journal of Consumer Protection and Food Safety 3(S2), 14-16.