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SCIENTIFIC OPINION

Scientific Opinion on an application (EFSA-GMO-NL-2010-80) for the placing on the market of herbicide-tolerant genetically modified maize NK603 × T25 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Single events NK603 and T25 were combined to produce the stack two-event maize NK603 × T25. The EFSA GMO Panel previously assessed the two single events and did not identify safety concerns in the context of their scope. No new data on single maize events leading to a modification of the original conclusions on their safety were identified. Agronomic and phenotypic characteristics, as well as compositional data of maize NK603 × T25, did not give rise to food/feed and environmental safety concerns. The EFSA GMO Panel considers that there is no reason to expect interactions between the single events that could impact on the food and feed safety and the nutritional properties of maize NK603 × T25. There are no indications of an increased likelihood of establishment and spread of feral maize plants. Considering the scope of application EFSA-GMO-NL-2010-80, potential interactions with the biotic and abiotic environment were not considered to be a relevant issue. The unlikely but theoretically possible transfer of the recombinant genes from maize NK603 × T25 to environmental bacteria does not give rise to any safety concern. The post-market environmental monitoring plan and reporting intervals are in line with the scope. In conclusion, the EFSA GMO Panel considers that the information available for maize NK603 × T25 addresses the scientific comments raised by Member States and that maize NK603 × T25, as described in this application, is as safe as its non-GM comparator and non-GM conventional maize varieties with respect to potential effects on human and animal health and the environment in the context of its scope.

KEY WORDS

GMO, maize (*Zea mays*), CP4 EPSPS, PAT, herbicide tolerant, stack

[1] On request from the Competent Authority of the Netherlands for an application (EFSA-GMO-NL-2010-80) submitted by Monsanto, Question No EFSA-Q-2010-00880, adopted on 24 June 2015.

[2] Panel members: Salvatore Arpaia, Andrew Nicholas Edmund Birch, Andrew Chesson, Patrick du Jardin, Achim Gathmann, Jürgen Gropp, Lieve Herman, Hilde-Gunn Hoen-Sorteberg, Huw Jones, József Kiss, Gijs Kleter, Martinus Lovik, Antoine Messéan, Hanspeter Naegeli, Kaare Magne Nielsen, Jaroslava Ovesna, Joe Perry, Nils Rostoks and Christoph Tebbe. Correspondence: gmo@efsa.europa.eu

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SUMMARY

Following the submission of application EFSA-GMO-NL-2010-80 under Regulation (EC) No 1829/2003 from 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 herbicide-tolerant genetically modified (GM) maize NK603 × T25 (Unique Identifier MONØØ6Ø3-6 × ACS-ZMØØ3-2). The scope of application EFSA-GMO-NL-2010-80 is for food and feed uses, import and processing, but excludes cultivation within the European Union (EU).

The single maize events NK603 (expressing CP4 EPSPS) and T25 (expressing PAT) were assessed previously and no concerns were identified for human and animal health or environmental safety. No safety issue has been identified by updated bioinformatic analyses, or reported by the applicant, concerning the two single events since the publication of those scientific opinions. Consequently, the EFSA GMO Panel considers that its previous conclusions on the safety of the single maize events remain valid.

The two-event stack maize NK603 × T25 was produced by conventional crossing to produce maize tolerant to glyphosate- and glufosinate-ammonium-based herbicides. The EFSA GMO Panel evaluated maize NK603 × T25 with reference to the scope and appropriate principles described in its guidelines for the risk assessment of genetically modified (GM) plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring of GM plants. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and analysis of the expression of the corresponding proteins. An evaluation of the comparative analyses of the compositional, agronomic and phenotypic characteristics was undertaken, and the safety of the newly expressed proteins and of the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. An evaluation of environmental impacts and the post-market environmental monitoring plan was also undertaken. In accordance with the EFSA GMO Panel guidance documents applicable to this application (EFSA, 2006, 2007), "*Where all single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to a) stability, b) expression of the events and c) potential interactions between the events*". Additional information received after May 2011 was assessed according to 2011 guidance (EFSA GMO Panel, 2011a).

The molecular data establish that the transformation events stacked in maize NK603 × T25 have the same molecular properties and characteristics as the single transformation events. Comparison of the levels of the CP4 EPSPS and PAT proteins between the stack and the corresponding single events did not reveal an interaction that manifests at protein or trait expression level. From the molecular characterisation, no indications of interactions between the events based on the biological functions of the newly expressed proteins were identified.

Based on the agronomic and phenotypic characteristics of maize NK603 × T25 under the tested conditions (not treated with the intended herbicide), some differences were observed in maize NK603 × T25 compared with its conventional counterpart. None of the significant differences observed needed further assessment for its potential environmental impact. Similarly, the EFSA GMO Panel concluded that none of the differences identified in the agronomic and phenotypic characteristics and in the composition of grain and forage obtained from maize NK603 × T25 needed further assessment regarding food and feed safety.

The safety assessment identified no concerns regarding the potential toxicity of the newly expressed proteins CP4 EPSPS and PAT in maize NK603 × T25. The EFSA GMO Panel found no reason to suggest that the presence of the two proteins in combination would result in interactions producing effects different from those of the individual proteins. Similarly, no indications of safety concerns were identified regarding allergenicity of the individual newly expressed proteins or their mixture in maize NK603 × T25, or regarding potential changes in its overall allergenicity. Maize NK603 × T25 is as nutritious as non-GM conventional maize varieties.

Considering the scope of application EFSA-GMO-NL-2010-80, there is no requirement for scientific information on possible environmental effects associated with the cultivation of maize NK603 × T25 in Europe. There are no indications of an increased likelihood of establishment and spread of feral maize NK603 × T25 plants in the event of accidental release into the environment of viable GM maize seeds. Potential interactions of maize NK603 × T25 with the biotic and abiotic environment were not considered to be a relevant issue by the EFSA GMO Panel. The unlikely but theoretically possible transfer of the recombinant genes from maize NK603 × T25 to environmental bacteria does not give rise to safety concerns owing to the lack of a selective advantage in the context of the scope of this application. The post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the scope of application EFSA-GMO- NL-2010-80.

In delivering its scientific opinion, the EFSA GMO Panel took into account application EFSA-GMO-NL-2010-80, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. In conclusion, the EFSA GMO Panel is of the opinion that the two-event stack maize NK603 × T25, as described in this application, is as safe as its non-GM comparator and non-GM conventional maize varieties with respect to potential effects on human and animal health and the environment in the context of its scope.

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BACKGROUND

On 21 May 2010, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2010-80, for authorisation of genetically modified (GM) maize NK603 × T25 submitted by Monsanto within the framework of Regulation (EC) No 1829/2003[4] for food and feed uses, import and processing.

After receiving the application EFSA-GMO-NL-2010-80 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed Member States and the European Commission, and made the summary of the application available to the public on the EFSA website.[5] 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 22 September 2010 EFSA received additional information (requested on 2 July 2010). On 12 October 2010, EFSA declared the application valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

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

The EFSA GMO Panel carried out an evaluation of the scientific risk assessment of maize NK603 × T25 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The EFSA GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed (EFSA, 2006), the environmental risk assessment of GM plants (EFSA GMO Panel, 2010) and on the post-market environmental monitoring of GM plants (EFSA GMO Panel, 2011b). Furthermore, the EFSA GMO Panel also took into consideration the scientific comments of Member States, the additional information provided by the applicant and relevant scientific publications.

On 16 December 2013, 8 July 2014, 28 November 2014, 16 February 2015 and 19 March 2015 the EFSA GMO Panel requested additional information from the applicant. The applicant provided the requested information on 3 February 2014, 9 September 2014, 13 January 2015, 13 May 2015, 19 May 2015 and on 27 May 2015.

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

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5).

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific assessment of maize NK603 × T25 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

[4] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.
[5] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2010-00880.
[6] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, p. 1–38.

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 an opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA GMO Panel did not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

ASSESSMENT

1. Introduction

Application EFSA-GMO-NL-2010-80 covers a two-event stack maize produced by conventional crossing. The scope of this application is for food and feed uses, import and processing, but excludes cultivation in the European Union (EU).

European Food Safety Authority (EFSA) guidance applicable to this application establishes that "*Where all single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to a) stability, b) expression of the events and c) potential interactions between the events*" (EFSA, 2006, 2007). Additional information received after May 2011 was assessed in accordance with 2011 guidance (EFSA GMO Panel, 2011a).

Maize NK603 × T25 was developed to confer tolerance to glyphosate (*N*-(phosphonomethyl)glycine)- and glufosinate-ammonium-based herbicides. Tolerance to glyphosate is achieved by expression of the CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS and CP4 EPSPS l214p—a variant of EPSPS with one amino acid substitution of proline for leucine at amino acid position 214). Tolerance to glufosinate-ammonium is achieved by the expression of the phosphinothricin acetyl transferase (PAT) protein.

The two single maize events NK603 and T25 have been previously assessed (see Table 1) on the basis of experimental data. No concerns for human and animal health or environmental safety were identified.

Table 1: Single maize events already assessed by the EFSA Panel on Genetically Modified Organisms (GMO Panel)

Event	Application or mandate	EFSA Scientific Opinion
NK603	CE/ES/00/01	EFSA (2003a)
	Article 4 of the Novel Food Regulation (EC) No 258/97	EFSA (2003b)
	EFSA-GMO-NL-2005-22	EFSA (2009a)
	EFSA-GMO-RX-NK603	
T25	EFSA-GMO-NL-2007-46	EFSA GMO Panel (2013)
	EFSA-GMO-RX-T25	

2. Issues raised by Member States

Issues raised by Member States on maize NK603 × T25 were considered in this scientific opinion and were addressed in detail in Annex G of the EFSA overall opinion.[7]

3. Updated information on single events

Since the publication of the scientific opinions on the single maize events by the EFSA GMO Panel (EFSA, 2003a, b, 2009a; EFSA GMO Panel, 2013), no safety issue pertaining to the two single events has been reported by the applicant.

Updated bioinformatic analyses on the junction regions for events NK603 and T25 confirmed that no known endogenous genes were disrupted by any of the inserts.[8] Updated bioinformatic analyses of the amino acid sequences of the newly expressed proteins and other open reading frames (ORFs) present within the insert and spanning the junction sites revealed no significant similarities to known toxins.[9] An updated search for similarity to allergens was performed using the criterion of 35 % identity of the

[7] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2015-00391
[8] Additional information: 19/5/2015.
[9] Additional information: 19/5/2015.

amino acid sequence of the newly expressed proteins and other ORFs to the amino acid sequence of known allergens in a window of 80 amino acids. Results did not indicate similarities of the newly expressed proteins with known allergens. Identity of over 35 % was found with ragweed (*Ambrosia artemisiifolia*) homologues of the Art v 1 allergen for an ORF within the NK603 insert. The putative translation product of this ORF would be generated from the reverse strand of the CP4 *epsps* transcriptional units. Considering that this ORF is not in the codon frame intended to be expressed, does not have known promoters upstream and in close proximity and does not include an ATG start codon at the N-terminal of the putative translation product, the likelihood that it is both transcribed and translated in maize NK603 × T25 is negligible.

Having assessed the updated information on maize NK603 × T25, the EFSA GMO Panel considers that its previous conclusions on the safety of the single maize events remain valid.

4. Risk assessment of the two-event stack maize NK603 × T25

4.1. Molecular characterisation

Possible interactions between the known biological functions conferred by the individual inserts and interactions that would manifest at protein expression level are considered.

4.1.1. Genetic elements and their biological functions

Maize NK603 and T25 are combined by conventional crossing to produce maize NK603 × T25. The structure of the inserts introduced into maize NK603 × T25 are described in detail in the EFSA GMO Panel scientific opinions, and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 2.

Table 2: Genetic elements in the expression cassettes of the events stacked in maize NK603 × T25

Event	Promoter	5′ Leader	Transit peptide	Coding region	Terminator
NK603	P-Ract1 (*Oryza sativa*)	I-Ract1 (*O. sativa*)	TS-CTP2 (*Arabidopsis thaliana*)	CP4 *epsps* (*Agrobacterium* sp.)	*nos* (*Agrobacterium tumefaciens*)
	P-e35S (CaMV)	I-Hsp70 (*Zea mays*)	TS-CTP2 (*Arabidopsis thaliana*)	CP4 *epsps l214p* (*Agrobacterium* sp.)	*nos* (*Agrobacterium tumefaciens*)
T25[(a)]	35S (CaMV)	–	–	*pat* (*Streptomyces viridochromogenes*)	35S (CaMV)

(a): The insert also contains the following elements: 616 bp of the pUC18 cloning vector including 5 bp of the *bla* gene at the 5′ of the expression cassette; and 1 841 bp of the pUC18 plasmid including a 665-bp 3′ fragment of the *bla* gene and the *ori*, and 346 bp of the 35S promoter at the 3′ end of the expression cassette. The remainder of the *bla* gene (about 25 %) is not present in the insert.

–, no element was specifically introduced to optimise expression.

There are two newly expressed proteins[10] in maize NK603 × T25, both of which are enzymes. Biological functions conferred by these proteins are summarised in Table 3.

[10] CP4 EPSP and PAT including the variant CP4 EPSP l214p.

Table 3: Biological functions of the events stacked in maize NK603 × T25

Event	Protein	Function in donor organism	Function in GM plant
NK603	CP4 EPSPS and CP4 EPSPS L214P	Donor organism: *Agrobacterium* sp. 5-enopyruvyl-shikimate-3-phosphate (EPSPS) synthase is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	CP4 EPSPS L214P is a form of CP4 EPSPS that contains a single amino acid substitution from leucine to proline at position 214. Both CP4 EPSPS proteins confer tolerance to glyphosate (Funke et al., 2006; Garg et al., 2014)
T25	PAT	Donor organism: *Streptomyces viridochromogenes* Phosphinothricin-acetyl-transferase (PAT) enzyme confers resistance to the antibiotic bialaphos (Strauch et al., 1988)	PAT acetylates L-glufosinate-ammonium and thereby confers tolerance to glufosinate-ammonium-based herbicides (Droge-Laser et al., 1994)

4.1.2. Integrity of the events in maize NK603 × T25

The genetic stability of the inserted DNA over multiple generations in the single maize events NK603 and T25 was demonstrated previously (EFSA, 2003a, b, 2009a; EFSA GMO Panel, 2013). Integrity of the events in maize NK603 × T25 was demonstrated by Southern analyses[11] in an F_1 generation representative of commercial seed production.

4.1.3. Information on the expression of the inserts[12]

Plants were grown at five locations (three replicate blocks each) under field conditions in 2008 in the USA. The levels of CP4 EPSPS and PAT proteins in maize NK603 × T25 and the two single events were quantified by enzyme-linked immunosorbent assay (ELISA). Protein levels were determined in leaves and root (growth stages V2–V4), whole plant (V10–V12), pollen, forage root and forage (early dent) and grain (maturity). The plants were treated with the intended herbicides (glyphosate and/or glufosinate-ammonium). Data on grain and forage are reported and discussed below (Table 4). CP4 EPSPS and PAT protein levels in the two-event stack maize were similar to the corresponding levels in the single-event maize plants.

Table 4: Means and standard deviations (upper row) and ranges (lower row) of protein levels (µg/g dry weight) in grain and forage from maize NK603 × T25 and from single maize events NK603 and T25

	Protein	NK603 × T25	NK603	T25
Grain	CP4 EPSPS [(a)]	8.1 ± 1.1 (6.2–10)	7.2 ± 1.4 (5.5–11)	NA
	PAT	0.59 ± 0.18 (0.28–0.83)	NA	0.43 ± 0.10 (0.29–0.68)
Forage	CP4 EPSPS[(a)]	53 ± 17 (25–96)	50 ± 15 (29–85)	NA
	PAT	14 ± 5.6 (6.7–25)	NA	14 ± 9.2 (6.1–39)

(a): The values given represent the sum of CP4 EPSPS and CP4 EPSPS L214P, as the ELISA analytical method recognises both these proteins expressed in NK603 × T25 and NK603.
NA, not applicable.

[11] Dossier: Part I—Section D2.
[12] Dossier: Part I—Section D3.

4.1.4. Conclusion

The molecular data establish that the transformation events stacked in maize NK603 × T25 have the same molecular properties and characteristics as the single transformation events. Comparison of the levels of the CP4 EPSPS and PAT proteins between the stack and the single events did not reveal an interaction that manifests at protein expression level. The molecular characterisation revealed no indications of interactions between the events based on the biological functions of the newly expressed proteins.

4.2. Comparative analysis

4.2.1. Evaluation of relevant scientific data

4.2.1.1. Choice of comparator and production of material for the comparative analysis[13]

Field trials were performed in order to compare phenotypic and agronomic characteristics of maize NK603 × T25, its conventional counterpart (LH283 × PSB3274[14]) and 20 non-genetically modified (GM) maize commercial varieties, and to produce forage and grain material for compositional analyses. The conventional counterpart had a genetic background similar to maize NK603 × T25. The field trials were performed at five sites in North America (one each in Iowa and Kansas and three in Illinois) in 2008, all sites located in the major maize-growing regions of the USA. At each field trial site the maize materials were grown in a randomised complete block design with three replications and included maize NK603 × T25 sprayed with glyphosate and glufosinate-ammonium on top of maintenance pesticides[15] (used for the compositional studies[16]), maize NK603 × T25 not treated with target herbicides on top of maintenance pesticides (used for the agronomic/phenotypic characterisation[17]), and the conventional counterpart and 4 of 20 non-GM maize commercial varieties[18] sprayed with the same maintenance pesticides. The maintenance pesticides were chosen depending on the local requirements. The identities of the maize materials included in the field trials were confirmed using chain-of-custody records and by characterisation of the CP4 *epsps* and *pat* coding regions in their hereditary material by event-specific polymerase chain reaction (PCR) analysis. This analysis identified that one of the non-GM maize commercial varieties at one of the field trial sites in Illinois possibly contained adventitious presence of one of the studied GM events. This maize commercial variety (MG 8122) was omitted from the compositional analysis.

4.2.1.2. Agronomic and phenotypic characteristics

In the analyses of agronomic and phenotypic characteristics of maize NK603 × T25, its conventional counterpart and 20 non-GM maize commercial varieties (all maize materials given maintenance pesticides according to local requirements), 14 endpoints were studied.[19] In addition to these agronomic and phenotypic characteristics, arthropod damage and plant response to abiotic stressors and disease damage were evaluated for their environmental interaction characteristics.

Data on agronomic and phenotypic endpoints were statistically analysed for potential differences between maize NK603 × T25 and its conventional counterpart using two models based on analysis of variance (ANOVA): an across-site ANOVA (all trial sites combined) followed by an individual-site

[13] Dossier: Part I—Sections A3.1–A3.2; additional information: 17/2/2014.
[14] The conventional counterpart was called TXN in some documents of the application.
[15] Additional information: 3/2/2014.
[16] The experimental design for the compositional analysis does not allow distinguishing the effects of the genetic modification from the herbicide treatments.
[17] The experimental design for the agronomic/phenotypic characterisation allows a direct comparison between the four-event stack maize and its conventional counterpart in the presence of maintenance herbicides.
[18] The non-GM maize reference varieties used in these studies were DKC63-78, RC772, DKC62-30, BT 6610, Burrus 645, Crows 5151, N76-H2, 33H25, 33M54, C 5303, MG 8122, NC+ 5411, MG 8403, Stewart S650, Stone M24, C 6501, RX910, 31P41, MG 87801 and FC 7864.
[19] Early stand count, final stand count, seedling vigour, days to 50 % silking, days to 50 % pollen shed, ear height, plant height, stay green, dropped ears, stalk lodging, root lodging, grain moisture, test weight and yield.

analysis. No statistical comparisons were made between maize NK603 × T25 and the set of non-GM maize commercial varieties.

In the across-sites analysis, no significant differences were found between maize NK603 × T25 and the conventional counterpart for any of the 14 agronomic and phenotypic endpoints studied. Six statistically significant differences were observed in the individual site analyses. Four differences occurred at one field site whereas one difference was observed at two sites.

Three abiotic stressors, three diseases and three arthropod pests were evaluated four times during the growing season at each field trial site. These ecological interactions were selected on the basis that they were either actively causing plant injury in the study area or likely to occur in maize during the study period. A difference in susceptibility or tolerance to abiotic stressors, diseases and arthropod pests on a particular observation time was declared significant if the range of injury or severity to maize NK603 × T25 did not overlap with the range of injury or severity to the conventional counterpart across all three replications. There was no difference in response to abiotic stress between maize NK603 × T25 and the conventional counterpart for 49 out of 50 individual site comparisons, and no difference in disease damage and arthropod damage for any of the 65 and 60 comparisons. The only difference observed between maize NK603 × T25 and the conventional counterpart was for hail damage during the first observation at one of the field trials in Illinois, where it was observed (slight) in maize NK603 × T25 and not in the conventional counterpart.

4.2.1.3. Compositional analysis[20]

Maize forage was harvested from field trials in the USA in 2008 and analysed for proximates (crude protein, crude fat, ash and moisture), carbohydrates by calculation, fibre fractions (acid detergent fibre (ADF), neutral detergent fibre (NDF)), calcium and phosphorus. Maize grain harvested from the same field trials was, in addition to proximates and fibre fractions (ADF, NDF and total dietary fibre), analysed for 18 amino acids,[21] 22 fatty acids,[22] nine minerals,[23] seven vitamins[24] and five secondary metabolites and/or anti-nutrients (furfural, *p*-coumaric acid, ferulic acid, phytic acid and raffinose). In total, 69 different compounds were analysed in the grain material and nine in the forage material, in accordance with OECD (2002). Fifteen grain constituents that occurred at levels below the limit of quantification in more than 50 % of the samples were omitted from the statistical analysis.[25]

For each endpoint, the potential differences in level between maize NK603 × T25 (sprayed with target herbicides) and its conventional counterpart (not sprayed with target herbicides) were investigated using two models: an across-site ANOVA (all trial sites combined) followed by an individual-site analysis. When a statistically significant difference was identified, the levels in maize NK603 × T25 and the conventional counterpart were compared with those observed in non-GM maize commercial varieties, obtained both from analytical data on the varieties included in the field trials and from published data in the scientific literature.

[20] Dossier: Part I—Section A3.3.

[21] Alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine.

[22] Caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), γ-linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4) and behenic acid (C22:0).

[23] Calcium, phosphorus, potassium, sodium, iron, copper, magnesium, manganese and zinc.

[24] Thiamine, riboflavin, niacin, pyridoxine, folic acid, β-carotene and vitamin E.

[25] The following constituents were excluded from the statistical analysis: caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), γ-linolenic acid (C18:3), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4) and furfural.

In the across-site analysis, statistically significant differences between maize NK603 × T25 (sprayed with target herbicides) and its conventional counterpart (not sprayed with the target herbicides) were identified for 11 compositional endpoints, two in forage and nine in grain (Table 5).

The two forage endpoints (moisture and crude protein) were well within the ranges of the non-GM commercial maize varieties. All the significant differences in grain compounds (Table 5) were within the range of the non-GM commercial maize varieties included in the study (except for palmitoleic acid and raffinose) or in the range reported in the literature (Watson, 1987; Autran et al., 2003; Herman et al., 2007), and were of small magnitude. Considering the known chemical and biological characteristics of the compounds concerned and the magnitudes of the changes observed, the EFSA GMO Panel did not identify a need to further consider any of these differences.

Table 5: Constituents occurring at different levels in forage and grain of maize NK603 × T25 and LH283 × PSB3274 (conventional counterpart) harvested from field trials in the USA in 2008. As a reference the range in the level of these constituents in non-GM maize commercial varieties grown in the same field trial is given

Constituents (units)	Means across locations (2008 field trials)		
	Maize NK603 × T25 [(a)]	Conventional counterpart (LH283 × PSB3274) [(a)]	Range of non-GM maize variety values
Forage			
Moisture (% of fresh weight)	70.21 ± 0.53	72.97 ± 0.53	67.40–76.30
Crude protein (% dw)	7.37 ± 0.29	7.85 ± 0.29	4.77–8.45
Grain [(a)]			
Ash (% of dw)	1.60 ± 0.05	1.53 ± 0.05	1.14–1.70
Potassium (% dw)	0.36 ± 0.008	0.35 ± 0.008	0.31–0.41
Palmitic acid (C16:0) [(b)]	9.48 ± 0.03	9.34 ± 0.03	9.13–13.42
Palmitoleic acid (C16:1) [(b)]	0.17 ± 0.003	0.16 ± 0.003	0.06–0.15
Oleic acid (C18:1) [(b)]	27.82 ± 0.62	28.24 ± 0.62	22.40–32.75
Linolenic acid (C18:3) [(b)]	0.94 ± 0.01	1.02 ± 0.01	0.85–1.30
β-carotene (mg/kg dw)	1.03 ± 0.04	1.08 ± 0.04	0.72–1.73
Thiamine (mg/kg dw)	3.28 ± 0.05	2.97 ± 0.05	2.76–4.56
Raffinose (% dw)	0.18 ± 0.015	0.20 ± 0.015	0.09–0.17

(a): Least-square mean ± standard error.
(b): Fatty acid proportions are given as percentages of total fatty acid content.
dw, dry weight.

4.2.2. Conclusion

Based on the agronomic and phenotypic characteristics of maize NK603 × T25 under the tested conditions (not treated with the intended herbicide), a difference was observed in maize NK603 × T25 compared with its conventional counterpart. The difference observed for hail damage did not need further assessment for its potential environmental impact.

The EFSA GMO Panel concluded that none of the differences identified in the agronomic and phenotypic characteristics and in the composition of grain and forage obtained from maize NK603 × T25 needed further assessment regarding food and feed safety.

4.3. Food and feed safety assessment

4.3.1. Effect of processing[26]

Maize NK603 × T25 will undergo existing methods of production and processing used for commercial maize. No novel method of production and processing is envisaged.

[26] Dossier: Part I—Section A3.5.

4.3.2. Toxicology

4.3.2.1. Toxicological assessment of newly expressed proteins

Two proteins (CP4 EPSPS[27] and PAT) are newly expressed in maize NK603 × T25. The EFSA GMO Panel has previously assessed these proteins individually in the context of the single events (see Table 1), and no safety concern was identified. The EFSA GMO Panel is not aware of any new information that would change these conclusions.

The two proteins are enzymes that catalyse distinctly different biochemical reactions and act on unrelated substrates. Consequently, the EFSA GMO Panel found no reason to suggest that the presence of the two proteins in combination would result in effects different from those of the individual proteins. As the individual proteins were considered safe for humans and animals (EFSA 2009a; EFSA GMO Panel, 2013), the same conclusion can be extended to the combination.

4.3.2.2. Toxicological assessment of components other than newly expressed proteins

The two-event stack maize did not show any compositional difference from its conventional counterpart that would require further assessment (see Section 4.2). No further food and feed safety assessment of components other than newly expressed proteins is required.

4.3.2.3. Animal studies with the food/feed derived from GM plants

A 42-day feeding study with a total of 800 day-old male and female chickens for fattening (Cobb 500) was provided.[28] The birds were randomly allocated to eight dietary treatments with 100 chickens per treatment (five pens/treatment per sex, initially 12 birds per pen and reduced to 10 birds per pen at day 7). Maize NK603 × T25 (verified by PCR), treated with the intended herbicide,[29] was compared with its conventional counterpart and with six non-GM commercial varieties (NK N64Z, Burrus 645, Golden Harvest, Middlekoop 3210, Asgrow RX715, Garst 8424). The starter and grower/finisher diets contained about 60 % and 64 % maize,[30] respectively. Other main components were soybean meal and corn gluten meal. Before feed formulation, grains of all maize varieties were analysed for proximates, amino acids, minerals and fatty acids, mycotoxins and pesticide residues. The diets were calculated to be isonitrogenous (confirmed by analysis) and isocaloric. The starter diets (about 22 % crude protein (CP), 3 080 kcal metabolisable energy (ME)/kg) were given until day 21, grower/finisher diets (about 21 % CP, 3 100 kcal ME/kg) from day 8 to day 21, and finisher diets (about 20 % CP, 3 135 kcal ME/kg) from day 22 until the end. Feed (starter as crumbles and grower/finisher as pellets) and water were provided for *ad libitum* intake.

Chickens were observed twice daily for clinical signs; deaths were recorded and necropsy performed on all birds found dead. Body weight per pen was measured at the start and the end. Feed intake was determined at day 21 and day 42. At days 43 (males) and 44 (female) all surviving birds were taken for carcass evaluation (dressing percentage, weight of thighs, breast, wings, drums, abdominal fat and whole liver). Data were statistically analysed by a two-factor ANOVA (diet and sex), and pair-wise comparison was made by Fischer's Least Significant Difference test. A mixed linear model was applied to compare the maize NK603 × T25 group with the mean of all non-GM varieties.

Overall mortality was low (< 3%) with no significant differences between the groups. No significant treatment × sex interaction was detected for performance characteristics. Overall, no significant difference was seen in final body weight (about 2.5 kg), feed intake (about 4.0 kg), or feed to gain ratio (about 1.61) between the maize NK603 × T25, the conventional counterpart or the non-GM commercial varieties. No significant differences were observed in carcass parameters (except that the

[27] Including its variant CP4 EPSP l214p.
[28] Dossier: Part I— CQR-09-010 (2010 & 2010b); Additional information: 12/6/2012.
[29] Addition information: 13/5/2015.
[30] Maize materials are derived from field trial 2008.

fat content of the pad was lower for maize NK603 × T25 than for the conventional counterpart, but essentially similar to all non-GM commercial varieties).

The study did not show unintended effects of maize NK603 × T25 at the inclusion level of 60 % in complete feed. The Panel concluded that maize NK603 × T25 is as nutritious as the conventional counterpart and six non-GM commercial varieties.

4.3.3. Allergenicity

For allergenicity assessment, a weight-of-evidence approach is followed, taking into account all of the information obtained on the newly expressed proteins, as no single piece of information or experimental method yields evidence to predict allergenicity (EFSA, 2006; Codex Alimentarius, 2009). In addition, when known functional aspects of the newly expressed protein or structural similarity to known adjuvants may indicate an adjuvant activity, the possible role of these proteins as adjuvants is considered. When newly expressed proteins with a potential adjuvant activity are expressed together, possible interactions increasing adjuvanticity and impacting the allergenicity of the GM crop are assessed.

4.3.3.1. Assessment of allergenicity of the newly expressed proteins[31]

For allergenicity, the EFSA GMO Panel previously evaluated the safety of the CP4 EPSPS and PAT proteins, and no concerns about allergenicity were identified in the context of the applications assessed (see Table 1). No new information on allergenicity of the single events that might change the previous conclusions of the EFSA GMO Panel has become available. Based on current knowledge, and as none of the newly expressed proteins showed allergenicity, no reasons for concern regarding the mixture of these newly expressed proteins in this two-event stack maize in terms of allergenicity were identified.

As regards adjuvanticity, no information available on the structure or function of the newly expressed CP4 EPSPS and PAT proteins would suggest an adjuvant effect of the individual proteins or their mixture in maize NK603 × T25 resulting in or increasing an eventual immunoglobulin E response to a bystander protein.

4.3.3.2. Assessment of allergenicity of the whole GM plant[32]

To date, maize has not been considered to be a common allergenic food[33] (OECD, 2002), and therefore the EFSA GMO Panel did not request experimental data to analyse the allergen repertoire of GM maize. The EFSA GMO Panel regularly reviews the available publications on food allergy to maize (e.g. EFSA GMO Panel, 2013).

In the context of the present application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins, the EFSA GMO Panel identified no indications of safety concerns regarding the overall allergenicity of maize NK603 × T25.

4.3.4. Nutritional assessment of GM food/feed

The intended trait of maize NK603 × T25 is herbicide tolerance, with no intention of altering the nutritional parameters. Comparison of maize NK603 × T25 composition with that of its conventional counterpart did not identify differences that would require a safety assessment (see Section 4.2). From these data, the nutritional characteristics of maize NK603 × T25-derived food and feed are not expected to differ from those of conventional maize varieties. This was confirmed by the results of a feeding study in chickens for fattening (see Section 4.3.2.3).

[31] Dossier: Part I—Section D7.9.1.
[32] Dossier: Part I—Section D7.9.2.
[33] Directive 2007/68/EC of the European Parliament and of the Council of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ L 310, 27.11.2007, p. 11–14.

4.3.5. Post-market monitoring of GM food/feed

The EFSA GMO Panel considers that post-market monitoring of GM food/feed is not necessary, given the absence of safety concerns identified for maize NK603 × T25.

4.3.6. Conclusion

The safety assessment identified no concerns regarding the potential toxicity of the newly expressed proteins CP4 EPSPS and PAT in maize NK603 × T25. The EFSA GMO Panel found no reason to suggest that the presence of the two proteins in combination would result in interactions producing effects different from those of the individual proteins. Similarly, no indications of safety concerns were identified regarding allergenicity of the individual newly expressed proteins or their mixture in maize NK603 × T25, or regarding potential changes in its overall allergenicity. Maize NK603 × T25 is as nutritious as non-GM conventional maize varieties.

4.4. Environmental risk assessment and monitoring plan

4.4.1. Evaluation of relevant scientific data

The scope of the application EFSA-GMO-NL-2010-80 is for food and feed uses, import and processing of maize NK603 × T25 expressing the CP4 EPSPS and PAT proteins for, respectively, glyphosate and glufosinate-ammonium herbicide resistance. The scope does not include cultivation and, therefore, the environmental risk assessment (ERA) of maize NK603 × T25 is concerned with (1) the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and those present in environments exposed to faecal material (manure and faeces) and (2) the accidental release into the environment of viable seeds of maize NK603 × T25 during transport and/or processing.

4.4.2. Environmental risk assessment

4.4.2.1. Potential unintended effects on plant fitness due to the genetic modification[34]

Fourteen agronomic and phenotypic characteristics as well as three abiotic stressors, three diseases and three arthropod pests were assessed on maize NK603 × T25 from field trials conducted in maize-growing areas in North America (five locations: one each in Iowa and Kansas and three in Illinois) during the 2008 growing season, in a randomised complete block design with three replications (for more details, see Section 4.2.1). The results show that the agronomic performance and phenotypic characteristics of maize NK603 × T25 are similar to those of the conventional counterpart in the across-sites analyses. Six statistically significant differences were observed in the individual site analyses. Four differences occurred at one field site, whereas one difference was observed at two sites. The EFSA GMO Panel considers it likely that these small and inconsistent differences were incidental. There were no differences in response to abiotic stress between maize NK603 × T25 and the conventional counterpart for 49 out of 50 comparisons, and no difference in disease and arthropod damage for any of the 65 and 60 comparisons. Hail damage was recorded as "slight" (i.e. symptoms not damaging to plant development) in maize NK603 × T25, whereas there was "none" in the conventional counterpart in the first observation at one of the field trials in Illinois and this slight hail damage was within the range observed in the reference varieties.

Based on the inserted traits, the EFSA GMO Panel considers that agronomic and phenotypic characteristics are unchanged in maize NK603 × T25. The EFSA GMO Panel concludes that there is very little likelihood that maize NK603 × T25 has any tendency towards increased persistence and invasiveness following accidental release into the environment of viable GM maize grains, as the presence of the intended herbicides would confer only a short-term selective advantage with no relevance to the development of longer term populations.

[34] Dossier: Part I—Sections D9.1 and D9.2.

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; furthermore, they have lost their ability to release grain 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 (e.g. mild winters). Observations made on cobs, cob fragments or isolated grain shed in the field during harvesting indicate that grain may survive and overwinter in some regions, resulting in volunteers in subsequent crops. The occurrence of maize volunteers has been 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).

Therefore, considering the scope of maize NK603 × T25, the outcomes of the molecular characterisation and the comparative analysis, and the poor ability of maize to survive outside cultivated land, the EFSA GMO Panel concludes that there are no indications that maize NK603 × T25 has increased fitness potential compared with its conventional counterpart.

4.4.2.2. Potential for gene transfer

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

(a) Plant-to-bacteria gene transfer[35]

Genomic plant DNA is a component of several food and feed products derived from maize. It is well documented that DNA present in food and feed becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to microorganisms, especially bacteria, in the digestive tract of humans, domesticated animals and other environments exposed to the GM plant or plant material is expected.

Current scientific knowledge of recombination processes in bacteria suggests that horizontal gene transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as from plants to bacteria) is not expected to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009b).

A successful horizontal gene transfer would require stable insertion of the recombinant DNA sequences into a bacterial genome and a selective advantage to be conferred on the transformed host. The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is homologous recombination. In the case of sequence identity between the transgenic DNA and the natural variants of the gene in bacteria, recombination could result in a gene replacement in bacteria. In the case of two pairs of sequences with sufficient length of identity and correct orientation, recombination could facilitate the transfer of insert sequences to bacterial recipients by double homologous recombination.

Maize NK603 × T25 contains several genetic elements of bacterial origin. These are (1) the coding sequence of the CP4 *epsps* gene from *Agrobacterium* sp. CP4; (2) the coding sequence of the *pat* gene from *Streptomyces viridochromogenes*; (3) two nopaline synthase (*nos*)-terminator sequences, each with a length of 300 bp, from the Ti plasmid of *A. tumefaciens*; (4) a sequence of 665 bp of the 3′ prime end of the *β-lactamase* gene as is present on plasmids of *Escherichia coli*; and (5) two sequences of 611 bp and 1 176 bp from the pUC cloning vector used in *E. coli*, the latter sequence including the origin of replication (*ori*). Bioinformatic analyses confirmed, except for the *pat* gene, high sequence identities between the above-mentioned sequences and the origin from which they were derived. Owing to codon optimisation, the *pat* gene showed insufficient sequence identity with bacterial sequences to facilitate homologous recombination.

[35] Dossier: Part I—Section D6a.

Whereas *E. coli* is considered to be prevalent in the main receiving environment, i.e. the gastrointestinal tract of humans or animals, *Agrobacterium* species, including *A. tumefaciens*, or its close relatives from the genus *Rhizobium*, are not expected to be prevalent in the gastrointestinal tract. However, occurrence of the recombinant genes outside the immediate receiving environment (through faecal material), in habitats where *E. coli* would be less prevalent but where *A. tumefaciens* could be more abundant, cannot be ruled out (Hart et al., 2009) and is therefore also taken into account for assessing the risks associated with a horizontal gene transfer.

On a theoretical basis (i.e. without any study providing experimental evidence for the occurrence of horizontal gene transfer in the case of GM food and feed derived from maize NK603 × T25 or any other GM plant), it can be assumed that, as an extremely rare event, homologous recombination may occur in the environment between nucleotide sequences of the recombinant CP4 *epsps* gene and their natural variants, as they may occur in *A. tumefaciens* CP4 or other strains.

The *nos*-terminator sequences present in maize NK603 × T25 may facilitate double homologous recombination with the corresponding *nos* gene on Ti plasmids of environmental *A. tumefaciens* strains. Theoretically, such recombination could result in the acquisition of the CP4 *epsps* gene on natural Ti plasmids. Likewise, the plant codon-optimised *pat* gene could be transferred by double homologous recombination into pUC or pUC-related plasmids as they may occur in *E. coli* or other bacteria. Owing to the presence of an *ori* within the recombinant gene cassette, an independent plasmid could theoretically also be formed in receiving bacteria and have the capacity for autonomous replication in bacteria that recognise the *ori*.

In addition to homology-based recombination processes, illegitimate recombination that does not require the presence of DNA similarity between the recombining DNA molecules is theoretically possible. However, the transformation rates for illegitimate recombination were considered to be 10^{10}-fold lower than those for homologous recombination (Hülter and Wackernagel, 2008; EFSA, 2009b). Illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM plant DNA (EFSA, 2009b). In comparison to the above-described homology-facilitated recombination processes, the contribution of illegitimate recombination is extremely low.

The following potential environmental implications are considered:

1) Substitutive recombination between the CP4 *epsps* gene with natural variants, as they may occur in habitats receiving DNA of maize NK603 × T25, would only replace natural variants (substitutive recombination) and are therefore unlikely to provide any new property connected to a selective advantage for the recipient organisms (EFSA, 2009b).
2) Double homologous recombination with terminator sequences of the *nos* gene would result in an insertion of the CP4 *epsps* gene into Ti plasmids and as a consequence confer resistance to glyphosate. CP4 EPSPS is already present in habitats receiving DNA of maize NK603 × T25 and introduction of the CP4 *epsps* gene into *A. tumefaciens* would be unlikely to provide a selective advantage for the recipient organism.
3) The *pat* gene could be transferred from DNA of NK603 × T25 by double homologous recombination onto pUC plasmids or other plasmids with corresponding sites of sequence identity. Alternatively, the *pat* gene could be transferred to bacterial strains with a capacity to recognise the *ori* within the recombinant gene cassette so that an independent replicating plasmid could be formed. Owing to the codon optimisation for expression in plant cells, it is, however, not expected that the *pat* gene would be as efficiently expressed as natural variants of similar genes occurring in bacteria. Even in the case of functionality and considering that *pat* genes originate from bacteria, e.g. *S. viridochromogenes* and other *Actinobacteria*, a transfer of the *pat* gene from NK603 × T25 would not confer a new trait to environmental bacterial communities.

Considering the intended uses (which exclude cultivation) of maize NK603 × T25, the EFSA GMO Panel concluded that the unlikely but theoretically possible horizontal gene transfer of recombinant genes from maize NK603 × T25 to bacteria does not give rise to any environmental safety concern.

(b) Plant-to-plant gene transfer[36]

Considering the scope of maize NK603 × T25 and the physical characteristics of maize grain, possible pathways of gene dispersal are (1) grain spillage during transport, and (2) processing and the dispersal of pollen from occasional feral GM maize plants originating from accidental grain spillage.

The extent of cross-pollination to other maize varieties will mainly depend on the scale of accidental release during transport and processing, and on successful establishment and subsequent flowering of the 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 during transport 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 neighbouring plants only at low levels (Palaudelmàs et al., 2009).

Although GM maize plants outside cropped areas have been reported in Korea as a result of grain spillage during transport 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, the absence of a dormancy phase, and susceptibility to plant pathogens, herbivores and frost. 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, even when treated with the intended herbicides.

The EFSA GMO Panel takes into account the fact that this application does not include cultivation of maize NK603 × T25 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. In conclusion, considering the scope of maize NK603 × T25, the mode of action of the introduced traits, the outcomes of the molecular characterisation and of the comparative analysis, and the poor ability of maize to survive outside cultivated land, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this GM maize in Europe will not differ from that of conventional maize varieties, even in the event of treatment with the intended herbicides.

4.4.2.3. Potential interactions of the GM plant with target organisms[37]

Interactions of maize NK603 × T25 with target organisms are not considered an issue by the EFSA GMO Panel, as there are no target organisms.

4.4.2.4. Potential interactions of the GM plant with non-target organisms[38]

Owing to the scope of maize NK603 × T25, which excludes cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered an issue by the EFSA GMO Panel.

[36] Dossier: Part I—Section D6b.
[37] Dossier: Part I—Section D9.4.
[38] Dossier: Part I—Section D9.5.

4.4.2.5. Potential interactions with the abiotic environment and biogeochemical cycles[39]

Considering the scope of maize NK603 × T25, which excludes cultivation, and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered a relevant issue by the EFSA GMO Panel.

4.4.3. Post-market environmental monitoring[40]

The objectives of a post-market environmental monitoring (PMEM) 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 ERA are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the ERA.

Monitoring is also related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of the EFSA GMO Panel. However, the EFSA GMO Panel gives its opinion on the scientific quality of the PMEM plan provided by the applicants (EFSA GMO Panel, 2011b).

The potential exposure to the environment of maize NK603 × T25 would be through ingestion by animals and their faecal material leading to exposure of the gastrointestinal tract and soil microbial populations to recombinant DNA, and through accidental release into the environment of viable NK603 × T25 seeds during transport and/or processing. As the ERA does not cover cultivation and no potential adverse effects have been identified, no case-specific monitoring is required.

The PMEM plan proposed by the applicant includes (1) the description of an approach involving operators (federations involved in maize import and processing) reporting to the applicant, 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 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 applicant proposes to submit a PMEM report on an annual basis.

The EFSA GMO Panel considers that the scope of the PMEM plan provided by the applicant is in line with the scope of maize NK603 × T25, as the ERA does not cover cultivation and no potential adverse effects have been identified. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plans.

4.4.4. Conclusion

Considering the scope of maize NK603 × T25, the outcomes of the molecular characterisation and of the comparative analysis, and the poor ability of maize to survive outside cultivated land, the EFSA GMO Panel concludes that there are no indications that maize NK603 × T25 has an increased fitness potential compared with its conventional counterpart. Risks associated with an unlikely but theoretically possible horizontal gene transfer of recombinant DNA from maize NK603×T25 to bacteria have not been identified. Considering the scope of the GM maize, interactions with the biotic and abiotic environment are not considered to be a relevant issue. Therefore, the EFSA GMO Panel concludes that no safety concerns are expected in the event of the accidental release of viable GM maize grains into the environment.

CONCLUSIONS AND RECOMMENDATIONS

No new data on the single maize events NK603 and T25 that would lead to a modification of the original conclusions on their safety were identified.

[39] Dossier: Part I—Section D10.
[40] Dossier: Part I—Section D11.

The combination of maize single events NK603 and T25 in the two-event stack maize NK603 × T25 did not give rise to issues—relating to molecular, agronomic, phenotypic or compositional characteristics—regarding food and feed safety. The EFSA GMO Panel considers that there is no reason to expect interactions that could impact on the food and feed safety and nutritional properties. The compositional data indicate that maize NK603 × T25 would be expected to deliver the same nutrition as its non-GM comparator.

Considering the scope of application EFSA-GMO-NL-2010-80, there are no indications of an increased likelihood of establishment and spread of feral maize NK603 × T25 plants in the case of accidental release into the environment of viable GM maize seeds. Potential interactions of maize NK603 × T25 with the biotic and abiotic environment were not considered a relevant issue by the EFSA GMO Panel. The unlikely but theoretically possible transfer of the recombinant genes from maize NK603 × T25 to environmental bacteria does not give rise to a safety concern owing to the lack of a selective advantage in the context of the scope of this application. The post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the scope of application EFSA-GMO-NL-2010-80.

In conclusion, the EFSA GMO Panel considers that the information available for maize NK603 × T25 addresses the scientific comments raised by Member States and that maize NK603 × T25, as described in this application, is as safe as its non-GM comparator and non-GM conventional maize varieties with respect to potential effects on human and animal health and the environment in the context of its scope.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from Competent Authority of the Netherlands received on 21 May 2010 concerning a request for authorisation for the placing on the market of maize NK603 × T25 (application EFSA-GMO-NL-2010-80) submitted in accordance with Regulation (EC) No 1829/2003 by Monsanto Europe S.A./N.V.

2. Acknowledgement letter dated 4 June 2010 from EFSA to the Competent Authority of the Netherlands.

3. Letter from EFSA to applicant dated 2 July 2010 requesting additional information under completeness check.

4. Letter from applicant to EFSA received on 1 September 2010 providing additional information under completeness check.

5. Letter from applicant to EFSA received on 22 September 2010 providing additional information under completeness check.

6. Letter from EFSA to applicant dated 12 October 2010 delivering the ‘Statement of Validity’ of application EFSA-GMO-NL-2010-80 (maize NK603 × T25) submitted by Monsanto Europe S.A./N.V under Regulation (EC) No 1829/2003.

7. Letter from EFSA to applicant dated 16 December 2010 stopping the clock due to single event.

8. Letter EFSA to applicant dated 23 September 2013 re-starting the clock due to single event.

9. Letter from EFSA to applicant dated 16 December 2013 requesting additional information and stopping the clock.

10. Letter from applicant to EFSA received on 3 February 2014 providing additional information.

11. Letter from EFSA to applicant dated 8 July 2014 requesting additional information and maintaining the clock stopped.

12. Letter from applicant to EFSA received on 9 September 2014 providing additional information.

13. Letter from EFSA to applicant dated 28 November 2014 requesting additional information and maintaining the clock stopped.

14. Letter from applicant to EFSA received on 13 January 2015 providing additional information.

15. Letter from EFSA to applicant dated 16 February 2015 requesting additional information and maintaining the clock stopped.

16. Letter from EFSA to applicant dated 19 March 2015 requesting additional information and maintaining the clock stopped.

17. Letter from applicant to EFSA received on 13 May 2015 providing additional information.

18. Letter from applicant to EFSA received on 19 May 2015 providing additional information.

19. Letter from applicant to EFSA received on 27 May 2015 providing complementary information to the additional information submitted.

20. Letter from EFSA to applicant dated 23 June 2015 re-starting the clock.

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