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Scientific Opinion on application EFSA-GMO-BE-2013-117 for authorisation of genetically modified maize MON 87427 x MON 89034 x NK603 and subcombinations independently of their origin, for food and feed uses, import and processing submitted under Regulation (EC) No 1829/2003 by Monsanto Company

Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda de Schrijver, Mikolaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, et al.

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Scientific Opinion on application EFSA-GMO-BE-2013-117 for authorisation of genetically modified maize MON 87427 × MON 89034 × NK603 and subcombinations independently of their origin, for food and feed uses, import and processing submitted under Regulation (EC) No 1829/2003 by Monsanto Company

EFSA Panel on Genetically Modified Organisms (GMO),
Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikołaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli, Jean-Michel Wal, Andrea Gennaro, Franco Maria Neri and Konstantinos Paraskevopoulos

Abstract

In this opinion, the EFSA Panel on Genetically Modified Organisms (GMO Panel) assessed the three-event stack maize MON 87427 × MON 89034 × NK603 and its three subcombinations, independently of their origin. The GMO Panel has previously assessed the three single events combined to produce this three-event stack maize and did not identify safety concerns. No new data on the single events, leading to modification of the original conclusions on their safety, were identified. Based on the molecular, agronomic, phenotypic and compositional characteristics, the combination of the single maize events and of the newly expressed proteins in the three-event stack maize did not give rise to issues regarding food and feed safety or nutrition. In the case of accidental release of viable grains of maize MON 87427 × MON 89034 × NK603 into the environment, the three-event stack maize would not raise environmental safety concerns. The GMO Panel concludes that the three-event stack maize is as safe and as nutritious as the non-GM comparator and the tested non-GM reference varieties in the context of its scope. The GMO Panel considered that its previous conclusions on the two-event stack maize MON 89034 × NK603 remain valid. For the two maize subcombinations for which no experimental data were provided the GMO Panel assessed the likelihood of interactions among the single events, and concluded that their combination would not raise safety concerns. These two subcombinations are therefore expected to be as safe as the single events, the previously assessed maize MON 89034 × NK603 and maize MON 87427 × MON 89034 × NK603. Since the post-market environmental monitoring plan for the three-event stack maize does not include any provisions for the two subcombinations not previously assessed, the GMO Panel recommended the applicant to revise the plan accordingly.

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Keywords: GMO, maize, MON 87427 × MON 89034 × NK603, herbicide tolerance, insect resistance, CP4 EPSPS, Cry1A.105, Cry2Ab2, Regulation (EC) No 1829/2003

Requestor: Competent Authority of Belgium

Question number: EFSA-Q-2013-00765

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Summary

Following the submission of application EFSA-GMO-BE-2013-117 under Regulation (EC) No 1829/2003 from Monsanto Company (referred to hereafter as the applicant), the Panel on Genetically Modified Organisms of the European Food Safety Authority(EFSA) (referred to hereafter as GMO Panel) was asked to deliver a Scientific Opinion on the safety of genetically modified glyphosate tolerant and insect resistant maize MON 87427 × MON 89034 × NK603 (referred to hereafter as 'three-event stack maize') and its subcombinations independently of their origin (referred to hereafter as 'subcombinations') according to the Commission Implementing Regulation (EU) No 503/2013. The scope of application EFSA-GMO-BE-2013-117 is for the placing on the market of maize MON 87427 × MON 89034 × NK603 and subcombinations MON 87427 × MON 89034, MON 87427 × NK603 and MON 89034 × NK603 independently of their origin for food and feed uses, import and processing.

The term 'subcombination' refers to any combination of two of the events present in the three-event stack maize. The safety of subcombinations occurring as segregating progeny in the harvested grains of maize MON 87427 × MON 89034 × NK603 is evaluated in the context of the assessment of the three-event stack maize in Section 3.3 of the present GMO Panel Scientific Opinion. The safety of subcombinations that have either been, or could be produced by conventional crossing through targeted breeding approaches, and which can be bred, produced and marketed independently of the three-event stack maize, are risk assessed in Section 3.4 of the present GMO Panel Scientific Opinion.

In delivering its Scientific Opinion, the GMO Panel considered the data available on the single events, the three-event stack maize and a subcombination, the scientific comments submitted by the Member States and the relevant scientific literature. The three-event stack maize was produced by conventional crossing to combine three single maize events: MON 87427, expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein for tolerance to glyphosate-containing herbicides; MON 89034, expressing the Cry1A.105 and Cry2Ab2 proteins which confer resistance to specific lepidopteran pests; and NK603, expressing the CP4 EPSPS protein and its variant CP4 EPSPS L214P for tolerance to glyphosate-containing herbicides.

The GMO Panel evaluated the three-event stack maize and its subcombinations with reference to the scope and appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed, the environmental risk assessment of GM plants and the post-market environmental monitoring of GM plants. The GMO Panel Guidance Documents establish the principle that where all single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to (a) stability of the inserts, (b) expression of the introduced genes and their products and (c) potential synergistic or antagonistic effects resulting from the combination of the events (EFSA GMO Panel, 2011a).

For application EFSA-GMO-BE-2013-117, the previous assessments of the three single maize events (MON 87427, MON 89034 and NK603) and of the two-event stack maize MON 89034 × NK603 provided a basis to evaluate the three-event stack maize and its subcombinations. Maize MON 87427, MON 89034, NK603 and MON 89034 × NK603 were previously assessed by the GMO Panel and no concerns on their safety were identified. No safety issue concerning the three single maize events was identified by the updated bioinformatic analyses, nor reported by the applicant since the publication of the previous GMO Panel Scientific Opinions. Therefore, the GMO Panel considers that its previous conclusions on the safety of the single maize events remain valid.

For the three-event stack maize, the risk assessment included the molecular characterisation of the inserted DNA and analysis of protein expression. An evaluation of the comparative analyses of agronomic, phenotypic and compositional characteristics was undertaken, and the safety of the newly expressed proteins and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional characteristics. An evaluation of environmental impacts and post-market environmental monitoring plans was also undertaken.

The molecular data establish that the events stacked in maize MON 87427 × MON 89034 × NK603 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the three-event stack maize and in the single events or, in the case of CP4 EPSPS, show expected changes resulting from the combination of the MON 87427 and NK603 single events, both producing the CP4 EPSPS protein. No indications of interactions that may affect the integrity of the events and the levels of the newly expressed proteins in the three-event stack maize were identified.

No relevant differences requiring further assessment for food/feed safety or environmental impact were identified between maize MON 87427 × MON 89034 × NK603 and the non-GM comparator in grain and forage composition and in agronomic and phenotypic characteristics.

Based on the molecular, agronomic, phenotypic and compositional characteristics, the combination of maize events MON 87427, MON 89034 and NK603 in the three-event stack maize did not give rise to issues regarding food and feed safety and nutrition. The combination of the newly expressed proteins in the three-event stack maize did not raise concerns for human and animal health.

Considering the combined events, the outcome of the comparative analysis, the routes of exposure and limited exposure levels, the GMO Panel concludes that the three-event stack maize would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment, irrespective of possible interactions between the individual events within the three-event stack maize.

The GMO Panel concludes that the three-event stack maize is as safe and as nutritious as the non-GM comparator and the tested non-GM reference varieties in the context of the scope of this application.

Since no new safety concerns were identified for the previously assessed two-event stack maize MON 89034 × NK603 and no new data leading to modification of the original conclusions on safety were identified, the GMO Panel considers that its previous conclusions on this subcombination remain valid. For the two subcombinations MON 87427 × MON 89034 and MON 87427 × NK603, for which no experimental data were provided, the GMO Panel assessed the possibility of interactions between the events, and concluded that different combinations of the events MON 87427, MON 89034 and NK603 would not raise safety concerns. These two subcombinations are therefore expected to be as safe as the single maize events, the previously assessed two-event stack maize and the three-event stack maize MON 87427 × MON 89034 × NK603.

Given that no safety concerns were identified for food and feed derived from maize MON 87427 × MON 89034 × NK603 and its subcombinations MON 89034 × NK603, MON 87427 × MON 89034 and MON 87427 × NK603, the GMO Panel considers that post-market monitoring of these products is not necessary. However, the post-market environmental monitoring (PMEM) plan submitted by the applicant for the three-event stack maize does not include any provisions for the two subcombinations that were not previously assessed. Therefore, the GMO Panel recommends the applicant to revise the plan accordingly.

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1. Introduction

1.1. Background

On 13 September 2013, the European Food Safety Authority (EFSA) received from the Competent Authority of Belgium application EFSA-GMO-BE-2013-117, for authorisation of genetically modified (GM) insect resistant and glyphosate tolerant maize MON 87427 × MON 89034 × NK603 (referred to hereafter as 'three-event stack maize'), submitted by Monsanto Europe S.A./N.V. (referred to hereafter as the applicant) within the framework of Regulation (EC) No 1829/2003[1], for food and feed uses, import and processing. The risk assessment of application EFSA-GMO-BE-2013-117 presented here is for the placing on the market of the three-event stack maize and its subcombinations,[2] independently of their origin, for food and feed uses, import and processing.

After receiving application EFSA-GMO-BE-2013-117 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.[3] 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. EFSA requested additional information under completeness check on 25 October 2013 and 13 December 2013 and received it on 22 November 2013 and 20 December 2013, respectively. On 22 January 2014, EFSA declared the application valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003. The clock of the application was stopped from 28 January 2014 to 27 May 2015 due to the pending assessment of the single-event maize MON 87427 (application reference EFSA-GMO-BE-2012-110).

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[4] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had 3 months after the date of receipt of the valid application (until 11 September 2015[5]) to make their opinion known.

The GMO Panel carried out the scientific risk assessment of the three-event stack maize and subcombinations MON 87427 × MON 89034, MON 87427 × NK603 and MON 89034 × NK603 (referred to as 'subcombinations independently of their origin' according to the Commission Implementing Regulation (EU) No 503/2013[6]). The GMO Panel requested additional information from the applicant on 16 June 2015, 15 September 2015, 15 December 2015, 9 November 2016 and 6 April 2017. The applicant provided the requested information on 1 September 2015, 1 October 2015, 17 June 2016, 12 December 2016 and 6 June 2017, respectively. The applicant provided additional information spontaneously on 13 December 2016.

In the frame of contracts OC/EFSA/UNIT/GMO/2013/01 and OC/EFSA/UNIT/GMO/2014/01, the contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing bioinformatic analyses and statistical analyses, respectively.

In giving its Scientific Opinion to the European Commission, 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 6 months from the acknowledgement of the valid application. As additional information was requested by the GMO Panel, the time limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, 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).

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[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities, L 268, 1–23.

[2] The subcombinations are MON 87427 × MON 89034, MON 87427 × NK603 and MON 89034 × NK603.

[3] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2013-00765

[4] 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.

[5] The Member States' commenting period of application EFSA-GMO-BE-2013-117 was suspended until the clock of the application was re-started following the adoption of the Scientific Opinion of application EFSA-GMO-BE-2012-110 (authorisation of GM maize MON 87427).

[6] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L 157, 8.6.2013, p. 1–48.

1.2. Terms of Reference as provided by the requestor

The GMO Panel was asked to carry out a scientific assessment of 'maize MON 87427 × MON 89034 × NK603 and all subcombinations of the individual events independently of their origin (as present in the segregating progeny as well as independent stacks to be placed on the market as such)', for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The risk assessment of application EFSA-GMO-BE-2013-117 presented here is for the placing on the market of glyphosate tolerant and insect resistant maize MON 87427 × MON 89034 × NK603 and subcombinations, independently of their origin, for food and feed uses, import and processing.

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 GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the 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.

2. Data and methodologies

2.1. Data

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-BE-2013-117, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2.2. Methodologies

The GMO Panel carried out a scientific risk assessment of maize MON 87427 × MON 89034 × NK603 and its subcombinations MON 87427 × MON 89034, MON 87427 × NK603 and MON 89034 × NK603, independently of their origin (Table 1), for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The 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 GMO Panel, 2011a), for the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010a) and for the post-market environmental monitoring (PMEM) of GM plants (EFSA GMO Panel, 2011b).

The comments raised by Member States are addressed in Annex G of EFSA's overall opinion[3] and were taken into consideration during the scientific risk assessment.

3. Assessment

3.1. Introduction

Application EFSA-GMO-BE-2013-117 covers the three-event stack maize MON 87427 × MON 89034 × NK603 and its subcombinations MON 87427 × MON 89034, MON 87427 × NK603 and MON 89034 × NK603 independently of their origin (Table 1). The scope of this application is for food and feed uses, import and processing, and excludes cultivation within the European Union (EU).

The term 'subcombination' refers to any combination of two of the events present in the three-event stack maize.

The safety of subcombinations occurring as segregating progeny in the harvested grains of maize MON 87427 × MON 89034 × NK603 is evaluated in the context of the assessment of the three-event stack maize in Section 3.3 of the present GMO Panel Scientific Opinion.

'Subcombination' also covers combinations of two of the three events MON 87427, MON 89034 or NK603 that have either been or could be produced by conventional crossing through targeted breeding approaches (EFSA GMO Panel, 2011a). These are maize stacks that can be bred,

produced and marketed independently of the three-event stack maize. These stacks are risk assessed in Section 3.4 of this GMO Panel Scientific Opinion.

The three-event stack maize was produced by conventional crossing to combine three single maize events: MON 87427 (expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein), MON 89034 (expressing the Cry1A.105 and Cry2Ab2 proteins) and NK603 (expressing the CP4 EPSPS protein and the variant CP4 EPSPS L214P).

Table 1: Two- and three-event maize stacks covered by the scope of application EFSA-GMO-BE-2013-117

Degree of stacking	Events	Unique identifiers
Three-event stack maize	MON 87427 × MON 89034 × NK603	MON–87427–7 × MON–89Ø34–3 × MON–ØØ6Ø3–6
Two-event stack maize	MON 89034 × NK603	MON–89Ø34–3 × MON–ØØ6Ø3–6
	MON 87427 × MON 89034	MON–87427–7 × MON–89Ø34–3
	MON 87427 × NK603	MON–87427–7 × MON–ØØ6Ø3–6

Herbicidal tolerance traits are achieved by the expression of CP4 EPSPS protein and its variant CP4 EPSPS L214P from *Agrobacterium* sp. strain CP4. Insect resistant traits are achieved by the expression of the Cry1A.105 and Cry2Ab2 proteins from *Bacillus thuringiensis* subsp. *kurstaki* and subsp. *aizawai*, which confer protection against specific lepidopteran pests (e.g. *Ostrinia nubilalis* (European corn borer)).

The single maize events MON 87427, MON 89034, NK603 and the two-event stack maize MON 89034 × NK603 have been previously assessed by the GMO Panel (Table 2), and no safety concerns were identified.

Table 2: Single maize events and two-event stack maize previously assessed by the GMO Panel

Event	Application or mandate	EFSA Scientific Opinion
MON 87427	EFSA-GMO-BE-2012-110	EFSA GMO Panel (2015)
MON 89034	EFSA-GMO-NL-2007-37	EFSA (2008)
MON 89034 × NK603	EFSA-GMO-NL-2007-38	EFSA GMO Panel (2009)
NK603	CE/ES/00/01 EFSA-GMO-NL-2005-22 EFSA-GMO-RX-NK603	EFSA (2004), EFSA (2007) EFSA (2009) EFSA (2009)

EFSA guidance establishes the principle that 'For GM plants containing a combination of transformation events (stacked events) the primary concern for risk assessment is to establish that the combination of events is stable and that no interactions between the stacked events, that may raise safety concerns compared to the single events, occur. The risk assessment of GM plants containing stacked events focuses on issues related to: (a) stability of the inserts, (b) expression of the introduced genes and their products and (c) potential synergistic or antagonistic effects resulting from the combination of the events' (EFSA GMO Panel, 2011a).

3.2. Updated information on the events

Since the publication of the scientific opinions on the single maize events by the GMO Panel (Table 2), no safety issue concerning the three single events has been reported by the applicant.

Updated bioinformatic analyses on the junction regions for events MON 87427, MON 89034 and NK603 confirmed that no known endogenous genes were disrupted by any of the inserts.[7] Updated bioinformatic analyses of the amino acid sequence of the newly expressed CP4 EPSPS, Cry1A.105 and Cry2Ab2 proteins revealed no significant similarities to toxins and allergens.[7] In addition, updated bioinformatics analyses of the newly created open reading frames (ORFs) within the inserts and at their junctions for events MON 87427, MON 89034 and NK603 to identify any ORFs with significant similarity to toxins or allergens that were not previously assessed revealed that, for event MON 89034, a single ORF exceeded the allergenicity assessment threshold of 35% identity using an 80 amino acid

[7] Additional information: 17/6/2016.

sliding window approach.[7] This ORF is found within the transcriptional unit of the Cry2Ab2 coding sequence driven by the Figwort Mosaic Virus 35S promoter. It is in the same orientation but in a different reading frame than the Cry2Ab2 ORF and does not contain any in-frame translational start codon.[8] In conclusion, these analyses indicated that the expression of an ORF showing significant similarities to toxins or allergens for any of the events in maize MON 87427 × MON 89034 × NK603 is highly unlikely.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis to microbial DNA for events MON 87427, MON 89034 and NK603.[7] The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.3.4.2.

Based on the above information, the GMO Panel considers that its previous conclusions on the safety of the single maize events remain valid.

3.3. Risk assessment of the three-event stack maize MON 87427 × MON 89034 × NK603

3.3.1. Molecular characterisation

Possible interactions affecting the integrity of the events, protein expression levels or the biological functions conferred by the individual inserts are considered.

3.3.1.1. Genetic elements and their biological function[9]

Maize events MON 87427, MON 89034 and NK603 were combined by conventional crossing to produce event MON 87427 × MON 89034 × NK603. The structure of the inserts introduced into maize MON 87427, MON 89034 and NK603 is described in detail in the respective EFSA scientific opinions (Table 2) and no new genetic modifications were involved. Genetic elements in the expression cassettes of the single events are summarised in Table 3.

Intended effects of the inserts in maize MON 87427 × MON 89034 × NK603 are summarised in Table 3.

Based on the known biological function of the newly expressed proteins (Table 4), the only foreseen interactions at the biological level are between the Cry1A.105 and Cry2Ab2 proteins in susceptible insects.

Table 3: Genetic elements in the expression cassettes of the events stacked in maize MON 87427 × MON 89034 × NK603

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
MON 87427	35S (CaMV)	–	CTP2 (*Arabidopsis thaliana*)	CP4 *epsps* (*Agrobacterium* sp.)	*nos* (*Agrobacterium tumefaciens*)
MON 89034	35S (CaMV)	CAB (*Triticum* sp.)	–	*cry1A.105* (*B. thuringiensis*)	*Hsp17* (*Triticum sp*)
	35S (FMV)	–	CTP (*Zea mays*)	*cry2Ab2* (*B. thuringiensis*)	*nos* (*A. tumefaciens*)
NK603	*act1* (*Oryza sativa*)	–	CTP2 (*A. thaliana*)	CP4 *epsps* (*Agrobacterium* sp.)	*nos* (*A. tumefaciens*)
	35S (CaMV)	–	CTP2 (*A. thaliana*)	CP4 *epsps* L214P (*Agrobacterium* sp.)	*nos* (*A. tumefaciens*)

CaMV: cauliflower mosaic virus; UTR: untranslated region; CTP: chloroplast transit peptide.
(–): When no element was specifically introduced to optimise expression.

[8] Additional information: 6/6/2017.
[9] Dossier: Part II – Section A2.2.2.

Table 4: Characteristics and intended effects of the events stacked in maize MON 87427 × MON 89034 × NK603

Event	Protein	Donor organism and biological function	Intended effects in GM plant
MON 87427	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4. 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	Expression of the bacterial CP4 EPSPS protein in maize MON 87427 confers tolerance to glyphosate-containing herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme
MON 89034	Cry1A.105	Based on a gene from *Bacillus thuringiensis* subsp. *kurstaki* and subsp. *aizawai*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998)	Event MON 89034 expresses a modified *Bacillus thuringiensis* Cry1A-type protein with overall amino acid sequence identity of 93.4%, 90% and 76.7% to the Cry1Ac, Cry1Ab and Cry1F, respectively. Cry1A.105 is a protein toxic to certain lepidopteran larvae feeding on maize
	Cry2Ab2	Based on a gene from *B. thuringiensis* subsp. *kurstaki*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes	Event MON 89034 expresses also the Cry2Ab2, a protein toxic to certain lepidopteran larvae feeding on maize
NK603	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4. 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms	The bacterial CP4 EPSPS protein expressed in maize NK603 confers tolerance to glyphosate-containing herbicides as it has lower affinity towards glyphosate than the plant endogenous enzyme
	CP4 EPSPS L214P	Donor organism: *Agrobacterium* sp. strain CP4. 5-enopyruvylshikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms	Event NK603 expresses also CP4 EPSPS L214P – this variant, compared to the CP4 EPSPS protein, contains a single amino acid substitution from leucine to proline at position 214. The two CP4 EPSPS protein variants are structurally and functionally equivalent

3.3.1.2. Integrity of the events in the three-event stack maize MON 87427 × MON 89034 × NK603[9]

The genetic stability of the inserted DNA over multiple generations in the single maize events MON 87427, MON 89034 and NK603 was demonstrated previously (Table 2). Integrity of these events in maize MON 87427 × MON 89034 × NK603 was demonstrated by Southern analyses.

3.3.1.3. Information on the expression of the inserts[10]

CP4 EPSPS, Cry1A.105 and Cry2Ab2 protein levels were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from replicated field trials at five locations in the USA in the 2010 growing season. Samples analysed included leaf (V2–V4), grain (R6), pollen (pollination), root (V2–V4 and R5), whole plant (V10–V12) and forage (R5) both those treated and not treated with glyphosate. Since grain and forage are the main raw commodities used for food and feed purposes, protein levels in these commodities from maize MON 87427 × MON 89034 × NK603 (the highest mean values, regardless of the treatment) are summarised in Table 5.

[10] Dossier: Part II – Section A2.2.3; additional information: 17/6/2016.

Table 5: Means, standard deviation and ranges of protein levels (μg/g dry weight) in grain (n = 20) and forage (n = 20) from maize MON 87427 × MON 89034 × NK603

Tissue/Developmental Stage	Protein	MON 87427 × MON 89034 × NK603
Grain (R6)	CP4 EPSPS[(a)]	9.2[(b)] ± 1.5[(c)] (6.2–12)[(d)]
	Cry1A.105	4.4 ± 0.85 (3.3–6.2)
	Cry2Ab2	1.6 ± 0.38 (1.2–2.5)
Forage (R5)	CP4 EPSPS	170 ± 45 (93–240)
	Cry1A.105	19 ± 5.3 (11–31)
	Cry2Ab2	17 ± 5.4 (9.0–30)

(a): CP4 EPSPS levels in the maize MON 87427 × MON 89034 × NK603 are the sum of two protein variants, CP4 EPSPS (expressed in MON 87427 and NK603) and CP4 EPSPS L214P (expressed in NK603).
(b): Mean.
(c): Standard deviation.
(d): Range.

In order to assess the changes in protein expression levels which may result from potential interactions between the events, protein levels were determined for the three-event stack maize and the corresponding single events in different parts of the plant.

The levels of all the newly expressed proteins in the three-event stack maize and the corresponding singles were similar in all tissues, except for the expected difference for the CP4 EPSPS protein levels resulting from the combination of single events MON 87427 and NK603 both producing CP4 EPSPS protein in the three-event stack maize (Appendix A). Therefore, there is no indication of interactions that may affect the levels of the newly expressed proteins in this stack.

3.3.1.4. Conclusions of the molecular characterisation

The molecular data establish that the events stacked in maize MON 87427 × MON 89034 × NK603 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the three-event stack maize and in the single events except for CP4 EPSPS, which showed in general the expected higher levels in the stack resulting from the combination of the single events MON 87427 and NK603. Therefore, there is no indication of an interaction that may affect the integrity of the events and the levels of the newly expressed proteins in this stack.

Based on the known biological function of the newly expressed proteins, the only foreseen interactions at the biological level are between the Cry1A.105 and Cry2Ab2 proteins in susceptible insects, which will be dealt with in Section 3.3.4.

3.3.2. Comparative analysis

3.3.2.1. Choice of comparator and production of material for the comparative analysis[11]

Application EFSA-GMO-BE-2013-117 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition, of the three-event stack maize MON 87427 × MON 89034 × NK603 derived from field trials performed at nine sites in the USA during the 2010 growing season. Seven field trials were used to collect both agronomic and phenotypic characteristics and tissues for the compositional analysis, while the two remaining trial sites were used to collect only one set of data each (agronomic and phenotypic characteristics or composition) (Table 6).

The three-event stack maize MON 87427 × MON 89034 × NK603 was obtained by conventional crossing of the three single events. Event MON 87427 was introgressed in the inbred line LH198, while events MON 89034 and NK603 were introgressed in LH287. As documented by the pedigree, the three single events, after backcrossing, were combined in a hybrid maize with a genetic background (F_1) of LH198 × LH287. The same two inbred lines (LH198 × LH287) were crossed to produce the non-GM

[11] Dossier: Part II – Sections A3.1 and A3.2; additional information: 1/9/2015 and 17/6/2016.

hybrid maize used as comparator (MPA636B). On the basis of the provided pedigree, the GMO Panel considers that MPA636B is a suitable non-GM comparator.

Field trials for the agronomic, phenotypic and compositional analysis of the three-event stack maize MON 87427 × MON 89034 × NK603 were conducted in major maize growing areas in the USA, representing regions of diverse agronomic practices and environmental conditions. At each site, the following materials were grown in a randomised complete block design with four replicates: the three-event stack maize MON 87427 × MON 89034 × NK603, the non-GM comparator (MPA636B) and four non-GM maize reference varieties, all treated (sprayed) with plant protection products (PPP) according to local requirements, and the three-event stack maize MON 87427 × MON 89034 × NK603 treated with glyphosate in addition to PPP. Across all the sites, nineteen and twenty-two non-GM maize reference varieties were used for the agronomic and phenotypic characterisation and for the compositional analysis, respectively (Table 6).

Table 6: Overview of comparative analysis studies for the three-event stack maize MON 87427 × MON 89034 × NK603

Study focus	Study details	Comparator	Commercial non-GM maize reference varieties[(a)]
Agronomic and phenotypic analysis	Field trials, 2010, USA, eight locations[(b)]	Maize MPA636B	Nineteen[(c)]
Compositional analysis	Field trials, 2010, USA, eight locations[(d)]	Maize MPA636B	Twenty-two[(e)]

GM: Genetically modified.
(a): Four different varieties were grown at each location.
(b): Jackson County, Arkansas; Clinton County, Illinois; Cumberland County, Illinois; Stark County, Illinois; Pawnee County, Kansas; Shelby County, Missouri; York County, Nebraska; and Miami County, Ohio.
(c): NC+ 5411; Burrus 645; DKC61-50; DKC62-30; Fielder's Choice NG6778; Fontanelle 4924; H-9180; Kruger K-0210; Legacy L6600; Lewis 6014; Lewis 7007; Midland Phillips 799; Midland Phillips 7B15P; Midwest Genetics 78130; Mycogen 2M746; NK N72-G8; Pioneer 32B81; Stewart S588; and Triumph 1416.
(d): Jackson County, Arkansas; Clinton County, Illinois; Jefferson County, Iowa; Stark County, Illinois; Pawnee County, Kansas; Shelby County, Missouri; York County, Nebraska; and Miami County, Ohio.
(e): Burrus 645; Cornbelt ×6043; DKC61-50; DKC62-30; Fielder's Choice NG6778; Fontanelle 4924; H-9180; Kruger K-0210; Legacy L6600; Legacy L6673; Lewis 6014; Lewis 7007; Midland Phillips 799; Midland Phillips 7B15P; Midwest Genetics 78130; Mycogen 2M746; NC+ 5411; NK N72-G8; Pioneer 32B81; Stewart S518; Stewart S588; and Triumph 1416.

Statistical analysis of field trials data

The statistical analysis of the agronomic, phenotypic and compositional data from the 2010 field trials followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, EFSA GMO Panel, 2011a). This includes, for each of the two treatments of maize MON 87427 × MON 89034 × NK603, the application of a difference test (between the GM maize and the non-GM comparator) and an equivalence test (between the GM maize and the set of non-GM commercial reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[12]

3.3.2.2. Agronomic and phenotypic characteristics[13]

The agronomic and phenotypic characteristics evaluated on the basis of data collected from the eight field trial sites in the USA during the 2010 growing season (Table 6) were: early stand count, days to 50% pollen shed, days to 50% silking, stay green, ear height, plant height, dropped ear count, stalk lodging, root lodging, final stand count, grain moisture, test weight, yield and endpoints related to environmental interactions (abiotic stressors, disease damage and arthropod damage).

The three-event stack maize MON 87427 × MON 89034 × NK603 not treated with glyphosate showed a statistically significant increase in ear height, plant height and stalk lodging, and a reduction in grain moisture. Ear height, plant height and grain moisture fell within the equivalence limits established by the non-GM reference varieties (equivalence category I), while for stalk lodging equivalence with the non-GM reference varieties was more likely than not (equivalence category II).

[12] In detail, the four outcomes are: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).
[13] Dossier: Part II – Section A3.4; additional information: 1/9/2015 and 17/6/2016.

For root lodging, a significant increase was observed but the endpoint could not be categorised for equivalence because the variation between the non-GM reference varieties was estimated to be zero.[14]

The three-event stack maize MON 87427 × MON 89034 × NK603 treated with glyphosate showed a statistically significant increase in ear and plant height. Both endpoints fell within the equivalence limits established by the non-GM reference varieties (equivalence category I).[15]

Given the magnitude of the observed differences, the outcomes of the equivalence test and the nature of the endpoints, the GMO Panel considered that none of the differences in agronomic and phenotypic characteristics between the three-event stack maize MON 87427 × MON 89034 × NK603 and the non-GM comparator were relevant for further assessment.

3.3.2.3. Compositional analysis[16]

Forage and grain harvested from the field trials in the USA in 2010 (Table 6) were analysed for 79 constituents (9 in forage and 70 in grain), including the key constituents recommended by the OECD (OECD, 2002). For 14 grain constituents,[17] more than 50% of the observations were below the limit of quantification (LOQ). The statistical analysis was applied to the remaining 65 constituents (9 in forage[18] and 56 in grain[19]).

The results of the statistical analysis were the following:

- For maize MON 87427 × MON 89034 × NK603 (not treated with glyphosate), statistically significant differences with the non-GM comparator were identified for 42 grain endpoints.[20] All the endpoints fell under equivalence category I or II except for levels of carbohydrates (category III) and calcium (category IV) (Table 7).
- For maize MON 87427 × MON 89034 × NK603 (treated with glyphosate), statistically significant differences with the non-GM comparator were identified for 16 grain endpoints and 2 forage endpoints.[21] All the endpoints fell under equivalence category I or II except for calcium levels (category IV) (Table 7).

[14] Estimated mean values for ear height (cm): 107.1 (GM maize) and 103.4 (non-GM comparator). Estimated mean values for plant height (cm): 229.1 (GM maize) and 222.6 (non-GM comparator). Estimated mean values for grain moisture (%): 14.6 (GM maize) and 14.9 (non-GM comparator). Estimated mean values for stalk lodging (plants/plot): 3.8 (GM maize) and 1.8 (non-GM comparator). Estimated mean values for root lodged (plants/plot): 1.7 (GM maize) and 0.4 (non-GM comparator).

[15] Estimated mean values for ear height (cm): 106.6 (GM maize) and 103.4 (non-GM comparator). Estimated mean values for plant height (cm): 229.0 (GM maize) and 222.6 (non-GM comparator).

[16] Dossier: Part II – Section A3.3; additional information: 31/8/2015 and 12/12/2016.

[17] Furfural and the fatty acids caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), myristoleic (C14:1), pentadecanoic (C15:0), pentadecenoic (C15:1), heptadecenoic (C17:1), γ-linolenic (C18:3), eicosadienoic (C20:2), eicosatrienoic (C20:3), arachidonic (C20:4) and erucic (C22:1).

[18] Protein, moisture, ash, calcium, phosphorus, carbohydrates by calculation, total fat, acid detergent fibre (ADF) and neutral detergent fibre (NDF)

[19] Proximates (moisture, protein, total fat, ash and carbohydrates by calculation), fibre fractions (acid detergent fibre (ADF), neutral detergent fibre (NDF) and total dietary fibre (TDF)), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), fatty acids (palmitic (C16:0), palmitoleic (C16:1), heptadecanoic (C17:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), eicosenoic (C20:1) and behenic (C22:0)) , vitamins (folic acid, niacin, β-carotene, thiamin, riboflavin, pyridoxine and α-tocopherol) , minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc) and other compounds (raffinose, ferulic acid, *p*-coumaric acid and phytic acid).

[20] The grain constituents with significantly different levels were ash, protein, total fat, carbohydrates by calculation, NDF, calcium, phosphorus, copper, iron, magnesium, manganese, potassium, zinc, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), stearic acid (C18:0), arachidic acid (C20:0), β-carotene, thiamin, α-tocopherol, raffinose, ferulic acid, *p*-coumaric acid and phytic acid.

[21] The forage constituents with significantly different levels were moisture and calcium. The grain constituents with significantly different levels were ADF, ash, calcium, magnesium, manganese, phosphorus, zinc, arginine, glycine, stearic acid (C18:0), niacin, α-tocopherol, ferulic acid and *p*-coumaric acid.

Table 7: Compositional endpoints from grain that were further considered based on the results of the statistical analysis: means (for the GM maize and the non-GM comparator) and equivalence limits (from the non-GM commercial reference varieties) estimated from USA 2010 field trials data

Endpoint	Non-GM comparator	Maize MON 87427 × MON 89034 × NK603		Equivalence limits from non-GM reference varieties
		Not treated[(a)]	Treated[(b)]	
Carbohydrates (by calculation) (% DM)	86.1	87.05*	86.16	(84.89, 86.95)
Calcium (mg/kg DM)	480	510*	530*	(270, 490)

DM: dry matter.
For the GM maize, significantly different entries are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: white (equivalence category I), light grey (equivalence category III) and dark grey (equivalence category IV).
(a): Treated with conventional herbicides only.
(b): Treated with conventional herbicides and with glyphosate.

The GMO Panel assessed all the compositional differences between maize MON 87427 × MON 89034 × NK603 and the non-GM comparator. After considering the known biological role of the compounds, the outcome of the equivalence test (Table 7) and the magnitude of the changes observed, the GMO Panel did not identify any need for further food/feed safety assessment.

3.3.2.4. Conclusions of the comparative analysis

The GMO Panel concludes that none of the differences identified in forage and grain composition between maize MON 87427 × MON 89034 × NK603 and the non-GM comparator, and none of those identified in the agronomic and phenotypic characteristics, needs further assessment regarding food and feed safety.

Based on the agronomic and phenotypic characteristics of the three-event stack maize MON 87427 × MON 89034 × NK603, none of the differences observed between maize MON 87427 × MON 89034 × NK603 and the non-GM comparator are further assessed for potential environmental impact.

3.3.3. Food and feed safety assessment

3.3.3.1. Effects of processing

Processed products[22]

Maize MON 87427 × MON 89034 × NK603 will undergo existing production processes used for conventional maize. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the three-event stack maize MON 87427 × MON 89034 × NK603 into food and feed products is not expected to result in products being different from those of conventional non-GM maize varieties.

Newly expressed proteins[23]

The effects of heat treatment on the newly expressed proteins Cry1A.105, Cry2Ab2, CP4 EPSPS (including the variant CP4 EPSPS L214P) have been previously assessed by the GMO Panel in the context of the single maize events (Table 2).

3.3.3.2. Toxicology

Toxicological assessment of newly expressed proteins[24]

Three proteins (Cry1A.105, Cry2Ab2 and CP4 EPSPS, including its variant CP4 EPSPS L214P) are newly expressed in the three-event stack maize MON 87427 × MON 89034 × NK603 (Section 3.3.1).

[22] Dossier: Part II – Section A3.5.
[23] Dossier: Part II – Section A4.2.3.
[24] Dossier: Part II – Section A4.2.

The GMO Panel has previously assessed these proteins in the context of the single events (Table 2), and no safety concerns were identified. The GMO Panel is of the opinion that no scientific data have emerged which call for a change of this conclusion.

The CP4 EPSPS proteins are enzymes acting on the shikimic acid pathway for the biosynthesis of aromatic amino acids in plants, showing high substrate specificity. The two insecticidal proteins (Cry1A.105 and Cry2Ab2) act through cellular receptors found in target insect species, and it is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with high specific affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015).

On the basis of the known biological function of the individual newly expressed proteins (Table 4), there is currently no expectation for possible interactions relevant to food and feed safety between the newly expressed proteins.

The GMO Panel concludes that there are no safety concerns to human and animal health related to the newly expressed proteins Cry1A.105, Cry2Ab2, CP4 EPSPS and its variant CP4 EPSPS L241P in the three-event stack maize MON 87427 × MON 89034 × NK603.

Toxicological assessment of components other than newly expressed proteins[25]

The three-event stack maize does not show any compositional difference with the non-GM comparator that would require further toxicological assessment (Section 3.3.2).

3.3.3.3. Animal studies with the food/feed derived from GM plants[26]

No animal studies with food/feed derived from maize MON 87427 × MON 89034 × NK603 were provided by the applicant (e.g. 90-day toxicity studies in rodents or feeding studies in young rapidly growing animal species).

No substantial modifications in the composition of maize MON 87427 × MON 89034 × NK603 (Section 3.3.2), no indication of possible unintended effects and no indication of interactions relevant for food/feed safety were identified. Therefore, animal studies on the food/feed derived from maize MON 87427 × MON 89034 × NK603 are not required (EFSA GMO Panel, 2011a).

3.3.3.4. Allergenicity

For the allergenicity assessment, a weight-of-evidence approach was followed, taking into account all the information obtained on the newly expressed proteins, as no single piece of information or experimental method yields sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a). 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.

Assessment of allergenicity of the newly expressed proteins[27]

For allergenicity, the GMO Panel has previously evaluated the safety of the proteins Cry1A.105, Cry2Ab2 and CP4 EPSPS (including the variant CP4 EPSPS L214P) individually, and no concerns on allergenicity were identified in the context of the applications assessed (Table 2). No new information on allergenicity of these proteins that might change the previous conclusions of the GMO Panel has become available. Based on the current knowledge, and as none of the newly expressed proteins showed allergenicity, no reasons for concerns regarding the simultaneous presence of these newly expressed proteins in this three-event stack maize affecting their allergenicity were identified.

For adjuvanticity, proteins derived from *B. thuringiensis* (Bt proteins) have been suggested to possess adjuvant activity based on animal studies on Cry1Ac when applied at relatively high doses (e.g. Vazquez et al., 1999). The GMO Panel has previously evaluated the safety of the Cry1A.105 and Cry2Ab2 proteins and no concerns on adjuvanticity in the context of the applications assessed were identified (Table 2). The levels of Bt proteins in this three-event stack maize are similar to those in the respective single maize events (Table 5). From the limited experimental evidence available, the GMO Panel did not find indications that the presence of the Bt proteins at the levels expressed in this

[25] Dossier: Part II – Section A4.3.
[26] Dossier: Part II – Section A4.5.
[27] Dossier: Part II – Sections A5.1 and A5.3.

three-event stack maize might act as adjuvants with the potential to enhance a specific immunoglobulin E (IgE) response and to favour the development of an allergic reaction.

Assessment of allergenicity of GM plant products[28]

The GMO Panel regularly reviews the available publications on food allergy to maize. However, to date, maize has not been considered to be a common allergenic food[29] (OECD, 2002). Therefore, the GMO Panel did not request experimental data to analyse the allergen repertoire of GM maize.

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins (Sections 3.3.1, 3.3.2 and 3.3.3.2), the GMO Panel identified no indications of a potentially increased allergenicity of food and feed derived from the three-event stack maize with respect to that derived from the non-GM comparator.

3.3.3.5. Nutritional assessment of GM food/feed[30]

The intended traits of maize MON 87427 × MON 89034 × NK603 are insect resistance and herbicide tolerance, with no intention to alter nutritional parameters. Comparison of nutrients and anti-nutrients of maize MON 87427 × MON 89034 × NK603 with the non-GM comparator and reference varieties did not identify differences that would require further safety assessment. From these data, the GMO Panel concludes that the nutritional impact of food and feed derived from maize MON 87427 × MON 89034 × NK603 is similar to that expected from the non-GM comparator and non-GM commercial reference varieties.

3.3.3.6. Conclusion of the food and feed safety assessment

The three newly expressed proteins in the three-event stack maize MON 87427 × MON 89034 × NK603 (Cry1A.105, Cry2Ab2 and CP4 EPSPS, including its variant CP4 EPSPS L214P) do not raise safety concerns for human and animal health. No interactions between these proteins relevant for food and feed safety were identified. Similarly, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed proteins in maize MON 87427 × MON 89034 × NK603, or regarding the overall allergenicity of the three-event stack maize. Maize MON 87427 × MON 89034 × NK603 is as nutritious as the non-GM comparator and the non-GM commercial varieties tested.

3.3.4. Environmental risk assessment

Considering the scope of application EFSA-GMO-BE-2013-117 (which excludes cultivation), the ERA of the three-event stack maize MON 87427 × MON 89034 × NK603 is mainly concerned with: (1) the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and bacteria present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable maize MON 87427 × MON 89034 × NK603 grains during transportation and/or storage and processing (EFSA GMO Panel, 2010a).

3.3.4.1. Persistence and invasiveness of the GM plant[31]

Maize is highly domesticated, not winter hardy in colder regions of Europe, and generally unable to survive in the European environment without appropriate management. Occasional feral GM maize plants may occur outside cultivation areas (e.g. Han et al., 2015; Pascher, 2016), but survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens, herbivores and cold climate conditions. In fields within the EU, maize volunteers may arise under some environmental conditions (mild winters). Field observations indicate that maize grain may survive and overwinter in some regions, resulting in volunteers in subsequent crops (e.g. Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize volunteers in the EU have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009).

[28] Dossier: Part II – Section A5.2.

[29] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

[30] Dossier: Part II – Section A6.

[31] Dossier: Part II - Section E3.1.

As described in Section 3.3.2, field trials were carried out in the USA in the 2010 growing season to assess the agronomic and phenotypic characteristics of the three-event stack maize in comparison with the non-GM comparator. The data showed no changes in agronomic and phenotypic characteristics that would indicate altered fitness, persistence and invasiveness of the three-event stack maize. The GMO Panel considers that the persistence and invasiveness of the three-event stack maize MON 87427 × MON 89034 × NK603 compared to conventional maize remains unchanged.

Considering the agronomic and phenotypic data on the three-event stack maize and the general characteristics of maize described above, there are no indications of an increased likelihood of establishment and spread of occasional feral GM maize plants harbouring any combination of the three events of which it is composed. Should these plants be exposed to glyphosate-containing herbicides or infested by insect pests that are susceptible to the Cry1A.105 or Cry2Ab2 proteins, they are likely to exhibit a selective advantage that could increase their local occurrence. However, considering maize vulnerability to several abiotic and biotic factors, this occurrence is expected to be transient and will not result in different environmental impacts compared to conventional maize.

In addition to the data presented by the applicant, the GMO Panel is not aware of any scientific report of increased spread, establishment and survival capacity of the three-event stack maize MON 87427 × MON 89034 × NK603 or maize with comparable properties.

Therefore, the GMO Panel concludes that it is unlikely that the three-event stack maize MON 87427 × MON 89034 × NK603 would differ from conventional maize varieties in its ability to survive until subsequent seasons under European environmental conditions, if there was accidental release of viable GM maize grains into the environment. The occurrence of GM maize plants in the environment will thus be limited. Further, the occurrence of GM maize plants will not result in different environmental impacts compared to conventional maize.

3.3.4.2. Potential for gene transfer[32]

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through HGT of DNA, or through vertical gene flow via cross-pollination from feral plants originating from spilled grains.

Plant-to-microorganism gene transfer

The potential for HGT of the recombinant DNA of the single events has been assessed in previous GMO Panel Scientific Opinions (Table 2) and no concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of animal fed GM material or other receiving environments was identified. The applicant submitted an updated bioinformatic analysis for each of the single events in order to assess possibility for HGT by HR.[7]

Bioinformatic analysis of event MON 87427 revealed two elements that could provide sufficient length and sequence identity which could facilitate HGT, i.e. the truncated left border of an *Agrobacterium tumefaciens* octopine plasmid and the T-*nos* terminator of an *A. tumefaciens* nopaline plasmid. Because these elements are located on different plasmids, the results of the bioinformatic analyses give no indication for facilitated double HR.

Bioinformatic analysis of event MON 89034 revealed three elements that could provide sufficient length and sequence identity which could facilitate HGT. These are the truncated left border at 3′ and the one at 5′ and the T-*nos* terminator. The homologies with *A. tumefaciens* at the left borders align to the same region of the target sequences in an *A. tumefaciens* octopine plasmid but are inserted in the plant genome in an opposite orientation. Therefore they are supporting double HR. The T-*nos* terminator gives homology with an *A. tumefaciens* nopaline plasmid and does not support double HR.

Bioinformatic analysis of the NK603 event revealed sequence identity with the two copies of the T-*nos* terminator from an *A. tumefaciens* nopaline Ti plasmid present in the same orientation. This increases the likelihood of insertion of the *CP4 epsps* gene in the nopaline synthase terminator of the Ti plasmid. This insertion would affect the production of nopaline after transfer of the T-DNA to the plant and would hamper the development of crown gall tumour and the further spread of this recombinant plasmid in the environment. Moreover this insertion will result in the integration of a plant codon optimised version of the *CP4 epsps* gene that is expected to be less efficiently translated in bacteria.

[32] Dossier: Part II - Sections E3.1 and E3.2.

Synergistic effects of the recombinant genes, for instance due to combinations of recombinogenic sequences, which would cause an increase in the likelihood for HGT or a selective advantage, were not identified.

Based on the above information, the GMO Panel did not identify an increased likelihood for horizontal transfer of recombinant genes to bacteria for the three-event stack maize. This is consistent with its previous assessment of maize events MON 87427, MON 89034 and NK603.

Plant-to-plant gene transfer

Considering the scope of application EFSA-GMO-BE-2013-117 and the biology of maize, the potential for occasional feral GM maize plants originating from grain import spills to transfer recombinant DNA to sexually cross-compatible plants is assessed. As pointed out above (Section 3.3.4.1), the occurrence of feral GM maize plants is expected to be limited.

The extent of cross-pollination from occasional feral GM maize to other maize species will mainly depend on accidental release during transportation and processing and on successful establishment and subsequent flowering of the GM maize plant. For maize, vertical gene transfer is limited to *Zea* species. Populations of sexually compatible wild relatives of maize outside cultivation are not known in Europe (Eastham and Sweet, 2002; OECD, 2003), therefore vertical gene transfer is not considered to be an environmental issue in the EU.

The flowering of occasional feral GM maize plants originating from accidental release during transportation and processing is unlikely to lead to dispersal of significant amounts of GM maize pollen onto 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). Thus, the likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low.

In conclusion, even if cross-pollination would occur, the GMO Panel is of the opinion that the likelihood of environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties.

3.3.4.3. Interactions of the GM plant with target organisms[33]

Interactions might occur between different Cry proteins. Whether such an interaction takes place depends on the arthropod species (EcoΣtat, 2014; De Schrijver et al., 2015). Considering the scope of application EFSA-GMO-BE-2013-117, potential interactions with target organisms of occasional feral three-event stack maize MON 87427 × MON 89034 × NK603 plants arising from grain import spills are not considered a relevant issue by the GMO Panel.

3.3.4.4. Interactions of the GM plant with non-target organisms[34]

As mentioned in Section 3.3.4.3, interactions between Cry proteins, leading to synergistic insecticidal effects, might occur in other susceptible non-target species. Considering that environmental exposure of non-target organisms to stored GM grains, spilled GM grains or GM plants arising from spilled GM grains is limited, potential exposure of non-target organisms sensitive to Cry1A.105 and/or Cry2Ab2 proteins is likely to be very low.

The GMO Panel also evaluated whether the expressed Cry1A.105 and Cry2Ab2 proteins might affect non-target organisms by entering the environment through faecal material of animals fed GM maize. Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only low amounts of intact Cry proteins would remain in the faeces. This was demonstrated for Cry1Ab (Einspanier et al., 2004; Lutz et al., 2005; Lutz et al., 2006; Wiedemann et al., 2006; Guertler et al., 2008; Paul et al., 2010). Further degradation of the protein in manure and faeces will take place because of microbiological proteolytic activity. In addition, there will be further degradation of Cry proteins in soil, reducing the possibility for exposure of potentially sensitive non-target organisms. Although Cry proteins may bind to clay minerals and organic 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 (Gruber et al., 2012; Valldor et al., 2015). The GMO Panel is not aware of evidence of released Cry proteins from GM plants causing significant negative effects on soil inhabiting organisms.

[33] Dossier : Part II – Section E3.3.
[34] Dossier : Part II – Section E3.4.

Considering the scope of the application, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1A.105 and Cry2Ab2 proteins is likely to be very low and the risk related to interactions with non-target organisms is therefore of no relevance.

3.3.4.5. Interactions with the abiotic environment and biogeochemical cycles[35]

Considering the scope of application EFSA-GMO-BE-2013-117 and the low level of exposure to the environment, potential interactions of spilled grains or occasional feral maize MON 87427 × MON 89034 × NK603 with the abiotic environment and biogeochemical cycles are not considered a relevant issue by the GMO Panel.

3.3.4.6. Conclusion of the environmental risk assessment

There are no indications of an increased likelihood of establishment and spread of occasional feral three-event stack maize MON 87427 × MON 89034 × NK603 plants in the case of accidental release into the environment of viable grains, unless these plants are infested by insect pests that are susceptible to the Cry1A.105 and/or Cry2Ab2 proteins or exposed to glyphosate-containing herbicides. However, the GMO Panel is of the opinion that the possible exposure of feral GM plants to these herbicides or susceptible pests would not result in different environmental impacts compared to conventional maize. Considering the scope of application EFSA-GMO-BE-2013-117, interactions with the biotic and abiotic environment are not considered to be relevant issues. Risks associated with an unlikely but theoretically possible horizontal gene transfer of recombinant DNA from the three-event stack maize to bacteria have not been identified. Therefore, considering the novel combination of events, the introduced traits, the outcome of the comparative analysis, the routes of exposure and the limited potential exposure levels, the GMO Panel concludes that the three-event stack maize MON 87427 × MON 89034 × NK603 would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

3.3.5. Conclusion on the three-event stack maize MON 87427 × MON 89034 × NK603

No new data on the single maize events MON 87427, MON 89034 and NK603 leading to a modification of the original conclusions on their safety were identified.

The combination of maize events MON 87427, MON 89034 and NK603 in the three-event stack maize did not give rise to issues pertaining to the molecular, agronomic/phenotypic or compositional characteristics of the three-event stack maize that would be of concern for food and feed safety and nutrition.

The newly expressed proteins in the three-event stack maize do not raise safety concerns for human and animal health and the environment in light of the scope of this application.

No indications of interactions between the events based on the biological functions of the newly expressed proteins that would raise a safety issue were identified in maize MON 87427 × MON 89034 × NK603. Comparison of the levels of the newly expressed proteins between the three-event stack maize and those of the single maize events did not reveal an interaction at protein expression level.

Considering the combined events and the outcome of the comparative analysis, the routes of exposure and limited exposure levels, the GMO Panel concludes that the three-event stack maize MON 87427 × MON 89034 × NK603 would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.

No scientific information that could change the conclusions on this three-event stack maize was retrieved in a literature search covering the period since the time of validity of the application. The GMO Panel concludes that the three-event stack maize is as safe and as nutritious as the non-GM comparator in the context of its scope.

3.4. Risk assessment of the subcombinations

The GMO Panel Guidance Documents establish the principle that where all single events have been assessed, the risk assessment of stacked events focuses on issues related to: (a) stability of the events; (b) expression of the events; and (c) potential interactions between the events (EFSA GMO Panel, 2011a).

[35] Dossier : Part II – Section E3.6.

For the two subcombinations (maize MON 87427 × MON 89034 and MON 87427 × NK603) for which no specific data have been submitted and which have not been previously assessed by the GMO Panel, the risk assessment takes as its starting point the assessment of the single maize events, and uses the data generated for the three-event stack maize, as well as all the additional data available on subcombinations previously assessed by the GMO Panel.

3.4.1. Subcombination previously assessed

The two-event stack maize MON 89034 × NK603 has been previously assessed by the GMO Panel and no safety concerns were identified (EFSA GMO Panel, 2009). No new scientific information relevant to the risk assessment of this two-event stack maize became available since the validation of application EFSA-GMO-BE-2013-117. Consequently, the GMO Panel considers that its previous conclusions on this subcombination remain valid.

3.4.2. Subcombinations not previously assessed

The two-event maize stacks MON 87427 × MON 89034 and MON 87427 × NK603 included in the scope of this application have not been previously assessed by the GMO Panel. No experimental data were provided for these two maize stacks.

3.4.2.1. Stability of the events

The genetic stability of the inserted DNA over multiple generations in the three single maize events was demonstrated previously (Table 2). Integrity of the events was demonstrated in the three-event stack maize MON 87427 × MON 89034 × NK603 (Section 3.3.1) and in the two-event stack maize MON 89034 × NK603 (EFSA GMO Panel, 2009). The GMO Panel finds no reasons to expect the loss of integrity of the events in the two-event maize stacks MON 87427 × MON 89034 and MON 87427 × NK603.

3.4.2.2. Expression of the events

The GMO Panel assessed whether any combination of the three events by conventional crossing could result in significant changes in expression levels of the newly expressed proteins, as this could indicate an unexpected interaction between the events. Based on current knowledge of the molecular elements introduced, there is no reason to expect interactions that would affect the levels of the newly expressed proteins in the two subcombinations compared with those in the single maize events. This assumption was confirmed by comparing the levels of the newly expressed proteins of each single maize event with those of the three-event stack maize. The levels were similar in the three-event stack maize and in the single events except for CP4 EPSPS, which showed, in general, the expected higher level in the stack resulting from the combination of the single events MON 87427 and NK603 (Section 3.3.1.3 and Appendix A). Therefore, there was no indication of an interaction at protein expression level. In addition, expression data from the two-event stack maize MON 89034 × NK603 were similar to those observed in each of the single maize events (EFSA GMO Panel, 2009). This confirms that interactions affecting the expression levels of the newly expressed proteins are not expected in the two subcombinations MON 87427 × MON 89034 and MON 87427 × NK603.

3.4.2.3. Potential interactions between the events

The GMO Panel assessed the potential interactions between events, due to their combination in the two-event stacks MON 87427 × MON 89034 and MON 87427 × NK603, taking into consideration intended traits and unintended effects.

Based on the known biological functions of the individual newly expressed proteins (Table 4), there is currently no expectation for possible interactions between these proteins in maize MON 87427 × MON 89034 or MON 87427 × NK603 relevant for food/feed or environmental safety. The GMO Panel took into account all the intended and potential unintended effects considered in the assessment of the three single events, the two-event stack maize MON 89034× NK603 and the three-event stack maize MON 87427 × MON 89034 × NK603. It was concluded that none of these effects would raise safety concerns when combined in any of the two subcombinations.[36] Therefore, the GMO Panel is of the opinion that no additional data are needed to complete the assessment of subcombinations of the three-event stack maize.

[36] 115th GMO Panel meeting (Annex 1 of the minutes: http://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf).

3.4.3. Conclusion

Since no new safety concerns were identified for the previously assessed two-event stack maize MON 89034 × NK603, the GMO Panel considers that its previous conclusions on this subcombination remain valid. For the two subcombinations MON 87427 × MON 89034 and MON 87427 × NK603, for which no experimental data have been provided, the GMO Panel assessed the possibility of interactions between the events, and concluded that these combinations would not raise safety concerns. These two subcombinations are therefore expected to be as safe as the single maize events, the previously assessed two-event stack maize MON 89034× NK603 and the three-event stack maize MON 87427 × MON 89034 × NK603.

3.5. Post-market monitoring

3.5.1. Post-market monitoring of GM food/feed

There was no indication that food/feed products derived from the three-event stack maize MON 87427 × MON 89034 × NK603 are less safe or nutritious than those derived from the non-GM comparator. Furthermore, the overall intake or exposure is not expected to change because of the introduction of maize MON 87427 × MON 89034 × NK603 into the market. The two-event stack maize MON 89034× NK603 has been previously assessed and no safety concerns were identified. The subcombinations MON 87427 × MON 89034 and MON 87427 × NK603 are expected to be as safe as the single maize events, the previously assessed two-event stack maize MON 89034× NK603 and the three-event stack maize MON 87427 × MON 89034 × NK603. Therefore, the GMO Panel considers that post-market monitoring of maize MON 87427 × MON 89034 × NK603 and its subcombinations is not necessary.

3.5.2. Post-market environmental monitoring[37]

The objectives of a PMEM plan, according to Annex VII of Directive 2001/18/EC, are to: (1) 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) identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the environmental risk assessment.

Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific content of the PMEM plan provided by the applicant (EFSA, 2006; EFSA GMO Panel, 2011b).

The PMEM plan proposed by the applicant includes: (1) the description of a monitoring approach involving operators (federations involved in 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 information recorded by the various operators (Lecoq et al., 2007; Windels et al., 2008); and (3) the review of relevant scientific publications retrieved from literature searches. The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the authorisation period. The GMO Panel considers that the scope of the post-market environmental monitoring plan provided by the applicant is consistent with the scope of maize MON 87427 × MON 89034 × NK603. As the ERA does not cover cultivation and did not identify any potential adverse environmental effect from maize MON 87427 ×MON 89034 × NK603, no case-specific monitoring is necessary. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan. However, the PMEM plan submitted by the applicant for the three-event stack maize does not include any provisions for the two subcombinations not previously assessed by the GMO Panel. Therefore, the GMO Panel recommends the applicant to revise the plan accordingly.

4. Overall conclusions and recommendations

No new data on the three single maize events MON 87427, MON 89034 and NK603 that would lead to a modification of the original conclusions on their safety were identified.

The combination of events MON 87427, MON 89034 and NK603 in the three-event stack maize did not give rise to food/feed safety issues related to molecular, agronomic/phenotypic or compositional characteristics. The newly expressed proteins in the three-event stack maize did not raise concerns for

[37] Dossier: Part II – Section E4; additional information: 13/12/2016.

human and animal health. Maize MON 87427 × MON 89034 × NK603 is expected to be as nutritious as the non-GM comparator and the tested non-GM maize commercial reference varieties.

Considering the combined events, the outcome of the comparative analysis and the routes and levels of exposure, the GMO Panel concludes that maize MON 87427 × MON 89034 × NK603 would not raise environmental safety concerns in the event of accidental release of viable GM maize grains into the environment.

The GMO Panel concludes that maize MON 87427 × MON 89034 × NK603 is as safe and as nutritious as the non-GM comparator and the tested non-GM maize reference varieties in the context of the scope of this application.

Since no new data on the previously assessed two-event stack maize MON 89034 × NK603 that would lead to a modification of the original conclusions on its safety were identified, the GMO Panel considers that its previous conclusions on this two-event stack maize remain valid. For the two subcombinations MON 87427 × MON 89034 and MON 87427 × NK603, for which no experimental data have been provided, the GMO Panel assessed possible interactions between the events, and concluded that combinations of the events MON 87427, MON 89034 and NK603 would not raise safety concerns in these maize subcombinations. The two subcombinations are therefore expected to be as safe and as nutritious as the single maize events, the previously assessed two-event stack maize MON 89034 × NK603 and the three-event stack maize MON 87427 × MON 89034 × NK603.

Given the absence of safety concerns for food and feed derived from the three-event stack maize MON 87427 × MON 89034 × NK603 and its subcombinations MON 89034× NK603, MON 87427 × MON 89034 and MON 87427 × NK603, the GMO Panel considers that post-market monitoring of these products is not necessary.

The GMO Panel considers that the scope of the PMEM plans provided by the applicant is consistent with the scope of the three-event stack maize and the already assessed two-event stack maize MON 89034 × NK603. The GMO Panel agrees with the reporting intervals proposed by the applicant in the PMEM plans. However, the PMEM plan submitted by the applicant for the three-event stack maize does not include any provisions for the two subcombinations that were not previously assessed. Therefore, the GMO Panel recommends the applicant to revise the plan accordingly.

Documentation as provided to EFSA

1) Letter from the Competent Authority of Belgium received on 13 September 2013 concerning a request for placing on the market of genetically modified maize MON 87427 × MON 89034 × NK603 submitted by Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003 (application reference EFSA-GMO-BE-2013-117).
2) Acknowledgement letter dated 24 September 2013 from EFSA to the Competent Authority of Belgium.
3) Letter from EFSA to applicant dated 25 October 2013 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 22 November 2013 providing additional information under completeness check.
5) Letter from EFSA to applicant dated 13 December 2013 requesting additional information under completeness check.
6) Letter from applicant to EFSA received on 20 December 2013 providing additional information under completeness check.
7) Letter from EFSA to applicant dated 22 January 2014 delivering the 'Statement of Validity' of application EFSA-GMO-BE-2013-117 for placing on the market of genetically modified maize MON 87427 × MON 89034 × NK603 submitted by Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003.
8) Letter from EFSA to applicant dated 28 January 2014 stopping the clock due to single event not finalised (MON 87427).
9) Letter from EFSA to applicant dated 4 June 2015 re-starting the clock on 27 May 2015 due to finalisation of single event (MON 87427).
10) Letter from EFSA to applicant dated 16 June 2015 requesting additional information and stopping the clock.
11) Letter from applicant to EFSA received on 1 September 2015 providing additional information.
12) Letter from EFSA to applicant dated 15 September 2015 requesting additional information and maintaining the clock stopped.

13) Letter from applicant to EFSA received on 1 October 2015 providing additional information.
14) Letter from EFSA to applicant dated 15 December 2015 requesting additional information and maintaining the clock stopped.
15) Letter from applicant to EFSA received on 17 June 2016 providing additional information.
16) Email from EFSA to applicant dated 20 June 2016 re-starting the clock on 17 June 2016.
17) Letter from EFSA to applicant dated 9 November 2016 requesting additional information and stopping the clock.
18) Letter from applicant to EFSA received on 12 December 2016 providing additional information.
19) Letter from applicant to EFSA received on 13 December 2016 providing additional information spontaneously.
20) Email from EFSA to applicant dated 13 December 2016 re-starting the clock on 12 December 2016.
21) Email from applicant to EFSA, dated 11 January 2017 requesting clarifications.
22) Letter from EFSA to applicant, dated 13 January 2017 providing clarifications.
23) Email from applicant to EFSA, dated 25 January 2017 requesting clarifications.
24) Letter from EFSA to applicant, dated 31 January 2017 providing clarifications.
25) Email from applicant to EFSA, dated 3 March 2017 requesting clarifications.
26) Letter from EFSA to applicant dated 6 April 2017 requesting additional information and stopping the clock.
27) Letter from applicant to EFSA received on 6 June 2017 providing additional information.
28) Email from EFSA to applicant dated 6 June 2017 re-starting the clock on 6 June 2017.

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Abbreviations

ADF	acid detergent fibre
CaMV	cauliflower mosaic virus
CTP	chloroplast transit peptide
DNA	deoxyribonucleic acid
DM	dry matter
ELISA	enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	environmental risk assessment
GM	genetically modified
GMO	genetically modified organism
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
HR	homologous recombination
IgE	immunoglobulin E
LOQ	limit of quantification
NDF	neutral detergent fibre
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PMEM	post-market environmental monitoring
PPP	plant protection products
TDF	total dietary fibre
T-DNA	transfer-deoxyribonucleic acid
UTR	untranslated region

Appendix A – Protein expression data

Means, standard deviation and ranges of protein levels (μg/g dry weight) from maize MON 87427 × MON 89034 × NK603 (treated with glyphosate), MON 87427 (treated with glyphosate), NK603 (treated with glyphosate) and MON 89034 (not treated) from field trials performed in the USA in 2010

Tissue (Developmental stage)	CP4 EPSPS			Cry1A.105		Cry2Ab2	
	Stack[a]	MON 87427	NK603	Stack	MON 89034	Stack	MON 89034
Leaf (V2–V4)	890[b]±270[c] (470–1300)[d]	570 ± 220 (250–970)	340 ± 110 (130–480)	190 ± 90 (51–280)	210 ± 110 (49–370)	140 ± 70 (53–330)	150 ± 67 (57–260)
Grain (R6)	9.2 ± 1.5 (6.2–12)	4.4 ± 1.2 (2.9–7.6)	5.6 ± 1.0 (3.8–7.3)	4.4 ± 0.85 (3.3–6.2)	3.9 ± 0.65 (2.7–5.6)	1.6 ± 0.38 (1.2–2.5)	1.7 ± 0.56 (0.47–3.1)
Pollen (pollination)	330 ± 82 (130–460)	< LOQ[e]	350 ± 76 (210–470)	6.8 ± 1.6 (4.7–11)	8.4 ± 7.5 (4.7–38)	0.60 ± 0.61 (0.29–2.7)	0.78 ± 0.50 (0.39–2.1)
Root (V2–V4)	180 ± 73 (89–330)	230 ± 62 (78–350)	160 ± 90 (59–320)	41 ± 12 (25–60)	47 ± 12 (36–88)	61 ± 14 (33–94)	50 ± 12 (35–77)
Root (R5)	87 ± 31 (33–130)	69 ± 17 (38–100)	25 ± 9.2 (10–39)	9.7 ± 2.1 (5.9–13)	9.6 ± 1.7 (6.8–14)	17 ± 5.6 (6.2–25)	22 ± 7.3 (5.1–36)
Whole Plant (V10–V12)	550 ± 160 (340–880)	390 ± 150 (65–650)	180 ± 52 (93–270)	50 ± 16) (28–82)	61 ± 18 (34–100)	25 ± 9.0 (11–48)	32 ± 15 (16–68)
Forage (R5)	170 ± 45 (93–240)	120 ± 23 (83–180)	56 ± 13 (32–80)	19 ± 5.3 (11–31)	21 ± 5.8 (10–31)	17 ± 5.4 (9.0–30)	19 ± 5.1 (8.5–29)

(a): 'Stack' refers to maize MON 87427 × MON 89034 × NK603.
(b): Mean.
(c): Standard deviation.
(d): Range.
(e): LOQ: limit of quantification; due to specific insert design, little to no CP4 EPSPS protein is expected to be produced in pollen.