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Assessment of genetically modified maize 1507 × 59122 × MON810 × NK603 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2011-92)

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Abstract

In this opinion, the GMO Panel assessed the four-event stack maize 1507 × 59122 × MON810 × NK603 and its ten subcombinations, independently of their origin. The GMO Panel previously assessed the four single events combined in this four-event stack maize and five of their combinations and did not identify safety concerns. No new data on the single events or their previously assessed combinations leading to modification of the original conclusions 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 four-event stack maize did not give rise to food and feed safety or nutritional issues. The GMO Panel concludes that the four-event stack maize is as safe and as nutritious as its non-GM comparator. In the case of accidental release of viable grains of maize 1507 × 59122 × MON810 × NK603 into the environment, this would not raise environmental safety concerns. For four of the subcombinations not previously assessed, protein expression data were provided and did not indicate an interaction affecting the levels of the newly expressed proteins in these subcombinations. The five subcombinations not previously assessed are expected to be as safe as the single maize events, the previously assessed subcombinations and the four-event stack maize. The GMO Panel considers that post-market monitoring of maize 1507 × 59122 ×MON810 × NK603 and its subcombinations is not necessary. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of maize 1507 × 59122 × MON810 ×NK603 and its subcombinations.

Keywords: GMO, maize (Zea mays), 1507 × 59122 × MON810 × NK603, insect resistant and herbicide tolerant, Regulation (EC) No 1829/2003

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Summary

Following the submission of application EFSA-GMO-NL-2011-92 under Regulation (EC) No 1829/2003 from Pioneer, the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as the GMO Panel) was asked to deliver a Scientific Opinion on the safety of genetically modified glufosinate- and glyphosate-tolerant and insect-resistant maize 1507 × 59122 × MON810 × NK603 and its subcombinations independently of their origin, according to Regulation (EU) No 503/2013 (referred to hereafter as 'subcombinations'). The scope of application EFSA-GMO-NL-2011-92 is for the placing on the market of maize 1507 × 59122 × MON810 × NK603 and all its subcombinations, independently of their origin, for food and feed uses, import and processing.

The term 'subcombination' refers to any combination of up to three of the events present in the four-event stack maize. The safety of subcombinations occurring as segregating progeny in the harvested grains of maize 1507 × 59122 × MON810 × NK603 is evaluated in the context of the assessment of the four-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 four-event stack, are risk assessed in the 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 four-event stack maize, a three-event and four two-event stack subcombinations, the scientific comments submitted by the Member States, and relevant scientific literature. The four-event stack maize was produced by conventional crossing to combine four maize events: 1507 expressing the Cry1F protein which confers protection against specific lepidopteran pests and phosphinothricin acetyl transferase (PAT) protein for tolerance to glufosinate-ammonium-containing herbicides; 59122 expressing the Cry34Ab1 and Cry35Ab1 proteins to confer protection against coleopteran pests belonging to the genus *Diabrotica* and the PAT protein for tolerance to glufosinate-ammonium-containing herbicides; MON810 expressing the Cry1Ab protein to confer protection against specific lepidopteran pests; and NK603 expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein and its variant CP4 EPSPS L214P for tolerance to glyphosate- containing herbicides.

The GMO Panel evaluated the four-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.

For application EFSA-GMO-NL-2011-92, previous assessments of the four single maize events (1507, 59122, MON810 and NK603), the three-event stack maize 59122 × 1507 × NK603 and four two-event stack maize (1507 × 59122, 1507 × NK603, 59122 × NK603 and NK603 × MON810) provided a basis to evaluate the four-event stack maize and all its subcombinations. Maize 1507, 59122, MON810, NK603, 1507 × 59122, 1507 × NK603, 59122 × NK603 and NK603 × MON810, and 59122 × 1507 × NK603 were previously assessed by the GMO Panel and no concerns on their safety were identified. No safety issue concerning the four 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 four-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 1507 × 59122 × MON810 × NK603 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the four-event stack maize and in the single events except for the expected difference in PAT protein levels resulting from the combination of 1507 and 59122 events, both producing PAT protein in the four-event stack. No indications of interactions that may affect the integrity of the events and the levels of the newly expressed proteins in this four-event stack maize were identified.

No relevant differences between maize 1507 × 59122 × MON810 × NK603 and the non-GM comparator requiring further assessment regarding food and feed safety and environmental impact were identified in grain and forage composition and in the tested agronomic and phenotypic characteristics.

Based on the molecular, agronomic, phenotypic or compositional characteristics, the combination of maize events 1507, 59122, MON810, and NK603 in the four-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 four-event stack maize did not raise concerns for human and animal health.

Considering the events combined, their potential interactions, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that maize 1507 × 59122 × MON810 × NK603 would not raise safety concerns in the case of accidental release of viable GM maize grains into the environment.

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

Maize 1507 × 59122 × MON810 × NK603 has 10 possible subcombinations of which five have been previously assessed. Since no safety concerns were identified for the previously assessed two-event stack maize 1507 × 59122, 1507 × NK603, 59122 × NK603 and NK603 × MON810, and the three-event stack maize 59122 × 1507 × NK603, and no new data leading to the modification of the original conclusions on safety were identified, the GMO Panel considers that its previous conclusions on these maize subcombinations remain valid. For four of the subcombinations not previously assessed, protein expression data were provided and did not indicate an interaction affecting the levels of the newly expressed proteins in these subcombinations. The GMO Panel assessed the possibility of interactions between the events in these five subcombinations and concluded that these combinations would not raise safety concerns. The five subcombinations not previously assessed are expected to be as safe as the single maize events, the previously assessed maize 1507 × 59122, 1507 × NK603, 59122 × NK603 and NK603 × MON810 and the three-event stack maize 59122 × 1507 × NK603, as well as the four-event stack maize 1507 × 59122 × MON810 × NK603.

Given the absence of safety concerns for food and feed derived from maize 1507 × 59122 × MON 810 × NK603 and its subcombinations, the GMO Panel considers that post-market monitoring of these products is not necessary. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of the four-event stack maize and its subcombinations.

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1. Introduction

1.1. Background

On 3 February 2011, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2011-92, for authorisation of genetically modified (GM) maize 1507 × 59122 × MON810 × NK603. This application was submitted by Pioneer Overseas Corporation (referred to hereafter as the applicant), within the framework of Regulation (EC) No 1829/2003[1], for food and feed uses, import and processing[2], in accordance with Articles 3(1) and 15(1) of Regulation (EC) 1829/2003. The risk assessment of application EFSA-GMO-NL-2011-92 presented here is for the placing on the market of four-event stack maize and all its subcombinations independently of their origin, for food and feed uses (see Table 1).

After receiving the application EFSA-GMO-NL-2011-92 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. 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 30 March 2011, 9 June 2011 and 22 November 2011, respectively; the applicant provided the information on 17 May 2011, 25 October 2011 and 9 January 2012 respectively. On 30 January 2012, 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[3] 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 (i.e., until 30 April 2012) to make their opinion known.

The EFSA GMO Panel carried out an evaluation of the scientific risk assessment of the four-event stack maize and its subcombinations, independently of their origin (Table 1) (referred to as 'subcombinations', according to the Regulation (EU) No 503/2013).

EFSA and the GMO Panel requested additional information on 1 March 2012 (EURL-GMFF), 13 April 2012, 11 July 2012, 24 September 2012, 9 January 2013, 7 January 2014, 14 March 2014, 13 June 2014, 27 October 2014, 15 December 2014, 11 December 2015, 1 June 2016, 3 August 2016, 17 October 2016, 8 March 2017 and 18 May 2017, respectively. The applicant provided the requested information on 31 May 2012, 2 October 2012, 10 December 2012, 11 December 2012 (EURL-GMFF), 20 February 2013, 6 May 2014, 6 August 2014, 18 August 2014, 5 February 2015, 19 November 2015, 25 November 2015, 23 May 2016, 7 July 2016, 12 October 2016, 23 January 2017, 9 June 2017 and 21 August 2017. The applicant also submitted spontaneous information on 9 June 2017.

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

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 EFSA GMO Panel, the time limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1) and 18(2) of Regulation (EC) No 1829/2003.

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

1.2. Terms of Reference as provided by the requestor

The EFSA GMO Panel was requested to carry out a scientific risk assessment of 'maize 1507 × 59122 × MON810 × NK603 and ten sub-combinations of the single events, 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.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed.
[2] In accordance with Part C of Directive 2001/18/EC.
[3] 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.

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.

2. Data and methodologies

2.1. Data

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2011-92, 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 1507 × 59122 × MON810 × NK603 and its ten subcombinations,[4] independently of their origin (Table 1), for food and feed uses, 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, 2006a, 2007a; EFSA GMO Panel, 2011a), the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010), and 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 and were taken into consideration during the scientific risk assessment.

3. Assessment

3.1. Introduction

Application EFSA-GMO-NL-2011-92 covers the four-event stack maize 1507 × 59122 × MON810 × NK603 and its 10 subcombinations independently of their origin (Table 1). The scope of this application is for food and feed uses, and excludes cultivation within the European Union (EU).

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

The safety of subcombinations occurring as segregating progeny in harvested grains of maize 1507 × 59122 × MON810 × NK603 is evaluated in the context of the assessment of the four-event stack maize in Section 3.3 of the present GMO Panel Scientific Opinion.

'Subcombination' also covers combinations of three or two of the four events 1507, 59122, MON810 and NK603 that have either been or could be produced by conventional crossing through targeted breeding approaches (EFSA GMO Panel, 2011a). These maize stacks, that can be bred, produced and marketed independently of the four-event stack maize, are assessed in the Section 3.4 of this GMO Panel Scientific Opinion.

The four-event stack maize was produced by conventional crossing to combine four maize events: 1507 (expressing the Cry1F and phosphinothricin acetyl transferase (PAT) proteins), 59122 (expressing the Cry34Ab1, Cry35Ab1 and PAT proteins), MON810 (expressing Cry1Ab), and NK603 (expressing the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) and its variant CP4 EPSPS L214P protein).

[4] For the risk assessment of subcombinations, the GMO Panel used the strategy indicated in its 115th GMO Panel meeting (Annex 1 of the minutes: http://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf).

Herbicidal tolerance traits are achieved by the expression of CP4 EPSPS proteins from *Agrobacterium* sp. strain CP4, and PAT protein from *Streptomyces viridochromogenes*. Insecticidal resistance traits are achieved by the expression of the Cry1F, Cry34Ab1, Cry35Ab1 and Cry1Ab proteins from *Bacillus thuringiensis*, which confer protection against specific lepidopteran (e.g. *Ostrinia nubilalis* (European corn borer) and species belonging to the genus *Sesamia*) and coleopteran pests (*Diabrotica* spp. (corn rootworm larvae)).

Table 1: Stacked maize events covered by the scope of application EFSA-GMO-NL-2011-92.

Degree of Stacking	Events	Unique Identifiers
Four-event stack maize	1507 × 59122 × MON810 × NK603	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-ØØ81Ø-6 × MON-ØØ6Ø3-6
Three-event stack maize	1507 × 59122 × MON810	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-ØØ81Ø-6
	1507 × MON810 × NK603	DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × MON-ØØ6Ø3-6
	59122 × 1507 × NK603	DAS-59122-7 × DAS-Ø15Ø7-1 × MON-ØØ6Ø3-6
	59122 × MON810 × NK603	DAS-59122-7 × MON-ØØ81Ø-6 × MON-ØØ6Ø3-6
Two-event stack maize	1507 × 59122	DAS-Ø15Ø7-1 × DAS-59122-7
	1507 MON810	DAS-Ø15Ø7-1 × MON-ØØ81Ø-6
	1507 × NK603	DAS-Ø15Ø7-1 × MON-ØØ6Ø3-6
	59122 × MON810	DAS-59122-7 × MON-ØØ81Ø-6
	59122 × NK603	DAS-59122-7 × MON-ØØ6Ø3-6
	NK603 × MON810	MON-ØØ6Ø3-6 × MON-ØØ81Ø-6

Table 2: Single maize events and subcombinations of maize 1507 × 59122 × MON810 × NK603 previously assessed by the GMO Panel

Events	Application or mandate	EFSA Scientific Opinions
1507	C/NL/00/10 C/ES/01/01 EFSA-GMO-NL-2004-02 EFSA-GMO-RX-1507	2004a 2005a 2005b 2009a
59122	EFSA-GMO-NL-2005-12 EFSA-GMO-NL-2005-23	2007b 2013
MON810	EFSA-GMO-RX-MON810	2009b
NK603	CE/ES/00/01 Article 4 of the Novel Food Regulation (EC) No 258/97 EFSA-GMO-NL-2005-22 EFSA-GMO-RX-NK603	2007c 2004b 2009c 2009c
1507 × 59122	EFSA-GMO-NL-2005-15	2009d
1507 × NK603	EFSA-GMO-UK-2004-05	2006b
59122 × NK603	EFSA-GMO-UK-2005-20	2008
NK603 × MON810	C/GB/02/M3/3 EFSA-GMO-UK-2004-01	2005c 2005d
59122 × 1507 × NK603	EFSA-GMO-UK-2005-21	2009e

All four single maize events, four two-event stacks (1507 × 59122, 1507 × NK603, 59122 × NK603 and NK603 × MON810) and one three-event stack (59122 × 1507 × NK603) have been previously assessed (see Table 2), and no safety concerns were identified.

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, 2007a, EFSA GMO Panel, 2011a).

3.2. Updated information on the events

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

The applicant clarified that the maize 59122 sequence reported in this application corresponds to the sequence submitted in the original application EFSA-GMO-NL-2005-12 of the single event (EFSA, 2007b), but corrected for sequencing errors affecting three single nucleotides.[5] In addition, the applicant clarified that the maize 1507 sequence reported in this application is identical to the corrected maize 1507 sequence.[6] Analysis of the corrected sequencing data and the bioinformatic analyses performed on these sequences did not give rise to safety issues (EFSA GMO Panel, 2016 and 2017 respectively).

Updated bioinformatic analyses of the flanking regions of events 1507, 59122, MON810 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 Cry1F, PAT, Cry34Ab1, Cry35Ab1, Cry1Ab and CP4 EPSPS proteins confirmed previous analyses indicating no significant similarities to toxins or allergens; updated bioinformatics analyses of the newly created open reading frames (ORFs) within the inserts, or spanning the junctions between the inserts and the flanking regions, confirmed previous analyses indicating that the expression of an ORF showing significant similarities to toxins or allergens is highly unlikely.[7]

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 maize 1507 × 59122 × MON810 × NK603

3.3.1. Molecular characterisation

Possible interactions that would affect the integrity of the events, protein expression level, or the biological function conferred by the individual inserts are considered below.

3.3.1.1. Genetics elements and their biological function[8]

Maize events 1507, 59122, MON810 and NK603 were combined by conventional crossing to produce the four event-stack 1507 × 59122 × MON810 × NK603. The structure of the inserts introduced into maize 1507, 59122, MON810 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 1507 × 59122 × MON810 × NK603 are summarised in Table 4.

Based on the known biological function of the newly expressed proteins (Table 4), the only foreseeable interactions at the biological level are between the Cry proteins in susceptible insects (see Section 3.3.4.4).

Table 3: Genetic elements in the expression cassettes of the events stacked in maize 1507 × 59122 × MON810 × NK603

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
1507[(a)]	*ubi*ZM1 (*Zea mays*)	–	–	*cry1F* (*Bacillus thuringiensis*)	ORF25PolyA (*Agrobacterium tumefaciens*)
	35S (CaMV)	–	–	*pat* (*Streptomyces viridochromogenes*)	35S (CaMV)

[5] Additional information: 12/10/2016 and 23/1/2017.
[6] Additional information: 21/8/2017.
[7] Additional information: 19/11/2016 and 9/6/2017.
[8] Part I—Section C.

Event	Promoter	5′ UTR	Transit peptide	Coding region	Terminator
59122	*ubi*ZM1 (*Z. mays*)	–	–	*cry34Ab1* (*B. thuringiensis*)	*pin*II (*Solanum tuberosum*)
	wheat peroxidase (*Triticum aestivum*)	–	–	*cry35Ab1* (*B. thuringiensis*)	*pin*II (*S. tuberosum*)
	35S (CaMV)	–	–	*pat* (*S. viridochromogenes*)	35S (CaMV)
MON810	35S (CaMV) (partial)	I-*Hsp70* (*Z. mays*)	–	*cry1Ab* (*B. thuringiensis*) (partial)	(deleted during integration)
NK603[(b)]	*ract1* (*Oryza sativa*)	*ract1* (*O. sativa*)	*ctp2* (*Arabidopsis thaliana*)	CP4 *epsps* (*Agrobacterium* sp)	*nos* (*A. tumefaciens*)
	35S (CaMV)	I-*Hsp70* (*Z. mays*)	*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.
(a): Maize 1507 also contains partial fragments of the cry1F and pat genes at a single locus in the nuclear genome.
(b): Maize NK603 also includes at the 3′ end an additional 217 bp DNA fragment of the rice actin promoter, lacking sequences needed for promoter activity.

Table 4: Characteristics and intended effects of the events stacked in maize 1507 × 59122 × MON810 × NK603

Event	Protein	Donor organism and biological function	Intended effects in GM plant
1507	Cry1F	Based on a gene from *B. thuringiensis* 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 1507 expresses a synthetic version of the truncated Cry1F protein. Cry1F is a protein toxic to certain lepidopteran larvae feeding on maize
	PAT	Based on a gene from *S. viridochromogenes* strain Tü494. Phosphinothricin-acetyl-transferase (PAT) enzyme confers resistance to the antibiotic bialaphos (Wohlleben et al., 1988)	Event 1507 expresses the PAT protein, which acetylates L-glufosinate-ammonium and thereby confers tolerance to glufosinate ammonium-based herbicides
59122	Cry34Ab1	Based on a gene from *B. thuringiensis* strain PS149B1. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event 59122 expresses a *cry34Ab1* gene which was modified to enhance expression in plants. The amino acid sequence was not modified. Cry34Ab1 is a protein toxic to certain coleopteran larvae feeding on maize
	Cry35Ab1	Based on a gene from *B. thuringiensis* strain PS149B1. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998; Ellis et al., 2002)	Event 59122 expresses a cry35Ab1 gene which was modified to enhance expression in plants. The amino acid sequence was not modified. Cry35Ab1 is a protein toxic to certain coleopteran larvae feeding on maize
	PAT	Based on a gene from *S. viridochromogenes*. Phosphinothricin-acetyl-transferase (PAT) enzyme confers resistance to the antibiotic bialaphos (Wohlleben et al., 1988)	Event 59122 expresses the PAT protein, which acetylates L-glufosinate-ammonium and thereby confers tolerance to glufosinate ammonium-based herbicides
MON810	Cry1Ab	Based on a gene from *Bacillus thuringiensis* subsp. *kumamotoensis*. *B. thuringiensis* is an insect pathogen; its insecticidal activity is attributed to the expression of crystal protein (*cry*) genes (Schnepf et al., 1998)	Event MON810 expresses a *cry1Ab* gene which was truncated at the 3′ end as a result of the integration process. Cry1Ab is a protein toxic to certain lepidopteran larvae feeding on maize

Event	Protein	Donor organism and biological function	Intended effects in GM plant
NK603	CP4 EPSPS	Based on a gene from *Agrobacterium* strain CP4. 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Herrmann, 1995)	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-enopyruvyl-shikimate-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 maize 1507 × 59122 × MON810 × NK603[9]

The genetic stability of the inserted DNA over multiple generations in the single maize events 1507, 59122, MON810 and NK603 was demonstrated previously (see Table 2). Integrity of these events in maize 1507 × 59122 × MON810 × NK603 (F_1 hybrid) was demonstrated by Southern analyses.

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

Protein levels of Cry1F, PAT, Cry34Ab1, Cry35Ab1, Cry1Ab and CP4 EPSPS were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from field trials across three locations in the USA during the 2008 growing season. Samples analysed included leaf (R_1), stalk (R_1), root (R_1), pollen (R_1) and grain (R_6), both treated and not treated with glyphosate and glufosinate ammonium.[11] Grains and forage are the two main raw commodities used for food and feed purposes. The GMO Panel requested protein expression data from forage, but the applicant did not provide this information. In the absence of these data, the data on the levels of the newly expressed proteins in leaves (R_1) were used to estimate the levels in forage. However, although leaves are part of forage, the developmental stage used for forage is usually not R_1, therefore this estimation has a certain degree of uncertainty. To reduce this uncertainty, the GMO Panel assessed the data available for protein expression in forage and leaves from already assessed subcombinations (Table 2). The analysis of these data showed that the levels of the newly expressed proteins in leaves (R_1) were comparable and in general higher than those in forage suggesting that, in this case, using data on expression levels in leaves (R_1) would not result in an underestimation of the expression levels in forage. The highest mean values, regardless of the treatment, of the protein levels in grains and leaves of maize 1507 × 59122 × MON810 × NK603 are summarised in Table 5.

Table 5: Highest mean values and corresponding standard deviations and ranges of protein levels (μg/g dry weight) in grain (n = 30) and leaves (n = 30) from maize 1507 × 59122 × MON810 × NK603

Protein	Tissue/Developmental stage	
	Grain/R6	Leaves/R1
Cry1F	4.3[(b)] ± 1.3[(c)] (NT) 2.5–8.7[(d)]	21 ± 7.2 (T) 13–35
PAT	0.099 ± 0.066 (T) <LOQ–0.36	33 ± 8.9 (T) 23–52
Cry34Ab1	33 ± 10 (T) 12–51	110 ± 46 (T) 53–190
Cry35Ab1	1.3 ± 0.35 (NT) 0.69–2.2	81 ± 20 (T) 42–130

[9] Part I – Sections D.2 and D.5.
[10] Part I – Section D.3.
[11] Part I – Section D.3 and Annexes 4e and 4f.

Protein	Tissue/Developmental stage	
	Grain/R6	Leaves/R1
Cry1Ab	0.24 ± 0.094 (NT) 0.096–0.54	24 ± 4.4 (NT) 17–33
CP4 EPSPS[(a)]	14 ± 3.2 (T) 6.3–20	190 ± 34 (T) 130–270

T/NT: treated / not treated; <LOQ: Values below the limit of quantification.
(a): CP4 EPSPS levels in the maize 1507 × 59122 × MON810 × NK603 are the sum of two protein variants, CP4 EPSPS and CP4 EPSPS L214P, expressed in NK603.
(b): Mean.
(c): Standard deviation.
(d): Range.

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

The levels of all the newly expressed proteins in the four-event stack and the corresponding singles were similar in all tissues except for the expected difference in PAT protein levels resulting from the presence of two copies of the *pat* coding genes in the four-event stack (see Appendix A). Therefore, there is no indication of interaction 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 1507 × 59122 × MON810 × NK603 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the four-event stack and in the single events except for PAT which showed the expected higher levels in the stack resulting from the combination of 1507 and 59122 (producing PAT) events. Therefore, there is no indication of an interaction between the events that may affect their integrity and the levels of the newly expressed proteins in this stack.

Based on the known biological function of the newly expressed proteins, the only foreseeable interactions at the biological level are between the Cry proteins in susceptible insects, which will be dealt with in Section 3.3.4.4.

3.3.2. Comparative analysis

3.3.2.1. Choice of comparator and production of material for the comparative assessment[12]

Application EFSA-GMO-NL-2011-92 presents data on agronomic and phenotypic characteristics, as well as on forage and grain composition of maize 1507 × 59122 × MON810 × NK603 from field trials performed at six sites in the USA during the 2008 growing season.

Maize 1507 × 59122 × MON810 × NK603 was obtained by conventional crossing of the four single events. Events 1507, 59122 and NK603 were introduced in the inbred line PH09B, while event MON810 was in inbred line PH581. As documented by the pedigree, the four single events were combined in a hybrid maize with a genetic background (F_1) of PH09B × PH581. The same two inbred lines (PH09B and PH581) were crossed to produce the non-GM hybrid maize used as comparator. On the basis of the provided pedigree, the GMO Panel considers that hybrid maize PH09B × PH581 is a suitable non-GM comparator.

Field trials for the agronomic, phenotypic and compositional assessment of maize 1507 × 59122 × MON810 × NK603 were conducted at six sites 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 three replicates: the four-event stack maize and the non-GM comparator, both sprayed with plant protection products (PPP) according to local requirements, and the four-event stack maize sprayed with the intended herbicides (glyphosate and glufosinate-ammonium) on top of PPP. In addition, two field trials were performed with non-GM commercial maize varieties only. One was performed at six locations in the USA in 2003

[12] Part I – Sections D.7.1 and D.7.2.

and included four[13] non-GM commercial maize varieties,[14] whereas the other was performed in the USA (five sites) and Canada (one site) in 2007 with four[15] commercial non-GM maize hybrids.[16] Also, in these two trials, a randomised complete block design with three replications was used. These field trials were used to collect agronomic, phenotypic and compositional data from non-GM commercial varieties.

3.3.2.2. Agronomic and phenotypic analysis[17,18]

Fourteen traits related to crop physiology, morphology, development, yield and biotic stress were measured.[19] Data collected for 11 of the 14 parameters were subject to a formal statistical analysis.[20] The other parameters (stalk lodging, root lodging and pollen viability) were not subject to a formal statistical analysis because of the very low variability of the data (more than 80% of the data points had the same value).

In the across-site analysis, no difference was observed between the four-event stack maize and the non-GM comparator for nine of the 11 agronomic and phenotypic parameters. A significant increase was observed for early population in maize 1507 × 59122 × MON810 × NK603 (for both herbicides regimes) compared to the non-GM comparator,[21] and plant height was higher in maize 1507 × 59122 × MON810 × NK603 (not treated with the intended herbicides) than in the non-GM comparator.[22]

The environmental impact of the observed differences in early population and plant height is assessed in Section 3.3.4.

3.3.2.3. Compositional analysis[23]

Maize grain was analysed for 81 parameters[24] and forage for 9 parameters,[25] including the key constituents recommended by the OECD (OECD, 2002). All the data were analysed statistically across locations.[26] When 80% or more of the analytical results for a specific constituent were below the limit of quantification, the constituent was omitted from the statistical analysis. In cases where a significant difference between maize 1507 × 59122 × MON810 × NK603 and the non-GM comparator was identified, the level of the parameter in the four-stack maize was compared to the levels occurring in the eight commercial non-GM maize varieties grown in the USA in 2003 and 2007.[14,15]

[13] Non-GM hybrid maize: 34M94, 33G26, 33J24 and 3394.
[14] Part I – Section D.7.2, Annex 7.
[15] Non-GM hybrid maize: 38B85, 37Y12, 34A15 and 34P88.
[16] Part I – Section D.7.2, Annex 8.
[17] Part I – Section D7.4.
[18] Additional information: 18/8/2014.
[19] The following parameters were analysed: early population count, final population count, seedling vigour, time to silking, time to pollen shed, stalk lodging, root lodging, stay green, disease incidence, insect damage, pollen viability (shape and colour), plant height and ear height.
[20] A linear mixed model was fitted: genotype was the fixed effect, and the random effects were location and block-within-location.
[21] Mean value for early population for the non-GM comparator: 52 plants/plot; mean value for the GM (both herbicide regimes): 57 plants/plot.
[22] Mean value for plant height for the non-GM comparator: 262 cm; mean value for the GM (sprayed with conventional herbicides only): 273 cm.
[23] Part I – Section D7.3, additional information: 18/8/2014.
[24] The following parameters were measured in grain: crude protein, crude fat, crude fibre, acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, carbohydrates, 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), heptadecadienoic acid (C17:2), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), (9,15) isomer of linoleic acid (C18:2), linolenic acid (C18:3), γ-linolenic acid (C18:3), lignoceric acid, nonadecanoic acid (C19:0), arachidic acid (C20:0), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), heneicosanoic acid (C21:0), behenic acid (C22:0), erucic acid (C22:1), tricosanoic acid (C23:0), lignoceric acid (C24:0), methionine, cystine, lysine, tryptophan, threonine, isoleucine, histidine, valine, leucine, arginine, phenylalanine, glycine, alanine, aspartic acid, glutamic acid, proline, serine, tyrosine calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc, beta-carotene, thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid, α-tocopherol, β-tocopherol, δ-tocopherol, γ-tocopherol, total tocopherols, inositol, furfural, *p*-coumaric acid, ferulic acid, phytic acid, raffinose and trypsin inhibitor.
[25] The following parameters were measured in forage: crude protein, crude fat, ADF, crude fibre, NDF, ash, carbohydrates, calcium and phosphorus.
[26] A linear mixed model was fitted: genotype was the fixed effect, and the random effects were location, block-within-location, and genotype-by-location.

In forage, significant differences were observed for calcium and phosphorus between maize 1507 × 59122 × MON810 × NK603 not treated with the intended herbicides (untreated, Table 6) and the non-GM comparator. Taking into account the well-known biological roles of these constituents, the magnitude of the changes, the variability observed among non-GM commercial varieties in the 2003/2007 field trials and the ranges for maize reported in the literature (OECD, 2002), the EFSA GMO Panel concludes that these differences do not pose food and feed safety concerns.

Grains of maize 1507 × 59122 × MON810 × NK603 not treated with the intended herbicides (untreated) were significantly different from those collected the non-GM comparator for seven parameters: the levels of linoleic acid and lignoceric acid were lower in the four-stack maize, while crude fat, oleic acid, phosphorous, potassium and inositol were higher in the four-stack maize (Table 6). The levels of all the significantly different parameters observed in the four-stack maize fell within the range of compositional values obtained from the 2003/2007 field trials[27] and from the literature (OECD, 2002). Taking into account the known biological roles of these constituents, the magnitude of the changes and the variability observed in the non-GM maize varieties, the GMO Panel concluded that no further food and feed safety assessment is required.

Grains of maize 1507 x 59122 x MON810 x NK603 sprayed with the intended herbicides (treated) were significantly different from those collected the non-GM comparator for eight compounds (Table 6). These were increased levels of crude fat, oleic acid, potassium and raffinose, and reduced levels of linoleic acid, lignoceric acid, manganese and zinc in maize 1507 × 59122 × MON810 × NK603 as compared to the non-GM comparator. The levels of all the significantly different parameters observed in the four-stack maize fell within the range of compositional values obtained from the 2003/2007 field trials[20] and from the literature (OECD, 2002). As for untreated maize 1507 × 59122 × MON810 × NK603, the EFSA GMO Panel concluded that the differences observed did not require further food and feed safety assessment.

Table 6: Compositional endpoints (means estimated from US 2008 field trials data) for which significant differences (*) were found between maize 1507 × 59122 × MON810 × NK603 and the non-GM comparator PH09B × PH581 in forage and grain

Component	Maize 1507 × 59122 × MON810 × NK603		Non-GM comparator
	Untreated	Treated	
Forage			
Calcium [mg/kg dw]	2450*	2430	2250
Phosphorus [mg/kg dw]	2640*	2570	2410
Grain			
Crude fat [% dw]	5.26*	5.28*	5.00
Phosphorus [mg/kg dw]	3260*	3150	3010
Potassium [mg/kg dw]	3210*	3110*	2920
Zinc [mg/kg dw]	16.5	16.1*	17.2
Manganese [mg/kg dw]	6.9	6.6*	7.3
Oleic acid [% of total fatty acids]	26.5*	26.0*	24.1
Linoleic acid [% of total fatty acids]	55.6*	55.8*	58.0
Lignoceric acid [% of total fatty acids]	0.212*	0.210*	0.242
Inositol [mg/kg dw]	204*	190	183
Raffinose [mg/kg dw]	1510	1620*	1270

dw: dry weight.

3.3.2.4. Conclusions of the comparative analysis

The EFSA GMO Panel concludes that none of the differences in agronomic and phenotypic characteristics and composition of grain and forage identified between maize 1507 × 59122 × MON810 × NK603 and the non-GM comparator requires further assessment regarding food and feed safety.

[27] Part I – Section D7.2, additional information: 18/8/2014 and 23/5/2016.

The differences in early population and plant height identified between maize 1507 × 59122 × MON810 × NK603 and the non-GM comparator are further assessed for their potential environmental impact in Section 3.3.4.

3.3.3. Food and feed safety assessment

3.3.3.1. Effects of processing[28]

Based on the outcome of the comparative assessment, processing of maize 1507 × 59122 × MON810 × NK603 into food and feed products is not expected to result in products being different from those of commercial non-GM maize varieties.

3.3.3.2. Toxicology[29]

Toxicological assessment of newly expressed proteins[30]

Six proteins (Cry1F, Cry1Ab, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS) are newly expressed in maize 1507 × 59122 × MON810 × NK603 (Table 4). The GMO Panel has previously assessed these proteins individually in the context of the single maize events and no safety concerns were identified for humans or animals (Table 2). The GMO Panel is not aware of any new information that would change these conclusions.

The potential for a functional interaction of these newly expressed proteins in maize 1507 × 59122 × MON810 × NK603 has been assessed with regard to human and animal health.

The two enzymatic proteins (PAT and CP4 EPSPS) catalyse distinct biochemical reactions and act on unrelated substrates in the plant. The four insecticidal proteins (Cry1Ab, Cry34Ab1, Cry35Ab1 and Cry1F) act through cellular receptors found in target insect species. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with specific high affinity to Cry proteins (Hammond et al., 2013; Koch et al., 2015).

On the basis of the known biological function of the newly expressed proteins (Table 4), there is currently no expectation for possible interactions relevant to the food and feed safety assessment of maize 1507 × 59122 × MON810 × NK603. No safety concerns were identified for the individual proteins for humans and animals and the same conclusion can be extended to their presence in maize 1507 × 59122 × MON810 × NK603.

The EFSA GMO Panel concludes that there are no safety concerns to human and animal health related to the newly expressed proteins Cry1Ab, Cry34Ab1, Cry35Ab1, Cry1F, PAT and CP4 EPSPS in maize 1507 × 59122 × MON810 × NK603.

Toxicological assessment of components other than newly expressed proteins[31]

Maize 1507 × 59122 × MON810 × NK603 did not show any compositional difference to its non-GM comparator that would require further assessment (see Section 3.3.2.3). No further food and feed safety assessment of components other than newly expressed proteins is therefore required.

3.3.3.3. Animal studies with the food/feed derived from GM plants[32]

No substantial modifications in the composition of the four-event stack maize, no indication for potential occurrence of unintended effects based on the preceding molecular, compositional or phenotypic analyses, and no indication of possible interactions between the events were identified (Sections 3.3.1, 3.3.2 and 3.3.3.2). Therefore, no animal studies on the food and feed derived from maize 1507 × 59122 × MON810 × NK603 are required (EFSA, 2006a). Nevertheless, the EFSA GMO Panel considered a 42-day study in broilers provided by the applicant.

A total of 720 (360 per sex) one-day old chickens for fattening (Ross 708 broilers) were randomly allocated to six dietary groups with 120 chicks per treatment (12 pens per treatment, 10 birds per pen, half for each sex) and fed diets containing milled grains (650–750 μm) from maize 1507 × 59122 × MON810 × NK603, either unsprayed (-Gly/Glu) or sprayed (+Gly/Glu) with a glyphosate-glufosinate mixture (test items), or from an appropriate non-GM comparator (control item) (Section 3.3.2.1) or one of three non-GM commercial varieties 33H25, 33M15 or 33D11 (reference items).

[28] Part I – Section D.7.6.
[29] Part I – Section D.7.8.
[30] Part I – Section D.7.8.1.
[31] Part I – Section D.7.8.2.
[32] Part I – Section D.7.8.4.

Event-specific PCR analysis on maize grains prior to diet formulation confirmed the identity for events 1507, 59122, MON810 and NK603 of test grains and the lack of contamination in the non-GM comparator and non-GM commercial varieties. Low levels of event NK603 were however detected in reference maize 33H25 and 33D11. Newly expressed proteins (Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1Ab and CP4 EPSPS) were also analysed in maize grains by ELISA. Test, control and reference materials were analysed for proximates, amino acids and mycotoxins and the metabolizable energy was calculated for each maize lot.

Starter (0–21 days), grower (22–35 days) and finisher (36–42 days) diets contained 63%, 67.5% and 74% milled maize grains, respectively, and were balanced to meet nutrient requirements of broilers. Newly expressed proteins (Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1Ab and CP4 EPSPS) in test diets were used to confirm (ELISA test) diet homogeneity and stability of these proteins. Diets were sampled for proximate analysis (including calcium and phosphorus), gross energy and amino acid analysis. Diets (as mash feed) and water were offered ad libitum from day of hatching. Chickens were monitored three times daily for health status and mortality; body weight and feed intake were recorded weekly; body weight gain, feed intake, and mortality-adjusted feed:gain ratio (feed efficiency) were calculated for Days 0–42.

At the end of the feeding period, all survived birds were sacrificed and eight birds per pen (half for each sex) were randomly selected and processed in order to determine liver and kidney weight and post-chilled carcass yield, both as percentages of whole live bird weight and cuttable parts (i.e. thighs, breasts, wings, legs, abdominal fat including fat around gizzard) yield, as the percentage of post-chill dressed carcass weight.

No significant differences in mortality (about 2%), final body weight, weight gain, feed to gain ratio, and yield of pre-chill organs and post-chilled carcass and cuttable part percentages assessed were observed between the groups fed diets containing the four-event stack maize (treated with the intended herbicide or not) or the non-GM comparator (tailored mixed models of analysis of variance).

The GMO Panel concludes that administration of diets containing up to 74% of maize grain 1507 × 59122 × MON810 × NK603 to broilers, up to 42 days, did not cause adverse effects. Moreover, the measured performance endpoints were similar between groups fed balanced diets containing GM and non-GM comparator.

3.3.3.4. Allergenicity[33]

For allergenicity assessment, a weight-of-evidence approach was followed, taking into account all of the information on the newly expressed proteins, since no single piece of information or experimental method yields sufficient evidence to predict allergenicity (EFSA, 2006a; 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 (EFSA GMO Panel, 2011a). 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[34]

The GMO Panel previously evaluated the safety of the Cry1F, Cry34Ab1, Cry35Ab1, PAT, Cry1Ab and CP4 EPSPS proteins individually, and no concerns on allergenicity were identified (Table 2). No new information on allergenicity of the single events that might change the previous conclusions has become available. Based on current knowledge and since none of the newly expressed proteins showed allergenicity, no reasons for concern regarding the presence of these newly expressed proteins in this four-event stack maize affecting 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 Panel has previously evaluated the safety of Cry1F, Cry34Ab1, Cry35Ab1 and Cry1Ab proteins and no concerns on adjuvanticity in the context of the applications assessed were identified (Table 2). The levels of Bt proteins in this four-event stack maize are similar to those in the respective single maize events (see 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 four-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.

[33] Part I – Section D.7.9.
[34] Part I – Section D.7.9.1.

Assessment of allergenicity of GM plant products[35]

The GMO Panel regularly reviews the available publications on food allergy to maize. However, to date, maize has not been considered a common allergenic food[36] (OECD, 2002). Therefore, the EFSA 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 3.3.2 and 3.3.3.2), the GMO Panel identified no indications of a potentially increased allergenicity of food and feed derived from the four-event stack maize compared to that derived from its non-GM comparator.

3.3.3.5. Nutritional assessment of GM food/feed[37]

The intended trait of the four-event stack maize 1507 × 59122 × MON810 × NK603 is insect resistance and herbicide tolerance, with no intention to alter nutritional parameters.

The comparison of grain and forage composition between the four-event stack with its non-GM comparator did not identify differences that would require a nutritional assessment as regards to food and feed (Section 3.3.2.4).

From these data, the nutritional characteristics of the food and feed derived from this four-event stack maize are not expected to differ from those of food and feed derived from its non-GM comparator and non-GM commercial varieties. This was confirmed by a feeding study in broilers (Section 3.3.3.3).

3.3.3.6. Conclusion of the food and feed safety assessment

The newly expressed proteins in the four-event stack maize 1507 × 59122 × MON810 × NK603 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 newly expressed proteins in this four-event stack maize, or regarding the overall allergenicity of this four-event stack maize. Maize 1507 × 59122 × MON810 × NK603 is as nutritious as the non-GM comparator.

3.3.4. Environmental risk assessment

Considering the scope of the application EFSA-GMO-NL-2011–92, which excludes cultivation, the environmental risk assessment (ERA) of maize 1507 × 59122 × MON810 × 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 1507 × 59122 × MON810 × NK603 grains during transportation and/or processing (EFSA GMO Panel, 2010).

3.3.4.1. Persistence and invasiveness of the GM plant[38]

Maize is highly domesticated, not winter hardy in colder regions of Europe, and generally unable to survive in the environment without appropriate management. Occasional feral GM maize plants may occur outside cultivation areas in the EU (e.g. 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 (OECD, 2002). Field observations indicate that maize grains may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g. Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.

It is unlikely that the intended traits of maize 1507 × 59122 × MON810 × NK603 will provide a selective advantage to maize plants, except when they are exposed to glufosinate-ammonium and/or glyphosate-containing herbicides or infested by insect pests that are susceptible to the Cry1Ab, Cry1F or Cry34/35Ab1 proteins.

The GMO Panel considers that the fitness advantage provided by the intended traits, and the observed agronomic and phenotypic differences in early population and plant height (see

[35] Part I – Section D.7.9.2

[36] 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.

[37] Part I – Section D.7.10.

[38] Part I—Section D.9.1

Section 3.3.2.2) will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits and other observed differences will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers very unlikely that maize 1507 × 59122 × MON810 × NK603 will differ from conventional maize hybrid varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable maize 1507 × 59122 × MON810 × NK603 grains.

3.3.4.2. Potential for gene transfer[39]

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

Plant-to-microorganism gene transfer

The potential for horizontal adverse effects of horizontal gene transfer of the recombinant DNA have been assessed for the single events in previous GMO Panel Scientific Opinions (see in Table 2). No concern as a result of an unlikely, but theoretically possible, horizontal gene transfer of the recombinant genes to bacteria in the gut of animal fed GM material or other receiving environments was identified. Synergistic effects of the recombinant genes, for instance due to combinations of recombinogenic sequences, which would cause an increase in the likelihood for horizontal gene transfer or a selective advantage were not identified. Therefore, the EFSA GMO Panel concludes that the unlikely, but theoretically possible, horizontal transfer of recombinant genes from this four-event stack maize to bacteria does not raise any environmental safety concern.

Plant-to plant-gene transfer

The potential for occasional feral GM maize 1507 × 59122 × MON810 × NK603 plants originating from grain import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

For plant-to-plant gene transfer to occur, imported GM maize grains need to germinate and develop into plants in areas containing sympatric wild relatives and/or cultivated maize with synchronous flowering and environmental conditions favouring cross-pollination.

Vertical gene transfer from maize is limited to *Zea* species. Wild relatives of maize outside cultivation are not known/reported in Europe (Eastham and Sweet, 2002; OECD, 2003; EFSA, 2016; Trtikova et al., 2017). Therefore, potential vertical gene transfer is restricted to maize and weedy *Zea* species, such as teosintes, and/or maize-teosinte hybrids, occurring in cultivated areas (EFSA, 2016, Trtikova et al., 2017).

The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.3.4.1). The likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low. Even if cross-pollination would occur, the GMO Panel is of the opinion that 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 for the reasons given in Section 3.3.4.1, even in the case of treatment with the intended herbicides.

3.3.4.3. Interactions of the GM plant with target organisms[40]

Taking the scope of the application EFSA-GMO-NL-2011-92 into account, potential interactions of occasional feral maize 1507 × 59122 × MON810 × NK603 plants arising from grain import spills with the target organisms are not considered a relevant issue by the GMO Panel.

3.3.4.4. Interactions of the GM plant with non-target organisms[41]

Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled GM grains is limited and because most proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of maize 1507 × 59122 × MON810 × NK603 with non-target organisms are not

[39] Part I—Section D 9.3 and additional information: 19/11/2015 and 09/6/2017.
[40] Part I – Section D9.4.
[41] Part I – Section E3.4.

considered a relevant issue by the GMO Panel. Interactions that may occur between the Cry proteins (as mentioned in Section 3.3.1.1) will not alter this conclusion.

3.3.4.5. Interactions with the abiotic environment and biogeochemical cycles[42]

Given that environmental exposure to spilled grains or occasional feral maize 1507 × 59122 × MON810 × NK603 plants arising from grain import spills is limited and because most proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions 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

The GMO Panel concludes that it is unlikely that maize 1507 × 59122 × MON810 × NK603 would differ from conventional maize varieties in its ability to persist under the EU environmental conditions. Considering the scope of the application EFSA-GMO-NL-2011-92, interactions of occasional feral maize 1507 × 59122 × MON810 × NK603 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of horizontal gene transfer from this four-stack maize to bacteria does not indicate a safety concern. Therefore, considering the combined traits and their interactions, the outcome of the comparative analysis, the routes and levels of exposure, the GMO Panel concludes that maize 1507 × 59122 × MON810 × 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 maize 1507 × 59122 × MON810 × NK603

No new data on the single maize events 1507, 59122, MON810 and NK603 leading to a modification of the original conclusions on their safety were identified.

The combination of events 1507, 59122, MON810 and NK603 in the four-event stack maize does not raise issues relating to molecular, agronomic/phenotypic or compositional characteristics that would require further investigation in terms of food and feed safety and nutrition.

The newly expressed proteins in the four-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 1507 × 59122 × MON810 × NK603. Comparison of the levels of the newly expressed proteins between the four-event stack and each of the single events did not reveal an interaction at protein expression level.

Considering the combined traits and the outcome of the comparative analysis, and routes and levels of exposure, the GMO Panel concludes that maize 1507 × 59122 × MON810 × 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 four-event stack was retrieved in a literature search covering the period since the time of validity of the application.[43] The GMO Panel concludes that the four-event stack maize is as safe and as nutritious as its non-GM comparator in the context of its scope.

3.4. Risk assessment of the subcombinations

Subcombinations previously assessed in the frame of other applications are discussed in Section 3.4.1.

The strategy followed for the assessment of those subcombinations for which no specific data have been submitted and which have not been previously assessed by the GMO Panel (Table 1), has been described by the GMO Panel.[44] In this case, the risk assessment takes as its starting point the assessment of the single maize events, and uses the data generated for the four-event stack, as well as all the additional data available on subcombinations previously assessed by the GMO Panel.

[42] Part I – Section E3.6.
[43] Additional information: 7/7/2016 and 9/6/2017.
[44] 115th GMO Panel meeting (Annex 1 of the minutes: http://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf).

3.4.1. Subcombinations previously assessed

The two-event stack maize 1507 × 59122, 1507 × NK603, 59122 × NK603 and NK603 × MON810, and the three-event stack maize 59122 × 1507 × NK603 have been assessed previously by the GMO Panel, and no safety concerns were identified (Table 1). A literature search revealed no new scientific information relevant to the risk assessment of these stack maize that became available since the validation of the application EFSA-GMO-NL-2011-92.[45] Consequently, the GMO Panel considers that its previous conclusions on these subcombinations remain valid.

3.4.2. Subcombinations not previously assessed

Five subcombinations of maize 1507 × 59122 × MON810 × NK603 have not been previously assessed: the two-event stack maize 1507 × MON810 and 59122 × MON810 and the three-event stacks 1507 × 59122 × MON810, 1507 × MON810 × NK603 and 59122 × MON810 × NK603. Experimental data for four of these subcombinations (1507 × MON810, 59122 × MON810, 1507 × 59122 × MON810, and 1507 × MON810 × NK603) were provided in this application and are further discussed in Section 3.4.2.2. A literature search revealed no scientific information relevant to the risk assessment of these subcombinations that became available since the validation of this application.[44]

3.4.2.1. Stability of the events

The genetic stability of the inserted DNA over multiple generations in the four single maize events was demonstrated previously (Table 2). Integrity of the events was demonstrated in maize 1507 × 59122 × MON810 × NK603 (Section 3.3.1.2) and in previously assessed maize subcombinations (Table 2). The GMO Panel finds no reasons to expect the loss of integrity of the events in the maize subcombinations not previously assessed.

3.4.2.2. Expression of the events

Protein expression levels were measured for the newly expressed proteins in two subcombinations, maize 59122 × MON810 and maize 1507 × MON810 × NK603, in material harvested from plants grown in the same field trials as maize 1507 × 59122 × MON810 × NK603 (described in Section 3.3.1.3). For all tissues, the levels of the newly expressed proteins in these two subcombinations were similar to the levels observed in the singles and those observed in the four-event stack maize except for PAT, which showed the expected higher levels in the four-event stack resulting from the combination of events 1507 and 59122 both producing PAT protein.[46] From these data, there is no indication of an interaction that may affect the levels of the newly expressed proteins in these subcombinations.

Protein expression data obtained from a greenhouse study were provided for maize 1507 × MON810, 59122 × MON810 and 1507 × 59122 × MON810. These data did not indicate any interactions that may affect protein expression level; however, the GMO Panel notes that greenhouse experiments are not considered as informative as field trials.[47]

The analysis of the levels of the newly expressed proteins in four out of the five subcombinations of maize 1507 × 59122 × MON810 × NK603 did not indicate interactions that may affect their expression. The GMO Panel considered that based on these data, and 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 subcombination for which no data were provided.

This assumption was further confirmed by comparing the levels of the newly expressed proteins of each single maize event with those of the four-event stack maize. The levels were similar in the four-stack maize and in the single events except for PAT, which showed the expected higher levels in the stack resulting from the combination of events 1507 and 59122 both producing PAT protein (Section 3.3.1.3 and Appendix A). Therefore, there was no indication of an interaction manifesting at protein expression level. In addition, expression data from the two-event stacks maize 1507 × 59122, 1507 × NK603, 59122 × NK603 and NK603 × MON810 (EFSA, 2005d, 2006b, 2008, 2009d) and the three-event stack maize 59122 × 1507 × NK603 (EFSA, 2009e) were similar to those observed in each of the single maize events or showed the expected higher levels for PAT resulting from the

[45] Additional information: 9/6/2017.
[46] Annexes 4a–d
[47] Annex 5

combination of 1507 and 59122 events both producing PAT protein. This confirms that interactions affecting expression levels of the newly expressed proteins are not expected in the five maize subcombinations not previously assessed and included in the scope of application EFSA-GMO-NL-2011-92.

3.4.2.3. Potential interactions between the events

The GMO Panel assessed the potential for interactions between events, due to their combination in the two-event stack maize 1507 × MON810 and 59122 × MON810; and the three-event stacks 1507 × 59122 × MON810, 1507 × MON810 × NK603 and 59122 × MON810 × NK603, taking into consideration the intended traits and potential 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 1507 × MON810; 59122 × MON810; 1507 × 59122 × MON810; 1507 × MON810 × NK603 and 59122 × MON810 × NK603 relevant for the food/feed or environmental safety.

The GMO Panel took into account the intended and any potential unintended effects considered in the assessment of the four single events, of the two-event stack maize 1507 × 59122, 1507 × NK603, 59122 × NK603 and NK603 × MON810 and the three-event stack maize 59122 × 1507 × NK603. It was concluded that none of these effects would raise safety concerns when combined in any of these maize subcombinations. Therefore, the GMO Panel is of the opinion that no additional data are needed to complete the assessment of subcombinations of the four-event stack maize.

3.4.3. Conclusion

Maize 1507 × 59122 × MON810 × NK603 has 10 possible subcombinations, of which five have been previously assessed (Table 2). Since no new safety concerns were identified for these previously assessed two-event and three-event stack maizes, the GMO Panel considers that its previous conclusions on these subcombinations remain valid.

The remaining five subcombinations included in the scope of application EFSA-GMO-NL-2011-92 were not previously assessed. For four of these subcombinations protein expression data were provided and did not indicate an interaction affecting the levels of the newly expressed proteins. The GMO Panel assessed the possibility of interactions between the events in these five subcombinations and concluded that these combinations would not raise safety concerns. The five subcombinations not previously assessed are expected to be as safe as the single maize events, the previously assessed two-event stack maize 1507 × 59122, 1507 × NK603, 59122 × NK603 and NK603 × MON810, the three-event stack maize 59122 × 1507 × NK603, as well as the four-event stack maize 1507 × 59122 × MON810 × NK603.

3.5. Post-market monitoring

3.5.1. Post-market monitoring of GM food/feed

No relevant compositional, agronomic and phenotypic changes were identified in maize 1507 × 59122 × MON810 × NK603 when compared with the non-GM comparator. Furthermore, the overall intake or exposure is not expected to change because of the introduction of maize 1507 × 59122 × MON810 × NK603 into the market.

The two-event maize stacks 1507 × 59122, 1507 × NK603, 59122 × NK603 and NK603 × MON810 and the three-event stack maize 59122 × 1507 × NK603 have been previously assessed and no safety concerns were identified. The five subcombinations not previously assessed and included in the scope of application EFSA-GMO-NL-2011-92 are expected to be as safe as the single maize events, the previously assessed maize subcombinations and the four-event stack maize 1507 × 59122 × MON810 × NK603. Therefore, the GMO Panel considers that post-market monitoring of maize 1507 × 59122 × MON810 × NK603 and its subcombinations is not necessary.

3.5.2. Post-market environmental monitoring

The objectives of a 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) 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 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 methodology of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b).

As the ERA did not identify potential adverse environmental effects from the maize 1507 × 59122 × MON810 × NK603, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for maize 1507 × 59122 × MON810 × NK603 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 and (3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the authorisation period.

The EFSA GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 1507 × 59122 × MON810 × NK603 and its subcombinations. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

4. Overall conclusions and recommendations

No new information on the four single maize events 1507, 59122, MON810 and NK603 leading to a modification of the original conclusions on their safety were identified.

The combination of events 1507, 59122, MON810 and NK603 in the four-event stack maize did not give rise to issues relating to molecular, agronomic/phenotypic and compositional characteristics regarding food and feed safety. The newly expressed proteins in the four-event stack maize did not raise concerns for human and animal health. The compositional data indicated that maize 1507 × 59122 × MON810 × NK603 is expected to be as nutritious as its non-GM comparator.

The GMO Panel concludes that maize 1507 × 59122 × MON810 × NK603 is as safe and as nutritious as its non-GM comparator in the context of the scope of this application.

The GMO Panel concluded that there is a very low likelihood of environmental effects resulting from the accidental release of viable grains from maize 1507 × 59122 × MON810 × NK603 into the environment.

Since no new data on the five previously assessed subcombinations of maize 1507 × 59122 × MON810 × NK603 that would lead to a modification of the original conclusions on their safety were identified, the GMO Panel considers that its previous conclusions on these maize stacks remain valid.

Five subcombinations were not previously assessed. For four of these subcombinations, protein expression data were provided and did not indicate an interaction affecting the levels of the newly expressed proteins. The GMO Panel assessed the possibility of interactions between the events in the five subcombinations not previously assessed and concluded that these combinations would not raise safety concerns. The five subcombinations not previously assessed are expected to be as safe as the single maize events, the previously assessed two-event stack maize 1507 × 59122, 1507 × NK603, 59122 × NK603 and NK603 × MON810, the three-event stack maize 59122 × 1507 × NK603, as well as the four-event stack maize 1507 × 59122 × MON810 × NK603.

Given the absence of safety concerns for food and feed derived from maize 1507 × 59122 × MON810 × NK603 and its subcombinations, the GMO Panel considers that post-market monitoring of these products is not necessary. The PMEM plan and reporting intervals are in line with the intended uses of maize 1507 × 59122 × MON810 × NK603 and its subcombinations.

Documentation provided to EFSA

1) Letter from the Competent Authority of the Netherlands received on 3 February 2011 concerning a request for placing on the market of genetically modified maize 1507 × 59122 × MON 810 × NK603 submitted by Pioneer Overseas Corporation in accordance with Regulation (EC) No 1829/2003 (application reference EFSA-GMO-NL-2011-92).
2) Acknowledgement letter dated 15 February 2011 from EFSA to the Competent Authority of the Netherlands.
3) Letter from EURL-GMFF dated 21 February 2011 requesting additional information under completeness check.

4) Letter from EFSA to applicant dated 30 March 2011 requesting additional information under completeness check.
5) Letter from applicant to EFSA received on 17 May 2011 providing additional information under completeness check.
6) Letter from EURL-GMFF dated 26 May 2011 requesting additional information under completeness check.
7) Letter from EFSA to applicant dated 9 June 2011 requesting additional information under completeness check.
8) Letter from applicant to EFSA received on 8 August 2011 providing clarifications.
9) Letter from EURL-GMFF dated 30 September 2011 requesting additional information under completeness check.
10) Letter from applicant to EFSA received on 25 October 2011 providing additional information under completeness check.
11) Letter from EFSA to applicant dated 22 November 2011 requesting additional information under completeness check.
12) Letter from applicant to EFSA received on 9 January 2012 providing additional information under completeness check.
13) Letter from EFSA to applicant dated 30 January 2012 delivering the 'Statement of Validity' of application EFSA-GMO-NL-2011-92 for placing on the market of genetically modified maize 1507 × 59122 × MON 810 × NK603 submitted by Pioneer Overseas Corporation in accordance with Regulation (EC) No 1829/2003.
14) Letter from EURL-GMFF to EFSA dated 22 February 2012 requesting EFSA to stop the clock.
15) Letter from EFSA to applicant dated 1 March 2012 requesting additional information (EURL-GMFF) and stopping the clock.
16) Letter from EFSA to applicant dated 13 April 2012 requesting additional information and maintaining the clock stopped.
17) Letter from applicant to EFSA received on 31 May 2012 providing additional information.
18) Letter from EFSA to applicant dated 11 July 2012 requesting additional information and maintaining the clock stopped.
19) Letter from applicant to EFSA received on 14 August 2012 extending the timeline for submission of responses.
20) Letter from EFSA to applicant dated 24 September 2012 requesting additional information and maintaining the clock stopped.
21) Letter from applicant to EFSA received on 2 October 2012 providing additional information.
22) Letter from applicant to EFSA received on 7 November 2012 extending the timeline for submission of responses.
23) Letter from applicant to EFSA received on 10 December 2012 providing additional information.
24) Letter from EURL-GMFF to EFSA dated 11 December 2012 requesting EFSA to re-start the clock.
25) Letter from EFSA to applicant dated 9 January 2013 requesting additional information and maintaining the clock stopped.
26) Letter from applicant to EFSA received on 20 February 2013 providing additional information.
27) Letter from EFSA to applicant, dated 23 October 2013, re-starting the clock.
28) Letter from EFSA to applicant dated 7 January 2014 requesting additional information and stopping the clock.
29) Letter from EFSA to applicant dated 14 March 2014 requesting additional information and maintaining the clock stopped.
30) Letter from applicant to EFSA received on 8 April 2014 extending the timeline for submission of responses.
31) Letter from applicant to EFSA received on 6 May 2014 providing additional information.
32) Letter from EFSA to applicant dated 13 June 2014 requesting additional information and maintaining the clock stopped.
33) Letter from applicant to EFSA received on 4 July 2014 extending the timeline for submission of responses.
34) Letter from applicant to EFSA received on 6 August 2014 providing additional information.
35) Letter from applicant to EFSA received on 18 August 2014 providing additional information.

36) Letter from EFSA to applicant dated 27 October 2014 requesting additional information and maintaining the clock stopped.
37) Letter from EFSA to applicant dated 15 December 2014 requesting additional information and maintaining the clock stopped.
38) Letter from applicant to EFSA received on 5 February 2015 providing additional information.
39) Letter from applicant to EFSA received on 7 April 2015 extending the timeline for submission of responses.
40) Letter from applicant to EFSA received on 7 October 2015 extending the timeline for submission of responses.
41) Letter from applicant to EFSA received on 19 November 2015 providing additional information.
42) Letter from applicant to EFSA received on 25 November 2015 providing additional information.
43) Letter from EFSA to applicant dated 11 December 2015 requesting additional information and maintaining the clock stopped.
44) Letter from applicant to EFSA dated 10 March 2016 extending the timeline for submission of responses.
45) Letter from applicant to EFSA dated 7 April 2016 extending the timeline for submission of responses.
46) Letter from applicant to EFSA received on 23 May 2016 providing additional information.
47) Email from EFSA to applicant, dated 31 May 2016, re-starting the clock from 23 May 2016.
48) Letter from EFSA to applicant dated 1 June 2016 requesting additional information and stopping the clock.
49) Letter from applicant to EFSA received on 7 July 2016 providing additional information.
50) Email from EFSA to applicant, dated 8 July 2016, re-starting the clock from 7 July 2016.
51) Letter from EFSA to applicant dated 07 July 2015 requesting additional information and stopping the clock.
52) Letter from EFSA to applicant dated 3 August 2016 requesting additional information and stopping the clock.
53) Letter from applicant to EFSA received on 12 October 2016 providing additional information.
54) Email from EFSA to applicant, dated 13 October 2016, re-starting the clock from 12 October 2016.
55) Letter from EFSA to applicant dated 17 October 2016 requesting additional information and stopping the clock.
56) Letter from applicant to EFSA dated 25 November 2016 extending the timeline for submission of responses.
57) Letter from applicant to EFSA received on 23 January 2017 providing additional information.
58) Email from EFSA to applicant, dated 24 January 2017, re-starting the clock from 23 January 2017.
59) Letter from EFSA to applicant dated 8 March 2017 requesting additional information and stopping the clock.
60) Letter from EFSA to applicant dated 18 May 2017 requesting additional information and maintaining the clock stopped.
61) Letter from applicant to EFSA received on 9 June 2017 providing additional information.
62) Letter from applicant to EFSA dated 30 June 2017 extending the timeline for submission of responses.
63) Letter from applicant to EFSA received on 21 August 2017 providing additional information.
64) Email from EFSA to applicant, dated 22 August 2017, re-starting the clock from 21 August 2017

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Abbreviations

CaMV	cauliflower mosaic virus
Cry	crystal protein
CTP	chloroplast transit peptide
dw	dry weight
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
IgE	immunoglobulin E
LOQ	limit of quantification
ORF	open reading frame
PAT	phosphinothricin acetyl transferase
PMEM	post-market environmental monitoring
PPP	plant protection product
UTR	untranslated region

Appendix A – Protein expression data

Means, standard deviation and ranges of protein levels (μg/g dry weight) from maize 1507 × 59122 × MON810 × NK603, 1507, 59122, MON810 and NK603 from field trials performed in USA in 2008[48]

	1507 × 59122 × MON810 × NK603	1507	59122	MON810	NK603
Cry1F					
Pollen (R1)	26[(a)] ± 3.7[(b)] 19–34[(c)]	26 ± 3.6 20–35			
Leaf (R1)	18 ± 4.1 11–25	19 ± 3.8 13–29			
Stalk (R1)	7.4 ± 0.83 6.4–9.4	7.7 ± 0.71 6.0–9.4			
Root (R1)	4.1 ± 1.7 1.2–6.6	4.0 ± 1.7 1.4–6.9			
Grain(R6)	4.3 ± 1.3 2.5–8.7	4.3 ± 1.4 <LOQ–6.9			
Cry34Ab1					
Pollen (R1)	58 ± 6.5 44–72		61 ± 8.3 50–90		
Leaf (R1)	85 ± 13 66–110		83 ± 13 60–110		
Stalk (R1)	51 ± 4.6 44–60		53 ± 8.5 44–82		
Root (R1)	33 ± 19 5.7–57		31 ± 15 7.8–54		
Grain(R6)	30 ± 9.0 14–54		31 ± 9.9 17–60		
Cry35Ab1					
Pollen (R1)	<LOQ		<LOQ		
Leaf (R1)	76 ± 11 57–96		71 ± 13 49–100		
Stalk (R1)	16 ± 1.9 13–20		23 ± 5.8 15–34		
Root (R1)	10 ± 5.2 2.4–23		11 ± 3.4 3.0–18		
Grain(R6)	1.3 ± 0.35 0.69–2.2		1.3 ± 3.2 0.84–2.0		
PAT					
Pollen (R1)	<LOQ	<LOQ	<LOQ		
Leaf (R1)	30 ± 5.1 20–43	8.5 ± 1.7 5.1–13	18 ± 3.9 9.0–30		
Stalk (R1)	0.31 ± 0.078 0.22–0.56	0.35 ± 0.35 <LOQ–1.1	0.95 ± 0.87 0.16–2.6		
Root (R1)	1.2 ± 0.87 <LOQ–2.4	0.28 ± 0.17 <LOQ–0.60	0.79 ± 0.53 <LOQ–1.5		
Grain(R6)	0.075 ± 0.019 <LOQ–0.15	<LOQ	0.072 ± 0.015 <LOQ–0.15		
Cry1Ab[1]					
Pollen (R1)	<LOQ			<LOQ	
Leaf (R1)	24 ± 4.4 17–33			24 ± 8.2 13–38	

[48] Part I – Section D.3 and Annex 4e.

	1507 × 59122 × MON810 × NK603	1507	59122	MON810	NK603
Stalk (R1)	5.9 ± 0.57 4.8–7.2			6.6 ± 0.98 4.8–7.8	
Root (R1)	5.2 ± 2.5 1.4–9.9			5.3 ± 1.7 1.4–7.5	
Grain(R6)	0.24 ± 0.094 0.096–0.54			0.26 ± 0.074 0.084–0.39	
CP4 EPSPS[1]					
Pollen (R1)	260 ± 20 230–290				260 ± 19 230–290
Leaf (R1)	180 ± 15 150–200				170 ± 38 110–240
Stalk (R1)	62 ± 4.5 52–74				63 ± 8.0 48–86
Root (R1)	43 ± 15 15–66				42 ± 16 6.9–63
Grain(R6)	8.9 ± 3.0 3.9–17				12 ± 3.0 7.2–20

[1]The 1507 × 59122 × MON810 × NK603 samples analysed for Cry1Ab and CP4 EPSPS come from plants treated with the intended herbicide.
(a): Mean.
(b): Standard deviation.
(c): Range.